

Chapter 7. Epidemiology/Human Data

Health Assessment for 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) and Related Compounds

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CHAPTER 7. EPIDEMIOLOGY/HUMAN DATA
PART A: CANCER EFFECTS

CONTENTS

7.1. INTRODUCTION	7-1
7.2. SCOPE	7-1
7.3. PREVIOUS EPA REVIEWS	7-4
7.4. REVIEW METHODS	7-5
7.5. FOLLOW-UP STUDIES OF CHEMICAL MANUFACTURING AND PROCESSING WORKERS	7-8
7.5.1. United States	7-8
7.5.2. Germany	7-18
7.5.3. Ten-Country Study by International Agency for Research on Cancer ...	7-25
7.5.4. Other Studies	7-28
7.5.5. Summary	7-34
7.6. CASE-CONTROL STUDIES IN GENERAL POPULATIONS	7-37
7.6.1. Sweden	7-38
7.6.2. United States	7-47
7.6.3. New Zealand	7-54
7.6.4. Italy	7-61
7.6.5. Summary	7-63
7.7. STUDIES OF PULP AND PAPER MILL WORKERS	7-65
7.8. OTHER STUDIES	7-67
7.8.1. Vietnam Veterans	7-68
7.8.2. Residents of Seveso, Italy	7-70
7.8.3. Rice Oil Poisonings in Taiwan and Japan Involving Compounds Structurally Related to Dioxin	7-74
7.9. CONCLUSIONS	7-77

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CONTENTS (continued)

REFERENCES FOR CHAPTER 7, PART A	7-82
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CHAPTER 7. EPIDEMIOLOGY/HUMAN DATA

PART A: CANCER EFFECTS

7.1. INTRODUCTION

Animal bioassay data provide substantial presumptive evidence of the human carcinogenicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) (see Chapter 6), but confirmation must come from well-designed human studies. TCDD is a multiorgan carcinogen in animals. Target organs include the liver, thyroid, lung, skin, and soft tissues. There is no assumption of target tissue concordance from animals to humans, although site concordance would add support to a causal interpretation. This chapter reports on the cancer epidemiology evidence of TCDD and its congeners.

This review and analysis of the epidemiologic literature on dioxins and cancer begins by defining the scope of chemical exposures, cancers, and research reports to be considered. Then, following a brief summary of previous EPA assessments of epidemiologic literature, a description is given of the methods used in the present review. The original research reports are then discussed in four groups: 1) follow-up studies of chemical manufacturing and processing workers, 2) case-control studies in general populations, 3) studies of pulp and paper mill workers, and 4) other studies (including studies of pesticide applicators, Vietnam veterans with potential exposure to Agent Orange, residents of Seveso, Italy, exposed to TCDD during an accidental explosion, and victims of contaminated rice oil poisonings). Because the discussions of the first two groups of studies are relatively lengthy, brief summaries are given at the end of each of those sections. Conclusions are drawn following an overall discussion of all the studies.

7.2. SCOPE

Epidemiologic studies of cancer among persons exposed to TCDD and other polychlorinated dibenzodioxins (PCDDs) and dibenzofurans (PCDFs) are included in this

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review. Primary emphasis is placed on studies with exposures to TCDD itself, occurring primarily in the manufacture and use of 2,4,5-trichlorophenol, hexachlorophene, and the herbicide 2,4,5-trichlorophenoxyacetic acid (2,4,5-T). Because exposures to 2,4,5-T and 2,4-dichloroacetic acid (2,4-D) often occur among the same groups who manufacture and use these herbicides, some studies of groups exposed only to 2,4-D are also included. Exposure to lower-chlorinated PCDDs (dichlorinated and trichlorinated isomers) may occur in the manufacture and use of 2,4-D. Also included are studies of groups exposed to higher-chlorinated PCDDs (i.e., the hexachlorinated, heptachlorinated, and octachlorinated isomers), occurring primarily in the manufacture and use of pentachlorophenol and in the paper and pulp industries.

A major weakness in nearly all of these studies is the lack of good exposure information. Most studies rely solely on interviews and questionnaires of work history to ascertain exposure surrogates. There is little, if any, verification of actual internal dose to these compounds. Some studies use chloracne as a surrogate for exposure to TCDD. Three of the recent cohort studies of production workers (Fingerhut et al., 1991; Manz et al., 1991; Zober et al., 1990) do provide estimates of TCDD exposure in cohort samples via serum blood levels taken decades after cessation of exposure. These can be used to determine possible dose-response trends and estimate the risk of cancer to populations with low-level exposure to TCDD (see Chapter 8). Measures of exposure by individual study will be discussed.

At the time of EPA's last review in 1988, evidence of human carcinogenicity of TCDD and the phenoxy herbicides focused on soft tissue sarcomas (STSs) and malignant lymphomas. Consequently, this report will update and analyze the evidence pertaining to these cancers. But also included are evaluations of the evidence of cancer at other sites. The case-control studies reviewed for EPA's last analysis generally considered herbicide applicators with potential exposures to both 2,4-D and 2,4,5-T. Recent case-control studies of U.S. farmer groups in which exposure to 2,4-D and 2,4,5-T can be separated (Hoar et al., 1986; Zahm et al., 1990; Cantor et al., 1992) provide a validation mechanism to separate

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potential effects of these herbicides and, possibly, their different PCDD contaminants. Thus, these and other recent studies (Hardell and Eriksson, 1988; Eriksson et al., 1990; Woods et al., 1987) will be reviewed and compared with those discussed in EPA's earlier reports.

Four recent cohort mortality studies (Fingerhut et al., 1991; Manz et al., 1991; Zober et al., 1990; Saracci et al., 1991) totaling over 23,000 workers potentially exposed to TCDD and/or phenoxy herbicides/chlorophenols provide a new important database for analyzing cancer effects, and these will be reviewed first. The first three of these, and especially the large U.S. study of Fingerhut et al. (1991), are considered to be the most important new studies in the field of TCDD cancer epidemiology because of their attention to cohort selection and to TCDD exposures or exposure surrogates (chloracne). The fourth study (Saracci et al., 1991), while having the largest cohort, has less information on the TCDD-exposed subcohort and no information that would allow a quantitative estimate of exposure. Two other studies of phenoxy acid manufacturers are included (Lynge, 1985, 1993; Coggon et al., 1986), but their usefulness is limited due to the low unsubstantiated exposure to TCDD. Also included is a study of occupationally exposed women (Kogevinas et al., 1993) who had probable exposure to TCDD.

Three recent cohort studies of workers in the pulp and paper mill industry are included (Robinson et al., 1986; Jäppinen et al., 1987; Henneberger et al., 1989) because of potential for worker exposure to higher-chlorinated PCDDs, but not hexa-, hepta-, or octaphenoxy herbicides. However, none of these studies provide any additional information about which PCDD exposures were likely, and these studies are not given much weight.

Studies of Vietnam veterans potentially exposed to TCDD in Agent Orange are reviewed briefly, with only one (Michalek et al., 1990) judged to have sufficient information on potential TCDD exposure to be useful for analysis. Also, the recent studies of the Seveso, Italy, residents are discussed (Bertazzi et al., 1992, 1993, 1989a, b; Mocarelli et al., 1991; Pesatori et al., 1992); these studies provide some exposure data, but the cancer response analysis is limited due to inadequate follow-up time.

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Finally, the studies of the rice oil poisonings of residents in Taiwan and Japan with polychlorinated biphenyl (PCB) and PCDF contaminants are reviewed (Kuratsune et al., 1988, 1975; Chen et al., 1980; Koda and Masuda, 1975; Rogan et al., 1988). Even though these poisoned oils did not contain TCDD, they did contain many TCDD-like congeners currently considered by EPA to have carcinogenic potential, which can be compared to TCDD. Also, certain dioxin-like PCBs are suspected carcinogens based on their receptor-binding characteristics and on animal studies.

Only follow-up and case-control studies are considered in this review. Case reports, other clinical observations, and prevalence surveys are excluded. The review is restricted to studies that have been published in full and that are available in the open scientific literature. Prepublication reports and studies published only in abbreviated form (as well as abstracts or letters to editors) are included only where they supplement the published articles. These restrictions limit the review to studies that have received at least a minimum of peer review and that have been described fully enough to permit a thorough assessment of materials, methods, and results.

7.3. PREVIOUS EPA REVIEWS

In the Health Assessment Document for Polychlorinated Dibenzo-*p*-Dioxins, dated September 1985 (U.S. EPA, 1985), the majority of the epidemiology studies pertained to groups of herbicide applicators with potential exposure to phenoxy acids and/or chlorophenols. In that report, the analysis emphasized case-control studies of soft tissue sarcomas and non-Hodgkin's lymphoma (NHL). That report concluded that the epidemiologic research available at that time provided "limited evidence for the carcinogenicity of phenoxy acids and/or chlorophenols in humans. However, with respect to the dioxin impurities contained therein, the evidence for the human carcinogenicity for 2,3,7,8-TCDD based on the epidemiologic studies is only suggestive because of the difficulty of evaluating the risk of 2,3,7,8-TCDD exposure in the presence of the confounding effects of phenoxy acids and/or chlorophenols." In its next report, the review draft dated June 1988

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of A Cancer Risk-Specific Dose Estimate for 2,3,7,8-TCDD (U.S. EPA, 1988), the focus was essentially the same, and EPA concluded that "the human evidence supporting an association between exposure to 2,3,7,8-TCDD and cancer is considered inadequate."

7.4. REVIEW METHODS

This review will follow the spirit of the EPA Risk Assessment Guidelines of 1986 (U.S. EPA, 1987) by considering alternative explanations for results observed in epidemiologic studies. These explanations fall into the general categories of causality, chance, bias, and confounding. The basic approach is akin to a process of elimination, by which one attempts to determine the direction and to quantify the magnitude of the influence that chance, bias, and confounding may have had on the results of each study. Wherever possible, the results of all studies will be reported in units of relative risk estimates and 95% confidence limits.

Most biases in epidemiologic studies can be placed into one of two categories: biases of classification and biases of selection. Classification biases can result from inaccurate ascertainment of exposure, disease, or confounders. Selection biases can result from nonrepresentative sampling of populations, as in the selection of controls in case-control studies, or from incomplete participation by study subjects. Any bias gains tenability as an explanation for an observed result if empirical evidence can be adduced to buttress the mere suggestion that the bias might have occurred. Only those biases considered to be potentially important will be addressed explicitly in this review.

When imprecise exposure estimates are available, such as with much of the epidemiologic data on dioxin, estimates of risk can be potentially biased toward the null. Misclassification, if random, could potentially lead to a masking of a true effect.

Classification biases are of two kinds: differential versus nondifferential. Differential misclassification will lead to either an exaggeration or an underestimation of an effect. Nondifferential misclassification occurs when the exposure or disease classification is incorrect in a portion of the subjects (cases or controls). This type of bias is generally

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toward the null, and the risk estimate may reflect this. This can happen when some subjects are classified as having exposure to dioxin when they really were not exposed. Similarly, some actually may have had exposure but were classified as not having had it. In studies where few or no effects were seen, researchers must seriously consider the problem of nondifferential misclassification. This can be the reason why nonpositive risk estimates or even disparate risk estimates are seen from different studies of the same effect. On the other hand, in studies with significant results, nondifferential misclassification is not likely to be a cause of a significant finding.

Recall bias may produce the opposite effect. Persons with a disease may tend to remember exposure to a substance better when it is known to them that such exposure might be associated with the disease. This could potentially lead to inflated risk estimates. In fact, it has been suggested that such biases are present in many of the case-control studies on dioxin. The Swedish studies by Hardell and colleagues have been particularly singled out for criticism in this respect. That some recall bias may be present is confirmed in a later case-control study (Hardell and Eriksson, 1988) in which some reduction of risk estimates was produced by the use of cancer controls.

Confounding bias is a tenable explanation for an association between an exposure and a disease if the hypothetical confounder can be named and if a good case can be made that it is a cause of the disease, that it was associated with the exposure in the study population, and that it was not adequately controlled in the study design or data analysis. This review will explicitly mention only those potential confounders that meet all of these criteria.

As stressed in previous EPA reviews (1985, 1988), concomitant exposures present a special problem of potential confounding in the literature on TCDD and related chemicals. As a noteworthy example, an association between 2,4,5-T exposure and a given cancer, if causal, could be due to 2,4,5-T itself, to TCDD, or to some other contaminant. The problem multiplies when it is recognized that, historically, many phenoxy acid herbicide preparations were mixtures of 2,4,5-T and 2,4-D and that many persons who manufactured, processed, and used these preparations were exposed to other chemicals as well.

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Nevertheless, it may be possible by examining studies of persons exposed to different combinations of chemicals to identify "threads" of commonality and differences in the results, especially when specific cancers are considered separately.

Publication bias, sometimes considered a form of selection bias, is the tendency for the results of a study to influence a judgment as to whether or not it will be published. The direction and magnitude of publication bias is difficult, if not impossible, to quantify. It is expected to be a much greater problem in literature reviews and in studies relying on existing records than in original research in which substantial resources are devoted to collection of data of relatively high quality. The level of effort required for such studies creates a strong incentive to publish the results. There is a tendency to publish studies with positive results as opposed to studies with nonpositive findings.

Strength of association, as measured by the magnitude of the estimated relative risk, is an important feature of a study's results. The stronger the association, the stronger a bias or confounding factor would have to be to explain it. Because questions of bias and confounding are study-specific, no defensible criteria can be set up in advance to place relative risk values into categories of strength of association.

Trends in increased risk by degree of exposure and by time since first exposure (latency) are also important. Different hypothetical causal mechanisms might predict different exposure-response and latency patterns. Hypotheses of steadily increasing effect with increasing exposure (i.e., monotonic exposure-response functions) and hypotheses of effects early in the carcinogenic process (e.g., for factors that operate at the initiation stage) predict that increases in risk will be greatest among persons with relatively high degrees of exposure and after relatively long latency periods. Hypotheses of tumor promotion and/or initiation will be discussed in the appropriate sections.

Replication of results is important in all scientific research. When several studies have shown a positive association of effect with the same exposure but were conducted under different circumstances, the possibility that an unknown confounder or chance produced the observed elevated effect is minimized. When different investigators working with different

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populations using different methods confirm an original finding, the results are more believable.

The statistical aggregation of results from different studies (meta-analysis) has become a popular feature of epidemiologic literature reviews. In this review, results from separate studies are aggregated only when all key methodologic features and results are reasonably similar. The method of aggregation used here is to take the ratio of the sum of the cause-specific observed deaths to the sum of the cause-specific expected deaths for the individual studies. Because investigators recognize the value of varying their methods to test methodologic hypotheses, and because results often differ appreciably, aggregation of results is not often indicated and is done here with caution.

7.5. FOLLOW-UP STUDIES OF CHEMICAL MANUFACTURING AND PROCESSING WORKERS

7.5.1. United States

Fingerhut and colleagues (1990, 1991) reported a study of 5,172 males who had worked at 12 plants in the United States in the production of chemicals contaminated with TCDD. Five thousand of the cohort members (97%) were identified in company records as having been "assigned to a production or maintenance job in a process involving TCDD contamination" (Fingerhut et al., 1991). The remaining 172 cohort members were "identified in a previously published study on the basis of exposure to TCDD" (Fingerhut et al., 1991). This cohort subsumed, and thereby supplanted, company-specific cohorts from Dow Chemical USA (Ott et al., 1987; Cook, 1981) and the Monsanto Company (Zack and Gaffey, 1983; Zack and Suskind, 1980) that had been the subject of previous reports. This study was initiated in 1978 to determine whether health effects were apparent in humans who were exposed to 2,4,5-T. In 1978, toxicological, teratogenic, and carcinogenic effects data were released that indicated a cancer effect in animals. There was a concern about the potential effects of exposure to Agent Orange on Vietnam veterans and workers who produced products that were contaminated with dioxin (Fingerhut et al., 1992). Follow-up

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began in 1940 or on the date of the "first systematically documented assignment to a process involving TCDD contamination" (Fingerhut et al., 1991), whichever was later, and closed at the end of 1987. Comparisons were made with the United States population.

The authors stated that approximately 13% of the cohort of 5,172 workers had records of chloracne. The presence of chloracne in a group of people is an indicator of relatively intense exposure to TCDD. It can be caused by higher-chlorinated PCDDs, PCDFs, and PCBs as well (O'Malley et al., 1990). It is a highly specific indicator of exposure because it virtually never occurs among unexposed persons. It is a nonsensitive indicator, however, because many highly exposed persons do not develop it (Manz et al., 1991; Caramaschi et al., 1981; Mocarelli et al., 1991). Exposure to other dioxin-like chemicals that can be found in the workplace may produce a form of chloracne that could be indistinguishable from that produced by dioxin (Ott et al., 1993). These chemicals can be dioxin-like in their effects and act through the aryl hydroxylase (Ah) receptor.

Although all members of the cohort had specific assignments to TCDD exposure areas in common, exposures to multiple chemicals are the rule rather than the exception in the chemical industry. At one plant, for instance, considerable overlap existed among persons involved in the production of chlorophenols, 2,4,5-T, and 2,4-D (Ott et al., 1987; Bond et al., 1988, 1989a), and who were thus exposed to TCDD and higher-chlorinated and lower-chlorinated PCDDs. Presumably, many persons throughout the cohort had contact with substantial numbers of other chemicals. Although comprehensive surveys of chemical exposures were conducted in plant-specific cohorts, the results are not available.

Special attention was paid to results for the 3,036 workers who were followed for at least 20 years after first exposure. This group was again divided into those with less than 1 year (N=1,516) and those with more than 1 year (1,520) of exposure (referred to below as the long duration/latency subcohort). One year was chosen as the criterion for duration of exposure because an analysis of 253 workers from 2 plants showed that every worker with 1 or more years of exposure had a lipid-adjusted serum TCDD level greater than the mean value (7 ppt) in a comparison group of unexposed workers (Fingerhut et al., 1990).

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Although the average level for all 253 workers was 233 ppt, the average increased to 418 ppt in those 119 who were exposed for 1 or more years (Fingerhut et al., 1991). The researchers described a plan to replace this duration-based exposure scale by using "a dioxin exposure matrix constructed from historic process descriptions, analytic measurements of TCDD and industrial hygiene data . . . to develop the relative ranking of workers exposed to TCDD" (Fingerhut et al., 1990).

The cohort as a whole experienced an estimated 15% (95% CI=1.0-1.3) elevation of mortality from all cancers combined, with a 46% elevation (95% CI=1.2-1.8) among those in the long duration/latency subcohort (Table 7-1). An excess of deaths from cancers of connective and soft tissues (STSs) was apparent in the total cohort (RR=3.4, CI=0.9-8.6) and in the long duration/latency subcohort (RR=9.2, CI=1.9-27.0), but these results were based on only four deaths and three deaths, respectively, from two different plants. A 40% overall elevation in deaths (CI=0.7-2.5) from non-Hodgkin's lymphoma was confined to workers in the total cohort and was not seen in the long duration/latency subcohort. Results for Hodgkin's disease were highly imprecise, based on only three deaths (vs. 2.5 expected) in the total cohort. Lung cancer was elevated by 10% overall but by 40% (CI=1.0-1.9) in the long duration/latency subcohort. A similar 40% excess of stomach cancer (CI=0.4-3.5) in this subcohort was based on only four deaths; no excess was seen in the total cohort.

The investigators conducted a special study of connective and soft tissue cancers. A review of all available hospital records and tissue specimens failed to confirm the indications of soft tissue sarcomas on two of the four death certificates that had been assigned to this cause-of-death category (Fingerhut et al., 1990). The review also provided evidence that two persons in plant 8 whose deaths had been assigned to other causes of death had actually had soft tissue sarcomas. Because only the exposed cohort's death certificates were subjected to detailed review, the analytic comparisons with the United States population were required to be based strictly on death certificate information. The basic and well-known rule in such situations is that, absent evidence to the contrary, erroneous information on death certificates must be considered to have been equally frequent in the two groups being compared. Suruda

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Table 7-1. Relative Risks of Selected Cancers in Study of Chemical Manufacturing Workers Exposed to TCDD in United States, by Exposure Duration and Latency

Cancer	Measure ^a	Latency ≥20 years			Total cohort
		Latency <20 years	Exposure <1 year	Exposure ≥1 year	
Connective and soft tissues	Observed deaths	1	0	3	4
	Relative risk	1.4	0.02	9.22	3.4
	95% confidence interval	0.1 - 7.0	0.0 - 15.0	1.90 - 27.0	0.9 - 8.6
Hodgkin's disease	Observed deaths	2	0	1	3
	Relative risk	1.1	0.0	2.8	1.2
	95% confidence interval	0.2 - 3.5	0.0 - 15.0	0.1 - 15.3	0.3 - 3.3
Non-Hodgkin's lymphomas	Observed deaths	6	2	2	10
	Relative risk	1.6	1.5	0.9	1.4
	95% confidence interval	0.7 - 3.4	0.2 - 4.9	0.1 - 3.30	0.7 - 2.5
Lung cancer	Observed deaths	32	17	40	89
	Relative risk	0.9	1.0	1.4	1.1
	95% confidence interval	0.7 - 1.3	0.6 - 1.5	1.0 - 1.9	0.9 - 1.4
Stomach cancer	Observed deaths	3	3	4	10
	Relative risk	0.6	1.8	1.4	1.0
	95% confidence interval	1.5 - 1.6	0.4 - 5.2	0.4 - 3.5	0.5 - 1.9
All combined	Observed deaths	103	48	114	265
	Relative risk	1.05	1.02	1.46	1.15
	95% confidence interval	0.8 - 1.2	0.76 - 1.4	1.2 - 1.8	1.0 - 1.3

^aRelative risks and confidence intervals are based on rounded values and may differ slightly from those in the original reports (Fingerhut et al., 1990, 1991).

Source: Fingerhut et al., 1991.

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et al. (1993), in a study of STS diagnoses in cohorts exposed to dioxins and chlorinated naphthalenes, found that death certificates are "relatively insensitive" for detecting STS and that the power of life table analysis to detect excess risks of STS may be reduced compared with its utility in correctly estimating the risk of other cancers, such as colon cancer or rectal cancer. However, the correct identification of STS as the underlying cause of death on death certificates appears to be much better (82%), based on medical records, than first thought according to the results of this study. Medical records on the remaining 18% could not be found. If a death certificate gives as an underlying cause of death a soft tissue sarcoma and it is coded as 171x, it is very likely correctly coded according to the authors. In an earlier study, this figure was estimated by Percy et al. (1981) to be 55%.

Four of the soft tissue sarcomas that are discussed in Fingerhut's study (two are included in Fingerhut's life table analysis while two others are discussed but did not qualify for inclusion in the life table analysis) are actually from the Nitro, West Virginia, plant. The remaining two cases that were included in Fingerhut's life table analysis are from a different plant in the study. One of these cases also suffered chloracne. The four cases from the Nitro, West Virginia, plant are also the subject of a later study by Collins et al. (1993). The two cases included in Fingerhut's life table analysis had previously suffered chloracne from the 1949 accident (Zack and Gaffey, 1983) in which 121 workers developed chloracne as a result of a trichlorophenol process accident on March 8, 1949. Collins included one more STS not previously discussed by Fingerhut from the same plant. The Collins study is reviewed later.

If the properly diagnosed STSs were correctly assigned to their appropriate plants and the two incorrectly diagnosed STSs were removed, there would be one in plant 9 and three in plant 8. No STSs were found at the other plants. This unusually skewed distribution might possibly be related to the accident that happened in plant 8 some 44 years earlier. Or perhaps physicians in Nitro, West Virginia, are more likely to diagnose STS than are physicians in other areas of the country. Because of the rarity of STS, there was inadequate statistical power to expect to see STS in the other individual plants. If this cancer has a long

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latent period and an excess risk is associated with exposure to 2,3,7,8-TCDD, then it might be several more years before this cohort will produce STSs at the other facilities that are part of the study.

In any case, this nondifferential misdiagnosis can potentially bias risk estimates downward.

Cases of soft tissue sarcoma include a diverse group of histological entities. All soft tissue sarcomas arise only from mesenchymal tissue and all share common features that make them alike in their basic intercellular and intracellular composition rather than different in their morphology and location. Characterizing them as fundamentally different because they are found in different sites of the body is perhaps inadequate for the determination of the risk of cancer (Enzinger and Weiss, 1988).

The histological classification of STS is centered on a dozen distinctly different classes of mesenchymal cells that form six relatively well-defined but widely distributed organ systems. By considering the growth pattern and cell morphology with an evaluation of intracellular and extracellular products of the tumor cells, fairly precise histogenetic classification of soft tissue sarcomas is possible (Hajdu, 1981). For human cancer risk assessment, all connective tissues developing from the same mesodermal tissue, expressing the same set of "proto-oncogenes" and surrounded by the same chemical milieu of the extracellular matrix, are expected to develop cancer following exposure to certain carcinogens. The grouping of these end-target organs together is therefore a necessary measure to evaluate 2,3,7,8-TCDD human carcinogenicity.

Confounding by cigarette smoking must be considered in interpreting the approximate 40% excess of lung cancer deaths in the long duration/latency subcohort (Table 7-2). For the United States as a whole, the authors (Fingerhut et al., 1990) computed age-adjusted proportions of 24% never smokers, 19% former smokers, and 57% current smokers in 1965 (roughly midway through the follow-up period). The corresponding proportions were 28% never smokers, 14% former smokers, and 59% current smokers among the 87 workers from the study of serum TCDD levels who were members of the long duration/latency subcohort

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Table 7-2. Relative Risks of Lung Cancer in Subcohort of Chemical Manufacturing Workers Exposed to TCDD in United States for at Least 1 Year and With at Least 20 Years Latency, Adjusted for Alternative Hypotheses About its Smoking Distribution

Proportion of subcohort (percent) ^a			Expected lung cancer deaths (40 observed)	Relative risk	95 % confidence interval
Never smokers	Former smokers	Current smokers			
24	19	57	28.8	1.4	1.0 - 1.9
28	14	59	29.2	1.4	1.0 - 1.8
25	10	65	30.5	1.3	0.9 - 1.8
20	15	65	31.2	1.3	0.9 - 1.7
15	15	70	33.2	1.2	0.9 - 1.6
10	20	70	33.9	1.2	0.9 - 1.6
15	10	75	34.4	1.2	0.8 - 1.6
10	15	75	35.1	1.1	0.8 - 1.5

^aThe first set of proportions assumes no difference between the subcohort and the United States population. The second set is based on 87 surviving members of the subcohort (Fingerhut et al., 1990). The remaining sets are hypothetical values used to test the sensitivity of the results. Relative risks of lung cancer are assumed to be 4.7 for former smokers and 10.9 for current smokers (Fingerhut et al., 1990).

Source: Fingerhut et al., 1991.

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as well. Assuming relative risks of lung cancer of 4.7 for former smokers and 10.9 for current smokers, the authors used a standard technique (Axelson and Steenland, 1988) to adjust the number of expected lung cancer deaths and found essentially no change in the results. It should be kept in mind that the sample of smoking histories was taken from only 2 plants, numbers 1 and 2, but the excess in lung cancer risk was chiefly in plants 8 and 10. The generalization of smoking habits of employees in 2 of the 12 participating plants to that of the entire cohort may not be representative of the true smoking impact of the risk of lung cancer to the entire cohort. Most of the lung cancers came from 3 facilities (56 of 89 observed lung cancer deaths), plant numbers 8, 9, and 10. The remaining 7 plants contributed the remaining 33 lung cancers to the total due to the small sizes of the respective subcohorts and insufficient latency. It then would be possible to evaluate the effect of smoking on the risk of lung cancer at each individual plant. It should be remembered that national U.S. rates were used to derive expected deaths in each of the 12 plants. It is possible that local or regional rates at the locality of each of the plants may be a more appropriate comparison population for the cancer sites examined by the authors, although local rates may be unstable. In addition, if biomonitoring could be extended to the remaining 10 plants, a better idea could be derived concerning the dose levels that are associated with those plants experiencing higher lung cancer rates. The authors point out that deaths from other diseases associated with smoking such as diseases of the heart and circulatory system were either not increased or significantly decreased in this cohort (Fingerhut et al., 1990). This led the authors to conclude that "cancers of the respiratory tract . . . may result from exposure to TCDD, although we cannot exclude the possible contribution of factors such as smoking and occupational exposure to other chemicals" (Fingerhut et al., 1991).

One possible explanation for the increase follows from the fact that the comparison of smoking habits between the United States population and the 87 surviving members of the long duration/latency subcohort did not show the well-known tendency for smoking to be more common among blue-collar workers than in the general population. A possible explanation for this result is that because smoking appreciably elevates the overall death rate,

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fewer and fewer smokers will remain in a fixed group of persons as time goes by. Thus, the use of the 87 *surviving* members of the long duration/latency subcohort may have underestimated the proportions of former and current smokers in the subcohort as a whole over the course of mortality follow-up. However, this same phenomenon is also present in the population from which expected deaths were generated, so the effect is probably nullified. As shown in Table 7-2, the actual proportions would not have to be inordinately high for smoking to have exerted appreciable confounding on the estimated relative risks of lung cancer. The lack of increased mortality from cardiovascular diseases as well as cancer of the buccal cavity and pharynx in this cohort, however, makes this explanation less likely. Furthermore, as the authors point out, mortality from nonmalignant respiratory disease (standard mortality ratio [SMR] = 96), which is often associated with smoking, was less than expected.

On the other hand, the authors report a correlation coefficient of 0.72 between length of exposure and serum TCDD tissue level. This negative bias is potentially much greater than the positive bias that could possibly be produced by smoking. And because nondifferential bias is the only type of bias that could occur in the Fingerhut study, risk estimates are more than likely to be lower than the true risk. The only way that dioxin exposure could have a positive bias is if it prevents cancer and the exposure classification is 100% wrong.

The authors of this study also report that two of these cancer deaths were of mesothelioma, a finding that indicates more than likely exposure to asbestos. Although these two mesotheliomas occurred at plants 9 and 12, it is not known whether these persons were exposed in the current job or perhaps in some previous job. If exposure occurred throughout this cohort, it is possible that asbestos also could be a confounding factor for lung cancer. The combination of smoking and exposure to asbestos fiber might have a synergistic confounding effect on the risk of lung cancer.

The positive association of lung cancer in male workers observed in this study is also consistent with an excess of pulmonary tumors found in male mice and rats exposed to

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TCDD (see Chapter 6). These same animal data suggest the possibility of a protective hormonal effect from TCDD and the risk of pulmonary cancer in female rats. Because this study dealt with only male workers, this hypothesis could not be verified in female workers. On the other hand, no elevated risk of liver cancer is evident in male workers even in the long duration/latency subcohort. This is also consistent with rat data where the tumors were only observed in female rats. If there is a promoting effect on liver cancer in females due to hormonal effects as suggested by the rat studies, it could not be verified.

In a recently published paper (Collins et al., 1993), it is suggested that there is an association of STS with exposure to 4-aminobiphenyl. The authors reported that workers who developed chloracne from an accident in which a chemical mix containing TCDD was scattered throughout a 2,4,5-trichlorophenol plant had increased mortality from soft tissue sarcoma, bladder cancer, and respiratory cancer. All individuals who were identified in this study of 754 chemical employees as having STSs or lung cancers, and who were employed at the time of the 1949 accident, were potentially exposed to 4-aminobiphenyl as well as to 2,3,7,8-TCDD. However, it is not known how many were actually working inside the plant when the accident occurred. 4-Aminobiphenyl is thought to be a bladder carcinogen from previous studies. However, this chemical has not been shown to be associated with STS or lung cancer in humans. Unfortunately, no tissue measurements are available to substantiate exposure to 2,3,7,8-TCDD or exposure to 4-aminobiphenyl. It is also of interest that no significant increase in STSs was noted in the "chloracne-free" subgroup exposed to 4-aminobiphenyl. However, the authors report that an additional 106 persons also had indications of chloracne-type conditions noted in their medical files, presumably as a result of exposure to 2,3,7,8-TCDD and not as a result of the accident. Although these individuals probably were heavily exposed to dioxin as well, they were included in the "no chloracne" subgroup for the purpose of analysis. It would be of some interest to see how much of an effect would occur to the risk estimates if they were included in the "chloracne" subgroup. Furthermore, plant employees who left work before March 8, 1949, or began work after November 22, 1949, were not included even if they had received exposure to dioxin or even

developed chloracne as a result of exposure. The major interest according to the authors was in the 122 workers who developed chloracne from the 1949 accident. The authors point out that the numbers are small and that confounding factors, such as misclassification of exposure, cannot be ruled out. This study presents an interesting theory that needs to be investigated further. However, at this time, it has not been substantiated anywhere.

It was also noted by Collins that toxicological evidence is available that supports the idea that STSs (i.e., angiosarcomas) have resulted from exposure to 4-aminobiphenyl (Schieferstein et al., 1985). Angiosarcomas and bladder cancer in females were found to be dose-related with oral consumption of 4-aminobiphenyl in drinking water. However, angiosarcomas of the liver arise mainly from the endothelial lining of blood vessels. This type of tissue is more likely to be susceptible to a hydrophilic carcinogen such as 4-aminobiphenyl during its passage through the blood vessel. Hydrophobic carcinogens such as 2,3,7,8-TCDD might be expected to exert an influence on mesenchymal tissue from which most STSs arise, i.e., fibrosarcoma, histiocytoma, liposarcoma, leiomyosarcoma, rhabdomyosarcoma, synovial sarcoma, schwannoma, myxoid neurogenic sarcoma, and others. Therefore, the author's assumption that 4-aminobiphenyl can cause other types of STSs remains unproven and highly unlikely.

7.5.2. Germany

Manz and colleagues (1991) reported a study of 1,583 persons (1,184 men and 399 women) employed at a German chemical manufacturing facility that produced 2,4,5-T and its precursor, 2,4,5-trichlorophenol. In 1954, a chloracne outbreak had occurred in the working population of the plant, and after that, production of the TCDD contaminant was reduced. Cohort members worked at least 3 months from 1952 through 1984. The start of follow-up was not stated in the report, but presumably began on the date of accumulation of 3 months of employment. The follow-up period closed at the end of 1989. The cohort's mortality experience was compared with that of the West German population and with that of a cohort of workers at a gas supply company. Because the results did not differ materially between

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the analyses, and because limited data on the gas workers forced that comparison to be based on a subset of the TCDD-exposed cohort, only the results of the comparisons with West Germany are reported here.

The cohort was postdivided by duration of employment and by a three-category exposure scale based on TCDD measurements "in nonsystematic samples of precursor materials, products, waste, and soil from the grounds of the plant, mainly after the plant had closed in 1984" (Manz et al., 1991). This scale was validated to some extent by adipose tissue TCDD levels in 48 volunteers (mean = 296 ng/kg in 37 persons from the highest group, 83 ng/kg in 11 persons from the other two groups). Based on these results, the low and intermediate groups are combined for the present analysis.

For the males, this study, with 75 total cancer deaths expected and 24 expected in the high-exposure subcohort, was considerably smaller than the study by Fingerhut et al. (1991), which had 230 total cancer deaths expected and 78 in its long duration/latency subcohort (Table 7-3). Manz et al. presented detailed analyses only for all cancers combined. The high-exposure subcohort, and especially those with longer employment duration, experienced an excess of total cancer deaths (RR=1.4, CI=1.0-2.0 for the high-exposure group and RR=2.6, CI=1.2-4.9 for the high exposed/long duration subcohort) (Table 7-3). The authors concluded that "the increase in (total) cancer risk of 1.24-1.39 . . . cannot be explained completely by confounding factors, and . . . is associated with exposure to TCDD" (Manz et al., 1991). In a later abstract of an update of this same paper, Dwyer (1992) reported that using a Cox regression analysis of nine major areas of employment within the plant, the area of work with the strongest relative risk for cancer mortality was found to be in 2,4,5-T production (RR=2.7, CI=1.7-4.2). These findings are similar to those of Fingerhut et al. (1991).

For the cohort as a whole, the estimated relative risk of lung cancer was 1.4 (CI=1.0-2.0, 30 observed deaths). Smoking as an explanation for the observed increase in lung cancer mortality is less likely because a comparison using the gas worker reference actually leads to an increased RR of 1.7 (CI=1.1-2.4). Although smoking histories were not

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Table 7-3. Relative Risks of all Cancers Combined in Study of Chemical Manufacturing Workers Exposed to TCDD in Germany, by Duration and Category of Exposure

Exposure duration	Measure	Exposure category (median adipose TCDD level)		
		Low and medium (60 ng/kg)	High (137 ng/kg)	Total
<20 years	Observed deaths	49	26	75
	Relative risk	1.1	1.2	1.1
	95 % confidence interval	0.8 - 1.4	0.8 - 1.8	0.9 - 1.4
≥20 years	Observed deaths	10	8	18
	Relative risk	1.5	2.6	1.9
	95 % confidence interval	0.8 - 2.7	1.2 - 4.9	1.1 - 2.9
Total	Observed deaths	59	34	93
	Relative risk	1.2	1.4	1.2
	95 % confidence interval	0.9 - 1.5	1.0 - 2.0	1.0 - 1.5

Source: Manz et al., 1991.

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available for the entire Boehringer cohort, of the 361 men, 73% reported that they smoked. Similarly, 76% of 2,860 gas workers smoked. Substantial confounding based on smoking does not appear to be present because smoking seems to be similar in both plants. The estimate for stomach cancer was 1.2 (CI=0.7-2.1, 12 observed deaths). Three deaths from non-Hodgkin's lymphomas and no connective and soft tissue cancer deaths were observed. (The authors described an additional three deaths from chronic lymphocytic leukemia as non-Hodgkin's lymphoma deaths, but these deaths would not have been classified as non-Hodgkin's lymphomas in the other studies in this review.) Expected numbers of deaths from these cancers were not given. Based on the proportions of expected cancer deaths in the Fingerhut study, one might estimate that approximately 2.4 non-Hodgkin's lymphoma deaths and 0.4 connective and soft tissue cancer deaths would have been expected in this cohort as a whole and about 0.1 connective and soft tissue cancer deaths in the high-exposure subcohort. (The numbers of expected deaths from lung cancer, stomach cancer, or non-Hodgkin's lymphoma in the high-exposure subcohort were not estimated because information is lacking on how many of the observed deaths from these cancers were in that subcohort.)

The authors reported exposure to other industrial chemicals, such as benzene and dimethylsulphite. In addition, manual laborers were exposed to asbestos "probably" to some extent. However, the authors maintain that this exposure explains neither the increased mortality from all cancers nor the patterns of associations with TCDD exposure groups.

Other possible sources of bias include a potential lack of comparability between cause of death ascertainment based on medical records in some, perhaps many, cohort members versus use of death certificates only for cause of death certification in the derivation of German national death rates. This is somewhat alleviated by the use of gas workers as a second comparison group. In these workers, the same methods were used for medical certification, making comparison of cause of death somewhat more comparable. However, this is offset by the fact that the gas workers may have somewhat better mortality experience because they had to work a minimum of 10 years in order to obtain entrance into the gas workers cohort, whereas the dioxin cohort had to work only a minimum of 3 months. This

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could have introduced survivorship bias in this group and consequently lower mortality and higher risk estimates when compared with dioxin workers.

Furthermore, the lack of an analysis of the mortality data by time since first exposure for individual causes such as lung cancer makes it impossible to assess latent effects.

Of the 399 female cohort members, only 7% worked in high-exposure departments. In total, there were 54 deaths and an overall $RR=0.8$ ($CI=0.6-1.0$). The RR for all cancers was 0.9 ($CI=0.6-1.4$), but the $RR=2.2$ ($CI=1.0-4.1$) for breast cancer was significantly increased based on 9 deaths. This is an interesting result in view of a suggestion of reduced mammary cancer based on mechanistic studies and animal bioassays. However, at this point the data do not provide a sufficient basis for any conclusions. Of all the worker follow-up studies with TCDD, this is the only one that reports on a cohort of females.

In another investigation in Germany, Zober and colleagues (1990) studied persons employed at a German chemical manufacturing facility where 2,4,5-trichlorophenol was produced. An uncontrolled decomposition reaction in 1953 and subsequent cleanup activities resulted in substantial TCDD exposures. The cohort contained 247 persons who had worked at the plant from 1953 through 1987, 51% of whom had developed chloracne or erythema (a skin condition suggestive of chloracne), with mortality follow-up covering the same calendar period. Seventy-eight persons had died, $RR=0.95$; 23 had died of cancer, $RR=1.2$ ($CI=0.8-1.7$). Expected deaths were based on national mortality rates in the Federal Republic of Germany. When workers with chloracne were looked at separately, the risk of cancer as expressed by the SMR rose to 1.4 ($CI=0.9-2.1$). Again, within this highly exposed subgroup, if the analysis is restricted to only those workers who were observed 20 or more years after first employment, the SMR was significant at 2.0 ($CI=1.2-3.2$). For lung cancer, the SMR is of borderline significance at 2.5 ($CI=1.0-5.3$). The authors report that the results "... do not support a strong association between cancer mortality and TCDD, but they do suggest that some hazard may have been produced."

Three subcohorts were defined on the basis of procedures by which cohort members were identified as having potential for varying degrees of exposure. Subcohort C1 contained

69 persons known to be exposed to TCDD during the accident period. Cohorts C2 (84 persons) and C3 (94 persons) contained workers thought to be exposed to lesser amounts of TCDD. Recent TCDD levels in blood samples from small numbers of persons in each group suggested that exposures had been higher in C1 (median 24.5 ppt, 11 samples) than in C2 (median 9.5 ppt, 7 samples) or C3 (median 8.4 ppt, 10 samples). Thus, C2 and C3 are grouped together in this review. A separate analysis by the authors divides the group into the 127 persons with chloracne (N=114) and erythema (N=13) versus those 120 persons with neither. The average serum TCDD levels in the two subcohorts are 15 ppt and 5.8 ppt, based on 16 samples (with chloracne) and 12 samples (without chloracne), respectively. The two stratifications provide similar results. Only the first is presented here. Section 8.5 provides an additional analysis.

This study, with only 20 expected cancer deaths in the total cohort and 4 expected cancer deaths in the members of subcohort C1 with 20 or more years of latency, is much smaller than the studies by Fingerhut et al. (1991) and Manz et al. (1991). The authors, however, did provide detailed analyses of data on specific cancers (Table 7-4). Elevated mortality rates from lung cancer, stomach cancer, and all cancers combined were confined largely to the members of subcohort C1 with long latency. The confidence intervals for the relative risk estimates are extremely wide, however. No deaths from cancers of connective and soft tissues or from non-Hodgkin's lymphomas were observed, and expected numbers of deaths from these cancers were not reported. However, one mesothelioma was reported in a plant supervisor with known asbestos exposure. Based on the proportions of all expected cancer deaths due to these cancers in the study by Fingerhut et al. (1991), one might estimate that approximately 0.6 non-Hodgkin's lymphoma deaths and 0.1 connective and soft tissue cancer deaths would have been expected in this cohort as a whole, and about 0.2 non-Hodgkin's lymphoma deaths and less than 0.1 connective and soft tissue cancer deaths among the members of subcohort C1 with long latency. This study lacks power to detect a significant site-specific cancer risk at most sites due to its small size.

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Table 7-4. Relative Risks of Selected Cancers in Study of Chemical Manufacturing Workers Exposed to TCDD in Germany, by Median Blood TCDD Level and Latency

Subcohort (median blood TCDD level)	Cancer	Measure	Time since first exposure		Total
			<20 years	≥20 years	
C1 (24.5 ppt)	Lung	Observed deaths	1	3	4
		Relative risk	1.2	2.5	2.0
		95% confidence interval	0.1 - 6.2	0.6 - 6.9	0.6 - 4.8
	Stomach	Observed deaths	1	2	3
		Relative risk	2.0	4.0	3.0
		95% confidence interval	0.1 - 9.7	0.7 - 13.2	0.8 - 8.1
	All combined	Observed deaths	2	7	9
		Relative risk	0.7	1.7	1.3
		95% confidence interval	0.1 - 2.4	0.7 - 3.3	0.6 - 2.4
C2 (9.5 ppt) and C3 (8.4 ppt)	Lung	Observed deaths	0	2	2
		Relative risk	0.0	1.0	0.5
		95% confidence interval	0.0 - 1.8	0.2 - 3.4	0.1 - 1.8
	Stomach	Observed deaths	0	0	0
		Relative risk	0.0	0.0	0.0
		95% confidence interval	0.0 - 3.2	0.0 - 3.9	0.0 - 1.7
	All combined	Observed deaths	5	9	14
		Relative risk	0.8	1.3	1.1
		95% confidence interval	0.3 - 1.9	0.6 - 2.4	0.6 - 1.8
Total cohort	Lung	Observed deaths	1	5	6
		Relative risk	0.4	1.6	1.1
		95% confidence interval	0.0 - 2.0	0.6 - 3.5	0.4 - 2.2
	Stomach	Observed deaths	1	2	3
		Relative risk	0.7	1.6	1.1
		95% confidence interval	0.0 - 3.4	0.3 - 5.2	0.3 - 3.0
	All combined	Observed deaths	7	16	23
		Relative risk	0.8	1.5	1.2
		95% confidence interval	0.4 - 1.6	0.9 - 2.3	0.8 - 1.7

Source: Zober et al., 1990.

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7.5.3. Ten-Country Study by International Agency for Research on Cancer

A historical cohort study of cancer mortality in 18,390 production workers or sprayers exposed to chlorophenoxy herbicides and/or chlorophenols was reported on by Saracci et al. (1991). Exposure was reconstructed through questionnaires, factory or spraying records, and job histories. Workers were classified as exposed (N=13,482), probably exposed (N=416), exposure unknown (N=541) and nonexposed (N=3,951). The exposed group contains everyone known to have sprayed chlorophenoxy herbicides and everyone who had worked in any of certain specified departments at factories producing chlorophenoxy herbicides. The criteria for duration or level of exposure required for selection was reported for only 3 of the 10 countries and only 4 of the 20 cohorts; these ranged from at least 1 month to 1 year. For all the other cohorts, the criterion for inclusion was to have ever been employed in production or spraying of these herbicides. The cohort contained 1,537 female workers, but results were not presented separately, except for female breast and genital organ cancers by phenoxy herbicide exposure. Average follow-up for the cohort was 17 years; 5% of eligible workers were lost to follow-up. Three of the cohorts comprising over 10,000 workers have also been reported in separate publications but for different follow-up periods (Lyng, 1985, 1987, 1993; Coggon et al., 1986; Kogevinas et al., 1993); these are discussed briefly in this report following this discussion.

Also included in the analysis was a division of the cohort (probable vs. unlikely) by whether or not exposure to TCDD occurred. No definition is given for that which is considered "probable" exposure to TCDD. Exposure to phenoxy herbicides does not necessarily imply exposure to TCDD in this study. The "probably exposed" category includes production workers at two plants producing PCP, 2-(2,4-dichlorophenoxy) propanoic acid (2,4-DP; dichlorprop), 4-(2,4-dichlorophenoxy) butanoic acid (2,4-DB), 4-chloro-2-methylphenoxyacetic acid (MCPA), or 2-(4-chloro-2-methylphenoxy)-propanoic acid (MCP; mecoprop). Those "unlikely exposed" appear to be so classified because they appeared to work in different factories. There were 181 cases of chloracne among workers in the cohort.

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The results are presented below by each of the two divisions of the total cohort: a) phenoxy herbicide (ph) and/or chlorophenols and b) probable TCDD exposure. For the cohort division by ph and chlorophenols, no excess was observed for all-cause mortality, for all malignant neoplasms, for most common epithelial cancers, or for lymphomas. The four STS deaths were all in the "exposed to phenoxy herbicides and chlorophenols" subcohort (RR=2.0, CI=0.5-5.2) and all appeared 10 to 19 years after first exposure (RR=6.1, CI=1.6-15.5), with the excess risk limited to exposed sprayers (RR=8.8, CI=1.8-25.8) based on three observed deaths. None were observed in the 20 years or more category. Increases were also noted in the exposed group for mortality from thyroid cancer (RR=3.7, CI=1.0-9.4) based on four deaths, cancer of the testis (RR=2.2, CI=0.9-4.6) based on seven deaths, other endocrine glands (RR=4.6, CI=0.9-13.5) based on three deaths, and nose and nasal cavities (RR=2.9, CI=0.6-8.5) based on three deaths. An increase in lung cancer mortality was limited to the "probably exposed" (to phenoxy herbicide and chlorophenols) group (RR=2.2, CI=1.1-4.0) based on 11 observed deaths.

The authors provided an additional analysis of STS, including five additional cases who were either alive at the end of follow-up or who had died from another cause. They concluded that the results suggest that STS in these workers "is compatible with a causal role for chlorophenoxy herbicides, though not specifically for those probably contaminated with TCDD."

The authors present only a limited analysis based on 215 and 294 expected total cancer deaths in the "probable" vs. "unlikely" exposed groups, respectively. There was a slight increase in mortality from all cancers for the probably versus the unlikely exposed groups (RR=1.1, CI=1.0-1.2 versus RR=0.9, CI=0.8-1.1), but no increase in either STS or NHL based on 4 and 11 total cases, respectively. There was also an increased mortality for testicular cancer in the group probably exposed to TCDD versus those probably not exposed (RR=3.0 vs. 1.6) based on seven total deaths and for thyroid cancer (RR=4.3 vs. 3.1) based on four total deaths. These latter two differences are not significant and, while

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interesting because of TCDD's known effects on these organs, add little to the information base.

While the Saracci et al. cohort is significantly larger than the other three worker cohorts (Fingerhut et al., 1991; Manz et al., 1991; Zober et al., 1990), the lack of both a clear definition of exposure and uniformity of exposure classification between and within plants makes the results difficult to interpret and lessens the confidence in these results. When several studies have shown a positive association of effect with the same exposure but were conducted under different circumstances, the possibility that an unknown confounder or chance produced the observed elevated effect is minimized. When different investigators working with different populations using different methods confirm an original finding, the results are more believable. TCDD tissue levels were available only from a sample of 9 of the 181 workers with chloracne (median = 340 ng/kg, range 98 to 659 ng/kg). For 17 external controls, the median was 16 ng/kg while the range was 0 to 23.3 ng/kg. This suggests that some of the controls were exposed to TCDD. Unfortunately, no further analysis was presented on these workers.

There are several problems with this study. A portion of the Saracci et al. cohort consists of Danish workers from the Lynge (1985) study. None of them are reported by Saracci et al. (1991) as having had any exposure to 2,4,5-T. Lynge indicates that 2,4,5-T was produced at the Kemisk Vaerk Køge (KVK) facility in Denmark from 1951 until the end of 1980. However, in a later update (Lynge, 1993), she maintains that the excess is due to exposure to phenoxy herbicides other than 2,4,5-T because only 5.3 tons of 2,4,5-T were produced in 1951-1952. This statement is somewhat contradicted in her methods paper (Lynge, 1987), where she discloses that 350 tons of 2,4,5-T esters were produced during the period 1951-1981 based on purchased 2,4,5-T acid. Perhaps there occurred more exposure to 2,4,5-T than was asserted by the author. This suggests the possibility that exposure misclassification may be present in the Saracci et al. study. There may be potentially as many as 3,844 workers who had exposure to 2,4,5-T and consequently 2,3,7,8-TCDD. Many or all of them were considered as unexposed in the Saracci et al. study.

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Lynge in her studies reported on five histologically confirmed cases of soft tissue sarcoma. These are listed as cases 2, 3, 4, 5, and 9 in Table IV of the Saracci et al. study. Two are considered alive for the purposes of the Saracci et al. study even though in the later 1993 Lynge study these same two are listed as deceased. The remaining three are reported by Saracci et al. as deceased. Two of these three are coded to cancer sites other than STS. Only one is correctly coded to STS. This suggests that underreporting of STS as the underlying cause of death is a problem in this study, which is consistent with the findings of Suruda (1993) that STS is underreported generally on death certificates. Added evidence of underreporting of STS is provided by the death certificate's cause of death for the two who were deceased after 1984. Both were coded to a cancer site other than STS. Altogether, four out of five of the confirmed STSs in the Lynge study were coded to causes other than STS.

7.5.4. Other Studies

Four studies containing portions of the same cohort reported above were reported elsewhere (Lynge, 1985, 1987, 1993; Coggon et al., 1986; Kogevinas et al., 1993; Bueno de Mesquita et al., 1993). Lynge (1985) reported a study of cancer incidence among persons employed in the manufacture of phenoxy herbicides in Denmark. The cohort consisted of 4,459 persons from two factories. One factory contributed 615 cohort members who had worked in the years 1951-1981. The only phenoxy acids manufactured and packaged at this plant were MCPA and mecoprop unlikely to contain TCDD. The other factory, Kemisk Vaerk Køge, contributed 3,844 cohort members who had worked in the years 1933-1981. At this plant, MCPA, 2,4-D, and lesser amounts of mecoprop, dichlorprop, and 2,4,5-T were manufactured and packaged. The investigators were unable to classify cohort members by the specific types of phenoxy herbicides to which they were exposed. However, in this plant, where exposure to TCDD-contaminated 2,4,5-T probably did occur, a significant excess risk of STS (4 observed vs. 1.00 expected, CI=1.09-10.24) was noted by the author

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in those workers who had achieved a minimum 10 years of latency. Unfortunately, individual tissue measurements of 2,3,7,8-TCDD were not included in this study.

In an update of the earlier study, Lynge continues to report an increase in the risk of STS with four cases reported to be in persons exposed to phenoxy herbicides ($SIR=2.3$, $CI=0.6-5.8$). Just as before, this excess occurred in workers employed for more than 1 year in the Kemisk Vaerk Køge factory ($SIR=6.4$, $CI=1.3-18.7$). The author concluded that her study continues to provide evidence that exposure to phenoxy herbicides increases the risk of STS.

However, Lynge maintains that only small amounts of 2,4-D and "negligible" amounts of 2,4,5-T were produced at the KVK factory. That this amount of 2,4,5-T was negligible is somewhat at odds with data from an earlier paper in which she discussed the design of her ongoing cohort study (Lynge, 1987). In the 1987 paper, she reported that although 5.3 tons of 2,4,5-trichlorophenol were produced in 1951 and 1952, 350 tons of 2,4,5-T esters were produced from 1951-1981 based on purchased 2,4,5-T acid. This varied from zero to as much as 63 tons in any one year. Very likely, the term "negligible" is used in a relative sense—relative to the amounts of the other herbicides produced in the KVK factory, which were produced in much larger amounts. Actual exposure to 2,4,5-T may have been greater than the impression given in Lynge's 1993 study. Most of the potential exposure was to MCPA, MCPP, 2,4-DP, and various dyes and pigments. MCPA, MCPP, 2,4-DP, and the nondioxin-containing phenoxy herbicides have not heretofore been seriously thought of as possible causes of cancer in humans.

Lynge also found that the risk of non-Hodgkin's lymphoma was not elevated in persons potentially exposed to phenoxy herbicides. She did find what she calls a "puzzling" 3.5-fold excess risk in employees of KVK employed in other manufacturing departments. No detailed information on production in these areas was included in the study.

Little additional information is provided concerning any increased risks of other forms of cancer, except for a statement that multiple myeloma and cervical cancer in women and malignant melanoma in men were significantly increased. No numbers are given for these

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statements. A significant excess risk of lung cancer seen in the earlier study is now borderline significant in this study (obs. =13, SIR=1.6, C.I.=0.9-2.8). The author is now arranging to have serum tissues in some of her subjects analyzed for their dioxin content.

Coggon and colleagues (1986) conducted a study of 5,754 workers at a British plant that manufactured and formulated MCPA from 1947 until 1982 and operated its own aerial and tractor-mounted spraying service from 1947 until 1972. The authors stated that other phenoxy acids were handled "at times" and that, "in comparison with MCPA, 2,4,5-T was handled only on a small scale."

Coggon et al. (1991) conducted a study of four British cohorts of manufacturers of phenoxy herbicides, including 2,4,5-T, comprising 2,239 men employed sometime during the period 1963 to 1985. All four of these cohorts were included in the Saracci et al. study previously discussed. Follow-up was to the end of 1987 through the National Health Service Central Register and the National Insurance Index. Comparisons were with the national population. Factory A produced 2,4,5-T only beginning in 1968, while the remaining three factories formulated it only beginning in 1959, 1960, and 1970, respectively. No tissue measurements were conducted on any members of the cohort. A slight excess of lung cancer was noted (19 observed, 14.2 expected). Two non-Hodgkin's lymphomas also were observed (0.87 expected). No STSs were observed (0.18 expected). Total cancer also was not increased (37 observed, 36.85 expected).

This cohort has not been followed for a sufficiently long enough time to expect latent effects to manifest themselves. The authors assumed that the slight increase in lung cancer was probably due to cigarette smoking or a chance occurrence based on the observation that most of the lung cancer deaths occurred less than 10 years after first exposure to phenoxy compounds. Phenoxy herbicides produced or formulated at these factories include 2,4-D, MCPA, 2,4-DP, MCPB, MCPP, PBA, PCPA, and PAA. In addition, other herbicides were also made here. The author says they were exposed to a multiplicity of chemicals. Except for the slight increase in lung cancer, which is in the same direction as the findings from the

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earlier cohort studies, this study contributes little to the elucidation of the risk of cancer from exposure to 2,3,7,8-TCDD.

Kogevinas and her colleagues (1993) studied a group of 701 occupationally exposed women who were enrolled in IARC's International Registry of Persons Exposed to Phenoxy Herbicides and Their Contaminants. These workers were also included in the earlier Saracci et al. (1991) study. The likelihood of exposure to TCDD was based on individual job histories, company records, and company exposure questionnaires. Actual measurements of TCDD serum levels in women were not available according to the authors, so that confirmation of exposure could not be performed. Both national cancer incidence rates and national death rates were used to generate expected cases and deaths utilizing the methods of the Saracci IARC study.

The overall cancer risk did not exceed expected ($SIR=96$, $CI=0.6-1.4$) based on 29 cases. However, the group with the greatest potential for exposure to TCDD-contaminated chlorophenoxy herbicides produced a significant excess risk of cancer of all sites ($SIR=222$, $CI=1.0-4.2$) based on nine cases. The risk was observed within the first 10 years of exposure, with no elevated risk appearing after the 10th year of observation. For those women who had probable exposure to TCDD, the risk of dying from cancer was slightly elevated as well ($SMR=165$, $CI=0.4-4.8$) based on three deaths.

This study suffers from many of the same problems as the Saracci et al. (1991) study. In addition, it is a study of a small population and as such cannot be considered sensitive to the detection of small risks. These same workers were also subject to exposure to other toxic chemicals in the workplace, which may also have an effect on the risk of cancer.

However, the elevated cancer risk in women exposed to TCDD-contaminated phenoxy herbicides is consistent with a hypothesis of overall increased cancer risk seen in other studies from exposure to TCDD or TCDD-like contaminants.

Recently, another study was published (Bueno de Mesquita et al., 1993) of a cohort of 2,310 workers in two plants involved in the manufacture and preparation of phenoxy herbicides (not necessarily 2,4,5-T) in the Netherlands. These workers were also included in

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the IARC International Registry of Persons Exposed to Phenoxy Herbicides and Their Contaminants and hence part of the Sarraci et al. (1991) study. Some 963 were considered by the author to be exposed to phenoxy herbicides, while 1,111 were considered not exposed. The follow-up periods were somewhat skewed between the two subcohorts as well. The workers of one plant were followed from 1955 to 1985, and those at the other were followed from 1965 to 1986.

Only a slight increase occurred in total cancer mortality based on 31 deaths (SMR=107, 95% CI=73-152) utilizing The Netherlands' national rates. A slightly higher risk of total cancer was seen based on 10 deaths (SMR=137, CI=66-252) in 139 workers probably exposed to dioxins during or immediately after a 1963 industrial accident in which dioxin was released into the atmosphere.

When compared with nonexposed workers, mortality due to all cancers was insignificantly elevated (RR=1.7, 95% CI=0.9-3.4) while that due to respiratory cancer was also insignificantly elevated (RR=1.7, 95% CI=0.5-6.3). This group was too small to provide enough power to detect significant site-specific cancers.

Although the size of the cohort seems large, actually only about 549 workers in Factory A had a potential for exposure to TCDD-contaminated 2,4,5-T and the higher chlorinated dioxins. No one at Factory B was exposed to 2,4,5-T because it was not produced there. At Factory A, the SMR for lung cancer was elevated insignificantly to 165 based on 6 deaths in the 20-year latent category. No serum dioxin measurements are available to substantiate exposure to dioxin. No soft tissue sarcomas were reported although only 0.28 were expected. However, the authors did conclude that the SMR of 73 for Factory A, the SMR of 118 for Factory B, and the SMR of 137 for the cohort exposed to the accident are not inconsistent with the possibility of a carcinogenic effect of TCDD in humans.

Wiklund and Holm (1986) studied a massive cohort of 354,620 Swedish men who were recorded as having an agriculture or forestry job according to the census of 1960 versus 1,725,845 Swedish men in all other industries. The primary exposure in those jobs was

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postulated to be MCPA; 2,4-D and 2,4,5-T were also used to a lesser extent. The authors found that the relative risk of STS was only 0.9. This study has several deficiencies that reduce its usefulness in determining the risk of STS due to exposure to 2,3,7,8-TCDD: (1) a lack of individualized exposure data, (2) only 15% of Swedish agricultural and forestry workers were estimated to be exposed to phenoxyacetic acids and 2% to chlorophenols, (3) Swedish agricultural workers have a decreased cancer risk and tend to use health services less frequently, (4) classifying workers according to a 1-week employment status in October of 1960 as reported in a census invites the possibility of misclassification, and (5) the crude rate of STS in agricultural and forestry workers based on data in the study is 5.45 per 100,000 person-years, and in the remaining workers it is 5.00 per 100,000 person-years. Both rates are high compared with rates from other nations (1 to 3 per 100,000 person-years).

A few years later, Wiklund et al. (1988, 1989) produced two new cohort studies that superficially appear to contradict the earlier findings of Hardell and Eriksson. Wiklund et al. followed some 20,245 licensed pesticide applicators in Sweden from date of license in 1965 or after until December 31, 1984. Some 72% were estimated to have been exposed to phenoxy herbicides 1 day or longer (based on questionnaire data sent to a random sample of 273 persons in the cohort). The relative risk for soft tissue sarcoma reported in the first study was found to be 0.9, with a mean follow-up time of 13.9 years. Even after a 10-year latency, the risk for soft tissue sarcoma was only 1.0 based on four deaths. With respect to the second study of all other cancer sites, major significant deficits were found in several sites followed for an average of 12.2 years until December 31, 1982. No report is given concerning loss to follow-up or vital status. The authors also report that "the cohort may have been observed for too short a time to reveal any excess risk"

The authors describe a major disadvantage of these studies to be a "lack of individual exposure data" and that information is available "for only a sample of the cohort." Even the length of exposure of individual applicators to phenoxy herbicides is not available. What is presented is information that herbicide use in the 1950s was only 19%, and in the 1960s it

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increased to just 49%. By the 1970s, however, it was up to 67%. On the other hand, pesticide use is reported to be 92% during the same period. This seems to indicate that perhaps less than half had any exposure to the phenoxy herbicides at the time of licensing and perhaps for many years afterwards. Furthermore, information is presented about the presence or absence of chloracne, another marker of exposure to 2,3,7,8-TCDD.

In addition to missing important information regarding the vital status of this cohort by the end of the follow-up, no information is available concerning the distribution of person-years "at risk" generated by the "lost to follow-up" group. Among the cancer sites reported to have significantly reduced risks are total cancer, liver, pancreas, lung, and kidney.

Furthermore, the extent of exposure to the agent of concern, 2,3,7,8-TCDD, may not be extensive among licensed applicators in Sweden. The entire discussion is centered on exposure to "phenoxy herbicides." The authors state that the most widely used phenoxy herbicides in Sweden are MCPA, mecoprop, and dichlorprop. None of these contain 2,3,7,8-TCDD as a contaminant. 2,4-D and 2,4,5-T have also been used to a "lesser extent" according to the authors. Sweden prohibited the use of 2,4,5-T in 1977. If, as the authors state, only 19% used herbicides in the 1950s, increasing to 49% in the 1960s, it suggests that far fewer applicators were exposed to small quantities of 2,3,7,8-TCDD for a long enough period of time to produce any effects. Furthermore, only 68.2% of the cohort could have attained the age of 59 by the close of the study in 1984. This hints at the likelihood that the full impact of exposure on mortality has not yet been achieved. In fact, the authors report that the "latency time may anyhow be too short to detect increasing risks of cancer"

7.5.5. Summary

The cohorts assembled by Fingerhut et al. (1991), Manz et al. (1991), and Zober et al. (1990) are important because they contain sizable proportions of persons with substantial TCDD exposures. These exposures were documented, at least in subsets of the cohorts, by blood and/or adipose tissue measurements, workplace measurements, and the

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occurrence of chloracne. The Saracci et al. cohort, while significantly larger, is assembled with nonuniform exposure criteria for TCDD exposure, leading to less confidence in the results.

The exposures and methods in the three former studies in males were similar enough to warrant aggregating the results. Within each study, relative risks were estimated by summing the observed and expected numbers of deaths across categories of age, race, and calendar time, and then dividing the totals to produce relative risk estimates in the form of standardized mortality ratios. Thus, aggregate relative risks can be obtained simply by summing the observed and expected numbers of deaths across the studies. Alternatively, the aggregate relative risk could have been derived by weighting the individual relative risks from each study by the inverse of the variance. A separate analysis of the three studies using estimates of lifetime dose intake is presented in Section 8.5.

As shown in Table 7-5, the studies by Manz et al. (1991) and Zober et al. (1990) add little to the information provided by the study by Fingerhut et al. (1991), except to increase the precision of the relative risk estimates (as indicated by a narrowing of the confidence intervals). Even when aggregated, however, the estimates for cancers of connective and soft tissues, non-Hodgkin's lymphoma, and stomach cancer are highly imprecise. With this important limitation, the results suggest little or no increase in the risk of non-Hodgkin's lymphomas. They also suggest increased risk—especially among persons with relatively high exposure and relatively long latency—for connective and soft tissue cancers, for lung cancer, and for all cancers combined. Some confounding from exposure to asbestos cannot be ruled out in the Fingerhut study.

The estimates of increased risk for lung cancer and for all cancers combined are considerably more precise (Table 7-5). The elevations for these cancers also appear to be more pronounced in the subcohorts of relatively high exposure and relatively long latency than in the total cohorts. Because they come from comparisons between blue-collar workers and national populations, it is reasonable to suspect that these estimates—especially for lung cancer—are inflated to some degree by confounding due to cigarette smoking, but the limited

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Table 7-5. Summary of Results for Selected Cancers From Follow-up Studies of Chemical Manufacturing and Processing Workers Exposed to TCDD

Cancer	Study	Total cohorts				Subcohorts with high exposure, long latency, or both			
		Observed deaths	Expected deaths	Relative risk	95% Confidence interval	Observed deaths	Expected deaths	Relative risk	95% Confidence interval
Connective and soft tissue cancers	Fingerhut (1991)	4	1.2	3.3	1.1 - 8.0	3	0.3	10.0	2.5 - 27.3
	Manz (1991)	0	0.4 ^a	0.0	0.0 - 7.5	0	0.1 ^a	0.0	0.0 - 30.0
	Zober (1990)	0	0.1 ^a	0.0	0.0 - 30.0	0	0.0 ^a	0.0	0.0 - 99.9
	Total	4	1.7	2.4	0.7 - 5.7	3	0.4	7.5	1.9 - 20.4
Non-Hodgkin's lymphomas	Fingerhut (1991)	10	7.3	1.4	0.7 - 2.4	2	2.1	1.0	0.2 - 3.1
	Manz (1991)	3	2.4 ^a	1.2	0.3 - 3.4	NA	NA	NA	NA
	Zober (1990)	0	0.6 ^a	0.0	0.0 - 5.0	0	0.2 ^a	0.0	0.0 - 15.0
	Total	13	10.3	1.3	0.7 - 2.1	2	2.3	0.9	0.1 - 2.9
Lung cancer	Fingerhut (1991)	89	80.1	1.1	0.9 - 1.4	40	28.8	1.4	1.0 - 1.9
	Manz (1991)	30	21.3	1.4	1.0 - 2.0	NA	NA	NA	NA
	Zober (1990)	6	5.6	1.1	0.4 - 2.2	3	1.2	2.5	0.6 - 6.8
	Total	125	107.0	1.2	1.0 - 1.4	43	30.0	1.4	1.1 - 1.9
Stomach cancer	Fingerhut (1991)	10	9.7	1.0	0.5 - 1.8	4	2.9	1.4	0.4 - 3.3
	Manz (1991)	12	9.9	1.2	0.7 - 2.1	NA	NA	NA	NA
	Zober (1990)	3	2.7	1.1	0.3 - 3.0	2	0.5	4.0	0.7 - 13.2
	Total	25	22.3	1.1	0.7 - 1.6	6	3.4	1.8	0.7 - 3.7
All cancers combined	Fingerhut (1991)	265	229.9	1.2	1.0 - 1.3	114	78.0	1.5	1.2 - 1.8
	Manz (1991)	93	75.2	1.2	1.0 - 1.5	34	23.9	1.4	1.0 - 2.0
	Zober (1990)	23	19.7	1.2	0.8 - 1.7	7	4.2	1.7	0.7 - 3.3
	Total	381	324.8	1.2	1.1 - 1.3	155	106.1	1.5	1.2 - 1.7

^aEstimated as a proportion of expected deaths from all cancers combined (see text).

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analyses presented suggest that the association is not a chance occurrence (Table 7-2). On the other hand, the counterinfluence of the healthy worker effect, exposure misclassification, and diagnostic error would tend to force risk estimates downward. The risk estimates may be expected to be too small in this event.

The results in males are consistent with results from animal studies to some degree. In Chapter 6, it was shown that in a lifetime TCDD bioassay male rats developed lung cancer, and in an initiation-promotion study ovariectomized rats exposed to TCDD developed lung tumors, while intact rats similarly exposed did not. Furthermore, the mice in the NCI study developed fibromas and fibrosarcomas. Also, TCDD affects the immune system and has been shown to be a tumor promotor in animal liver and skin assays. Either or both of these actions could lead to increased total cancer. With respect to health effects in females, the study by Saracci et al. (1991) suggests a possible increase in breast cancer, but the results are considered preliminary in view of the small numbers and less certain exposure. On the other hand, Kogevinas et al. (1993) reported an increase in cancer incidence, all causes, among women who were exposed to chlorophenoxy herbicides contaminated with TCDD. However, no excess was observed for breast cancer. Still, in another study by Bertazzi et al. (1993) to be discussed later in the section on Seveso, Italy, a deficit of breast cancer and endometrial cancer was seen in women living in geographical areas contaminated by dioxin. TCDD exposure might be expected to result in decreased breast cancer in females, based on similar observations in rats and on TCDD's action on downregulation of the estrogen receptor. However, this is very species-, tissue-, and age-specific.

7.6. CASE-CONTROL STUDIES IN GENERAL POPULATIONS

In this section on case-control studies, the discussion will focus chiefly on the cancer sites, i.e., STS and non-Hodgkin's lymphoma, that have been suggested by the earlier Swedish studies as being associated with exposure to the phenoxy herbicides. This is a reflection of the interest shown in these two cancer sites over the last decade. Few, if any, case-control studies have been completed on other cancer sites. And, of course, if other

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cancer sites are not studied, the risk of cancer cannot be evaluated. It is meaningless to do case-control studies on total cancer.

7.6.1. Sweden

Hardell, Eriksson, and colleagues conducted four studies of soft tissue sarcomas (Hardell and Sandström, 1979; Eriksson et al., 1981, 1990; Hardell and Eriksson, 1988) and one study of malignant lymphomas (Hardell et al., 1981) among men living in different parts of Sweden. In all studies, cases and their matched controls were considered exposed if they reported phenoxy acid or chlorophenol exposures lasting at least 1 day and occurring at least 5 years before the case's date of diagnosis.

The nature of the phenoxy acid exposures differed across the Swedish study locales. In northern Sweden, most exposures occurred in the use of 2,4,5-T and 2,4-D in combination in forestry applications, often by knapsack spraying (Hardell and Sandström, 1979; Hardell and Eriksson, 1988; Hardell et al., 1981; Hardell, 1981a, b). Phenoxy acid exposures not involving 2,4,5-T became progressively more common, on a proportional basis, in the central and southern regions in which agricultural herbicide uses predominated (Eriksson et al., 1981, 1990). Whereas exposures not involving 2,4,5-T made up only 22 percent of all phenoxy acid exposures in the first northern sarcoma study (Hardell and Sandström, 1979; Hardell, 1981a, b), they accounted for 27 percent in the study in central Sweden (Eriksson et al., 1990) and 58 percent in the study in southern Sweden (Eriksson et al., 1981). Exposures defined only as phenoxy acid exposures are therefore less useful as indicators of exposure to TCDD and related compounds in southern Sweden than in the central and northern parts of the country. Furthermore, none of these studies provide information dealing with how much exposure each subject may have had.

The reports contain little information on the specific chlorophenol preparations to which the cases and controls were exposed. Occasional statements in some of the manuscripts suggest that most chlorophenol exposures occurred in the sawmill and pulp industries, that they primarily involved pentachlorophenol, and that they seldom involved

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trichlorophenols. Thus, most of the reported chlorophenol exposures entailed exposures to the higher-chlorinated PCDDs and PCDFs but not to TCDD.

Because exposure prevalences were generally low and because phenoxy acids and chlorophenols tend to be used in different occupations, very few persons reported joint exposures. Thus, it is efficient to control potential confounding in the analysis of data from these studies by comparing each exposure category (phenoxy acids vs. chlorophenols) with the category composed of all persons who reported no exposure to phenoxy acids or chlorophenols. Based on this method of analysis, relative risk estimates from all five studies are presented in Table 7-6, with the sarcoma studies arranged in order of publication. The measure of effect here is the "odds ratio" that is an estimate of the relative risk when the cancer is relatively rare and henceforth will be called the relative risk.

The results for 2,4,5-T are the only results pertinent to TCDD exposures only. Because of the small number of cases and controls reporting 2,4,5-T use in southern Sweden, the confidence interval for the relative risk estimate from the study in that part of the country (Eriksson et al., 1981) is extremely wide. A separate relative risk estimate for 2,4,5-T could not be computed from the data in the second northern sarcoma study (Hardell and Eriksson, 1988). The published report, however, did state that all of the cases and most of the controls exposed to phenoxy acids were exposed to preparations including 2,4,5-T (Hardell and Eriksson, 1988). The report also gave a relative risk of 3.5 for TCDD exposure, but no confidence interval or counts of cases and controls was provided.

The studies were conducted in two phases. The lymphoma study (Hardell et al., 1981), the first northern sarcoma study (Hardell and Sandström, 1979), and the southern sarcoma study (Eriksson et al., 1981) were published between 1979 and 1981. The remaining two sarcoma studies (Hardell and Eriksson, 1988; Eriksson et al., 1990) appeared about a decade later. The relative risk estimates from these more recent studies are consistently lower than those from the earlier studies (Table 7-6). Thus, systematic differences between the two sets of studies may be an explanation for the heterogeneity of results.

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Table 7-6. Relative Risks of Soft Tissue Sarcomas and Malignant Lymphomas in Relation to Phenoxy Acid and Chlorophenol Exposures in Five Case-Control Studies in Sweden

Exposure category and measure	Malignant lymphoma Northern Sweden 1974-1978 (Hardell et al., 1981)	Soft tissue sarcoma Northern Sweden 1970-1977 (Hardell and Sandström, 1979)	Soft tissue sarcoma Southern Sweden 1974-1978 (Eriksson et al., 1981)	Soft tissue sarcoma Northern Sweden 1978-1983 (Hardell and Eriksson, 1988)	Soft tissue sarcoma Central Sweden 1978-1986 (Eriksson et al., 1990)
<u>Not exposed to phenoxy acids or chlorophenols</u>					
Cases	108	33	85	41 ^a	171 ^b
Controls	303	187	206	255 ^a	179 ^b
<u>Exposed to phenoxy acids, chlorophenols, or both</u>					
Cases	61	19	25	13 ^a	47 ^b
Controls	32	19	13	56 ^a	33 ^b
Relative risk (95% CI)	5.3 (3.3 - 8.7)	5.7 (2.7 - 11.8)	4.7 (2.3 - 9.5)	1.4 (0.7 - 2.9)	1.5 (0.9 - 2.4)
<u>Exposed to phenoxy acids</u>					
Cases	41	13	14	9	23
Controls	24	14	5	22	18
Relative risk (95% CI)	4.8 (2.8 - 8.3)	5.3 (2.3 - 12.2)	6.8 (2.4 - 19.4)	2.5 (1.1 - 5.9)	1.3 (0.7 - 2.6)
<u>Exposed to 2,4,5-T</u>					
Cases	29	11	7	NA	19
Controls	23	10	1	NA	11
Relative risk (95% CI)	3.5 (2.0 - 6.4)	6.2 (2.5 - 15.8)	17.0 (2.1 - 140.0)	NA	1.8 (0.8 - 3.9)
<u>Exposed to chlorophenols</u>					
Cases	50	7	11	4	15
Controls	35	6	8	34	3
Relative risk (95% CI)	4.0 (2.5 - 6.5)	6.6 (2.1 - 20.9)	3.3 (1.3 - 8.6)	0.7 (0.2 - 2.2)	5.2 (1.5 - 18.4)

^aAssuming no joint exposure to phenoxy acids and chlorophenols.

^bComputed from Table 3 in the original report (Eriksson et al., 1990) with the assumption of no phenoxy acid exposures among persons with low-grade chlorophenol exposures.

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The first set of studies received a considerable amount of criticism concerning the methods by which the exposure information had been obtained (Hardell, 1981b; Cole, 1980). The basic concern was the possibility of bias from differential exposure misclassification between cases and controls (sometimes called "observational bias" or "interviewer and recall bias"), with false-negative reports of exposure suspected as being more common among the controls and false-positive reports more common among the cases. Much of the discussion focused on telephone interviews that were conducted by research staff who were aware of the purpose of the study and of the case or control status of the respondents. These interviews were conducted with selected participants to confirm reported exposures and to resolve uncertainties on postal questionnaires, which were the primary sources of exposure information. For living cases, controls were selected from the Swedish National Population Registry. For deceased cases, controls were selected from the Swedish National Registry for Causes of Death. As the criticisms of these procedures have echoed through the years (Bond et al., 1989b; Colton, 1986), no quantitative analysis has been made of the degree of bias that would have been required to produce the very strong associations reported in the first three studies (Table 7-6). Of greater importance, analyses by Hardell based solely on the questionnaire information (Hardell, 1981b) have been largely overlooked. These analyses produced relative risk estimates very similar to those obtained when the information from the supplemental interviews was used.

Hardell also went so far as to enroll a series of colon cancer patients (Hardell, 1981) as a sort of "positive control" group. In contrast to soft tissue sarcomas and malignant lymphomas, colon cancer turned out not to be associated strongly with phenoxy acid or chlorophenol exposures. Hardell and Eriksson made a similar finding in one of the newer studies (Hardell and Eriksson, 1988) when they included a control group consisting of a variety of cancers along with a set of general population controls. The cancer controls were drawn at random from the Swedish Regional Cancer Registry.

Despite Hardell's conclusion that "the previously reported associations . . . cannot to any essential degree be explained by observational bias in the studies" (Hardell, 1981b), he

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and his colleagues imposed procedures designed to reduce the potential for such bias in their subsequent studies (Eriksson et al., 1990; Hardell et al., 1981). When lower relative risk estimates were produced (Table 7-6), the researchers suggested that one explanation might have been the improved methods of exposure assessment. Again, controls were from national population registries.

The investigators suggested that another explanation for the reduced relative risk estimate for phenoxy acids and soft tissue sarcomas in the study in central Sweden (Table 7-6) "could be the decade in which exposure occurred" (Eriksson et al., 1990), with the implication that exposures were higher in earlier decades. They supported this suggestion with an analysis in which only those phenoxy acid exposures occurring in the 1950s were considered. This analysis yielded a higher relative risk estimate of 2.3 (95% CI=1.0-5.4). Unfortunately, no basis of comparison exists because analyses by calendar time of exposure were not conducted in any of the other studies.

As an alternative explanation for the lack of an elevated relative risk estimate in connection with chlorophenols in the second northern sarcoma study (Table 7-6), the authors offered "random variation" due to a low number of exposed subjects (Hardell and Eriksson, 1988). The prevalence of chlorophenol exposures among the controls in that study (10.9%) was several times *higher* than in the earlier northern sarcoma study (2.9%) (Hardell and Sandström, 1979) and virtually identical to the prevalence in the lymphoma study (10.4%) (Hardell et al., 1981). The phenoxy acid exposure prevalences were highly uniform in all three northern studies: 7.2% in the lymphoma study (Hardell et al., 1981), 6.8% in the first sarcoma study (Hardell and Sandström, 1979), and 7.1% in the second sarcoma study (Hardell and Eriksson, 1988). The chlorophenol exposure prevalence among the controls in the first northern sarcoma study (Hardell and Sandström, 1979) seems to have been low. However, the overall consistency of some excess risk is perhaps more important than actual levels of the excess.

For three of the studies by Hardell and colleagues, relative risk estimates can be computed restricting the data to persons who had worked in agriculture and the other

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occupational categories in which the exposures of interest tend predominantly to occur (Table 7-7). For the lymphoma study (Hardell et al., 1981) and the sarcoma study in southern Sweden (Eriksson et al., 1981), the results for exposure to phenoxy acids, chlorophenols, or both in the restricted analyses are virtually identical to those obtained with the data for all subjects (Table 7-6). For the sarcoma study in central Sweden (Eriksson et al., 1990), however, the relative risk for phenoxy acids was higher (2.3) within the special occupational categories than among all subjects.

Interestingly, Eriksson and his colleagues stated that the association with the risk of STS seemed to strengthen with exposure to the higher-chlorinated dioxin isomers. His conclusion was that not only may 2,3,7,8-TCDD be a risk factor for STS but also that other higher-chlorinated dioxins may be risk factors.

The risk calculated for exposure to 2,4,5-T in the 1950s was somewhat higher at 2.94 (95% CI=1.1-8.0). However, exposure to 2,4,5-T during the span of the study was nonsignificant at 1.8 (95% CI=0.9-3.9) excluding the chlorophenols. Exposure to dioxin-containing phenoxyacetic acids or chlorophenols, excluding nondioxin-containing herbicides, produced a significant risk estimate of 2.4 (95% CI=1.3-4.5). Exposure to high-grade pentachlorophenols produced a risk ratio of 3.9 (95% CI=1.2-12.9).

These analyses are important because many of the mechanisms by which biases might occur would be related to occupation. For instance, biases in case identification, control selection, or nonparticipation that might be related to occupational status (e.g., by its link to socioeconomic status) would not be expected to be as great in analyses conducted within the occupational categories as in analyses of the overall data. The potential for confounding by occupational exposures encountered in the same lines of work would also be reduced in the occupationally restricted analyses. Several researchers and reviewers (Johnson, 1990; Pearce et al., 1985; Blair et al., 1985) have noted reports of farmers being at increased risk of malignant lymphomas and other cancers and have mentioned a wide range of potentially responsible exposures, "including pesticides, solvents, oils and fuels, dusts, paints, welding fumes, zoonotic viruses, microbes, and fungi" (Blair et al., 1985). (Studies of farmers and

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Table 7-7. Relative Risks of Malignant Lymphoma and Soft Tissue Sarcomas in Relation to Phenoxy Acid and Chlorophenol Exposures in Three Case-Control Studies in Sweden^a

Cancer and study locale	Exposure category	Cases	Controls	Relative risk (95% confidence interval)
Malignant lymphoma, northern Sweden (Hardell et al., 1981; Hardell, 1981) ^b	Exposed to phenoxy acids, chlorophenols, or both	51	28	1.0
	Unexposed	49	145	5.4 (3.1 - 9.5)
Soft tissue sarcoma, southern Sweden (Eriksson et al., 1981) ^c	Exposed to phenoxy acids, chlorophenols, or both	14	8	1.0
	Unexposed	17	39	4.0 (1.4 - 11.3)
Soft tissue sarcoma, central Sweden (Eriksson et al., 1990) ^d	Exposed to phenoxy acids	22	15	1.0
	Unexposed	33	51	2.3 (1.0 - 5.0)

^aRestricted to persons who worked in the occupational categories in which these exposures predominantly occur.

^bAnalysis restricted to persons employed in agriculture, forestry, or the wood products industry.

^cAnalysis restricted to persons employed in agriculture or forestry.

^dAnalysis restricted to persons employed in agriculture, horticulture, or forestry.

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other agricultural workers are not included in this review because mere membership in these occupational categories is insufficient as an indicator of exposure to such substances as 2,4,5-T or chlorophenols.)

In the original reports, occasional attempts to assess exposure-response trends produced mixed results. In general, the reported exposure periods were short in all of the studies. In the first northern sarcoma study, for instance, 93% of all reported phenoxy acid exposures lasted 1 year or less, 74% lasted 6 months or less, and 33% lasted 30 days or less (Hardell and Sandström, 1979; Hardell, 1981b). Reported exposures in southern Sweden were even briefer, with 53% lasting 30 days or less (Eriksson et al., 1981).

Hardell et al. recently aggregated the four soft tissue sarcoma studies in a re-analysis examining exposures to herbicides contaminated with TCDD and other dioxins (Hardell et al., 1991). Increasing trends in risk with duration of exposure (< 1 year and ≥ 1 year) and "latency" (5-19 years and ≥ 20 years since first exposure) were numerically impressive, being based on the totals of 434 cases and 948 controls from all the studies (Table 7-8). The problem of concomitant exposures was not solved in these analyses, however, and an analysis of the aggregated data obscured the pronounced heterogeneity of results among the individual studies (Table 7-6).

Regardless of the exposure definition, considerable heterogeneity exists among the relative risk estimates from the four soft tissue sarcoma studies (Table 7-6). (Tests of homogeneity yield two-tailed p-values of 0.002 for phenoxy acids, chlorophenols, or both; 0.02 for phenoxy acids; 0.03 for 2,4,5-T; and 0.01 for chlorophenols.) In this circumstance, aggregation of results across studies is not indicated and, instead, a search should be made for explanations for the heterogeneity.

Two additional studies of malignant lymphomas and one study of soft tissue sarcomas were conducted by independent research teams in southern Sweden. Olsson and Brandt's (1988) study consisted of 167 men diagnosed with non-Hodgkin's lymphoma in the years 1978-1981 and 140 controls from the Swedish National Population Registry. Men who reported handling phenoxy acids or chlorophenols for at least 1 day were considered

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Table 7-8. Mantel-Haenszel Odds Ratios for Soft Tissue Sarcoma Among Persons Exposed to All Dioxins, TCDD, and Dioxins Other Than TCDD in Four Case-Control Studies Involving 434 Cases and 948 Controls^a

Substance and variable	No exposure	Exposure < 1 Yr ^b		Exposure ≥ 1 Yr	
		Latency 5 - 19 Yr	Latency ≥ 20 Yr	Latency 5 - 19 Yr	Latency ≥ 20 Yr
All dioxins					
No. of cases	352	24	34	3	21
No. of controls	865	22	52	0	9
OR	1.0	2.4		6.4	
90% CI	—	1.7 - 3.4		3.5 - 12	
TCDD					
No. of cases	352	18	22	1	5
No. of controls	865	14	25	0	2
OR	1.0	3.0		7.2	
90% CI	—	2.0 - 4.5		2.6 - 20	
Other dioxins					
No. of cases	352	6	12	2	16
No. of controls	865	8	27	0	7
OR	1.0	1.7		6.2	
90% CI	—	0.98 - 2.9		2.9 - 13	

^aOR denotes odds ratio and CI confidence interval.

^bAll subjects were exposed for at least 1 day. Data for latency periods were combined to determine the odds ratios.

Source: Hardell et al. (1991).

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exposed. However, the main focus of the study was to evaluate the contribution of organic solvent exposure to the risk of non-Hodgkin's lymphoma. Persson et al. (1989) studied 54 cases of Hodgkin's disease, 106 cases of non-Hodgkin's lymphoma, and 275 controls of both genders from the population registry of Sweden. The cases were diagnosed in the years 1964-1986, but only those who were still alive in 1986 were included. The authors did not ask specific questions about phenoxy acid use. Wingren et al. (1990) studied 96 men with soft tissue sarcomas diagnosed in the years 1975-1982, 450 general population controls, and 200 cancer controls from the regional cancer registry. Because the results did not differ substantially between the two control groups, only those obtained from analyses with the general population controls are reported here. The authors had to resort to job-associated uses, of which one was called "unspecified chemical work, potential exposure to phenoxy herbicides and chlorophenols," because only limited information could be obtained about specific chemical exposures from postal questionnaires and selected, supplemental telephone interviews.

Results from these three studies are summarized in Table 7-9. Persson et al. (1989) found strong associations, Wingren et al. (1990) found an association of intermediate strength, and Olsson and Brandt (1988) found very little association. These studies are limited by the lack of specificity in their exposure information.

7.6.2. United States

Zahm, Cantor, and colleagues from the National Cancer Institute have reported results from three case-control studies of exposure to 2,4,5-T or 2,4-T as well as other pesticides and herbicides in four Great Plains states (Hoar et al., 1986; Zahm et al., 1990; Cantor et al., 1992). The first study was conducted in Kansas (Hoar et al., 1986). It included soft tissue sarcomas, Hodgkin's disease, and non-Hodgkin's lymphomas, but detailed analyses were confined to the non-Hodgkin's lymphomas. The two subsequent studies, one conducted in eastern Nebraska (Zahm et al., 1990) and the other in Iowa and Minnesota (Cantor et al., 1992), evaluated non-Hodgkin's lymphomas. Cancer risks at other sites from exposure to

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Table 7-9. Relative Risks of Soft Tissue Sarcomas, Non-Hodgkin's Lymphomas, and Hodgkin's Disease in Relation to Phenoxy Acid and Chlorophenol Exposures in Two Case-Control Studies in Southern Sweden

Authors, study period	Cancer	Exposure	Relative risk (confidence interval) ^a
Olsson and Brandt, 1978-1981 (Olsson and Brandt, 1988)	Non-Hodgkin's lymphomas	Phenoxy acids	1.3 (0.8 - 2.1)
		Chlorophenols	1.2 (0.7 - 2.0)
Persson et al., 1964-1986 (Persson et al., 1989)	Non-Hodgkin's lymphomas	Herbicides	4.9 (1.3 - 18)
	Hodgkin's disease	Herbicides	3.8 (0.7 - 21)
Wingren et al., 1975-1982 (Wingren et al., 1990)	Soft tissue sarcomas	Unspecified chemical work, potential exposure to phenoxy herbicides or chlorophenols	1.6 (0.8 - 3.3)

^aConfidence intervals are 95% in the Olsson and Brandt study and 90% in the other two studies.

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2,4-D calculated from these groups are the subject of later studies. These studies did not consider chlorophenol exposures, and only those persons who ever lived or worked on a farm were asked questions about pesticide exposures. Farmers and nonfarmers were asked about home and garden use of pesticides. Thus, all nonfarmers were considered unexposed. As in southern Sweden (Table 7-6), the vast majority of phenoxy acid exposures did not involve 2,4,5-T and those that did virtually always involved 2,4-D as well. The relevance of these studies to the focus on TCDD and related compounds in this review is therefore somewhat limited.

Results for 2,4,5-T from the three studies are summarized in Table 7-10. Among all subjects and among farmers, only the study in eastern Nebraska (Zahm et al., 1990) suggests an increase in risk. All three studies were conducted with virtually identical methods and no information on herbicide application methods in any of the reports indicate any exposure conditions peculiar to eastern Nebraska.

The third set of relative risk estimates in Table 7-10 were computed using the investigators' procedure of including only the exposed farmers and the unexposed nonfarmers, with the unexposed farmers excluded. Comparing exposed farmers with unexposed nonfarmers shows that it is possible that risk estimates could be influenced by potential confounding effects that are germane to farmers, i.e., exposure to other pesticides or herbicides in large quantities. Furthermore, if the results of follow-up efforts are markedly different in farmers versus follow-up efforts in nonfarmers, then this difference also might add some uncertainty to the accuracy of risk estimates. In these analyses, the relative risk estimates from the studies in Kansas, Iowa, and Minnesota are somewhat higher and the estimate from the eastern Nebraska study is somewhat lower than in the two more conventional analyses.

Formal homogeneity tests across the three studies yield two-tailed p-values of 0.4 in the analysis of all subjects, 0.3 in the analysis restricted to farmers, and 0.6 in the third analysis. Ordinarily, especially considering the virtually identical methods used in the three studies, these results would be considered sufficient justification to compute summary

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Table 7-10. Relative Risks of Non-Hodgkin's Lymphomas in Relation to Farm Use of 2,4,5-T in Case-Control Studies in Kansas, Eastern Nebraska, Iowa, and Minnesota

Occupational category	Exposure category and measure	<u>Kansas (Hoar et al., 1986)</u>		<u>Eastern Nebraska (Zahm et al., 1990)</u>		<u>Iowa and Minnesota (Cantor et al., 1992)</u>	
		Cases	Controls	Cases	Controls	Cases	Controls
All subjects	Exposed	3	18	13	27	25	48
	Unexposed	167	930	188	696	597	1,197
	Relative risk 95 % confidence interval	0.9 0.3 - 3.2		1.8 0.9 - 3.5		1.0 0.6 - 1.7	
Farmers	Exposed	3	18	13	27	25	48
	Unexposed	130	644	134	512	331	650
	Relative risk 95 % confidence interval	0.8 0.2 - 2.8		1.8 0.9 - 3.7		1.0 0.6 - 1.7	
Exposed farmers and unexposed nonfarmers	Exposed	3	18	13	27	25	48
	Unexposed	37	286	54	184	266	547
	Relative risk 95 % confidence interval	1.3 0.4 - 4.6		1.6 0.8 - 3.4		1.1 0.6 - 1.8	

Source: Hoar et al., 1986; Zahm et al., 1990; Cantor et al., 1992.

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estimates. Summary (maximum likelihood) estimates of relative risk are virtually identical in all three groups of subjects, with point estimates of 1.2, lower 95% confidence limits of 0.8, and upper 95% confidence limits of 1.7 to 1.8.

Woods and colleagues (1987) conducted a study of soft tissue sarcomas and non-Hodgkin's lymphomas in western Washington State. In this study, the principal method of phenoxy acid and chlorophenol exposure assessment was to place job titles, activities, and chemical preparations reported during interviews into categories of potential exposure. The categories were created "in consultation with local industrial and university representatives who had long-term experience with forestry, wood products, and agricultural industries in the Pacific Northwest" (Woods et al., 1987). No statement is given about the relative prevalence of 2,4-D and 2,4,5-T among the phenoxy acids used in this region. The authors did not present any information regarding tissue levels of TCDD in either cases or controls.

The results (Table 7-11) show no association between soft tissue sarcomas or non-Hodgkin's lymphomas and estimated potential for exposure to phenoxy acids or chlorophenols. The authors report, however, that the relative risk of non-Hodgkin's lymphomas associated with more than 15 years of potential exposure to phenoxy acids increased with time since the accumulation of that exposure. The relative estimates were 1.3 (95% CI=0.9-2.2) for exposures more than 5 years before diagnosis, 1.7 (95% CI=1.0-2.8) for exposures more than 15 years before, and 2.5 (95% CI=0.5-13.0) for exposures more than 25 years before. The authors stated that similar trends were not seen in any of the analyses of soft tissue sarcomas and phenoxy acids or of either cancer in connection with chlorophenol exposures. It is not possible with the available data from this study to conduct analyses restricted to persons who worked in forestry, agriculture, and the wood products industry, and in which the exposed persons are those who reported specific exposures to phenoxy acids or chlorophenols.

The western Washington State study reported two unique results. One consisted of elevated relative risks in connection with a history of chloracne based on personal interviews: 3.3 (95% CI=0.8-14.0) for soft tissue sarcomas and 2.1 (95% CI=0.6-7.0) for non-

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Table 7-11. Relative Risks of Soft Tissue Sarcomas and Non-Hodgkin's Lymphomas in Relation to Phenoxyacetic Acid and Chlorophenol Exposure in a Case-Control Study in Western Washington State, 1981-1984

Exposure measure	Soft tissue sarcomas	Non-Hodgkin's lymphomas
<u>Estimated potential for phenoxyacetic acid exposure^a</u>		
Low	0.6 (0.3 - 1.1)	0.9 (0.6 - 1.3)
Medium	1.0 (0.6 - 1.7)	0.9 (0.7 - 1.3)
High	0.9 (0.4 - 1.9)	1.2 (0.8 - 1.9)
Any	0.8 (0.5 - 1.2)	1.1 (0.8 - 1.4)
<u>Estimated potential for chlorophenol exposure^a</u>		
Low	0.9 (0.5 - 1.6)	1.0 (0.7 - 1.3)
Medium	0.9 (0.6 - 1.5)	0.9 (0.7 - 1.2)
High	0.9 (0.5 - 1.8)	0.9 (0.9 - 1.4) ^b
Any	1.0 (0.7 - 1.5)	1.0 (0.8 - 1.2)

^aThe reference category had no estimated potential for exposure (relative risk 1.0 by definition).

^bEither the point estimate or the lower confidence limit appears to have been a typographical error in the original report. (On a logarithmic scale, the point estimate should be centered between the two confidence limits.)

Source: Woods et al., 1987.

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Hodgkin's lymphomas. However, the diagnoses were not medically confirmed and because only 1% of all cases and controls reported chloracne histories, the confidence intervals were extremely wide. The other intriguing result consisted of elevated relative risks of soft tissue sarcomas among persons with Scandinavian surnames (12% of the cases and controls). The estimates from this analysis were 2.8 (95% CI=0.5-15.6) for "high" estimated potential for phenoxy acid exposure and 7.2 (95% CI=2.1-24.7) for "high" estimated potential for exposure to chlorophenols. The authors noted that similarly elevated relative risks were not found for non-Hodgkin's lymphomas.

In a later study of the same study population, Woods and Polissar (1989) found a significant excess risk (OR=1.33, 95% CI=1.03-1.7) of non-Hodgkin's lymphoma among farmers compared with nonfarmers. When further examined to determine if 2,4-D or 2,4,5-T was responsible, both tended to be nonsignificantly decreased. However, frequency of use of these herbicides was not considered by the authors.

Brown et al. (1990) conducted a population-based, case-control interview study of 578 white males with leukemia in Iowa and Minnesota matched to 1,245 controls living in those same states. The purpose of the study was to investigate potential agricultural hazards that may be related to a diagnosis of leukemia. The cases were derived from the Iowa Tumor Registry and a network of hospitals and pathology laboratories in Nebraska between March 1981 and October 1983. Areas with little farm activity were excluded from the study. There was a slight but marginally significant elevation of leukemia risk (OR=1.2, CI=1.0-1.5) in farmers versus nonfarmers. But for those who mixed, handled, or applied 2,4,5-T, the risk was slightly but nonsignificantly elevated (OR=1.3, CI=0.7-2.2).

In another case-control study of Iowa agricultural influences on multiple myeloma, Brown et al. (1993), using similar methodology as in her earlier study, matched 173 white males with multiple myeloma to 650 controls from Iowa. Although a slight nonsignificant elevated risk (OR=1.2, CI=0.8-1.7) was seen in farmers, the risk of multiple myeloma from exposure to 2,4,5-T was found to be nonsignificant (OR=0.9, CI=0.4-2.1). The same was true for numerous other herbicides, pesticides, and insecticides.

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The authors concluded that there was little evidence to suggest any association of multiple myeloma with farming or pesticides. Neither of these studies on leukemia or multiple myeloma has shown that exposure to dioxin occurred. This is only presumed. No actual measurements were taken. Both of these studies could be considered hypothesis-generating studies because they involved multiple exposures to many different chemicals used in farming.

The major problem with U.S. case-control studies is that specific exposure to TCDD and related compounds is not identified or quantified, although information on the use of 2,4,5-T and 2,4-D is available in some studies. In some, only potential exposure to phenoxy herbicides is the exposure surrogate. This limits the usefulness of these studies.

7.6.3. New Zealand

Smith, Pearce, and colleagues conducted two studies of soft tissue sarcomas (Smith et al., 1982a, 1983, 1984; Smith and Pearce, 1986) and one study of non-Hodgkin's lymphomas (Pearce et al., 1986, 1987) among men in New Zealand. In these studies, persons were first asked whether or not they "had worked in particular occupations in which there was potential for exposure to phenoxyherbicides or chlorophenols" (Smith et al., 1984). If the response was affirmative, "a series of subsidiary questions were asked to clarify the work done and the actual potential for exposure, firstly in general terms, and then in specific terms, seeking the identity of the chemicals used" (Pearce et al., 1986). The authors indicated that 2,4,5-T was widely used as a phenoxy acid herbicide in New Zealand over the years pertinent to these studies (i.e., prior to the early 1980s) (Smith et al., 1984). Thus, in these studies, the phenoxy acid exposure designation was considered a suitable indicator of exposure to 2,4,5-T and, thus, to TCDD. Typical uses of 2,4,5-T were in the spraying of gorse, blackberry, pasture, cereal, and peas. No actual measurements of 2,3,7,8-TCDD were made in these studies.

In the analyses of phenoxy acids, the authors distinguished between "potential" and "probable or definite" exposure. The latter category was created by deleting persons with

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only "possible" exposures from those with "potential" exposures. It is not clear whether the "probable or definite" designation included inferences from job titles, activities, and the like, or whether it was based solely on affirmative responses to specific questions about phenoxy acid exposures. For chlorophenols, only the "potential" designation was employed.

In these studies, the controls were patients diagnosed with other cancers. As opposed to a control group selected from the entire study population, a cancer control group offers less certainty about the degree to which its exposure distribution represents that of the study population, but greater certainty that bias from differential exposure misclassification through elimination of interviewer bias and recall bias is negligible. However, inclusion of cancer sites in the controls that may be associated with the exposure could potentially bias the risk estimate toward the null. In these particular studies, the cancer controls had an additional advantage in minimizing any bias that might have resulted from the inability of the researchers to include patients diagnosed at private hospitals. Private hospitals in New Zealand have only recently been contributing to the National Cancer Registry. In an interim report of the non-Hodgkin's lymphoma study, a second control group was drawn from the New Zealand electoral roll. The authors concluded that this control group "gave very similar findings to those obtained with the main control group of other cancer patients" (Pearce et al., 1986).

Another unique feature of the New Zealand soft tissue sarcoma studies is that, like the mortality follow-up studies of chemical manufacturing and processing workers previously reviewed, they included only those cases classified to the International Classification of Diseases (World Health Organization, 1977) category 171, malignant neoplasms of the soft and connective tissues. This category, which does not include soft tissue sarcomas occurring in parenchymatous organs such as the stomach or uterus, accounted for about 60% of the soft tissue sarcoma cases in the studies in Sweden (Fingerhut et al., 1984). There is no indication from the Swedish studies, however, that the associations with phenoxy acids or chlorophenols differed between soft tissue sarcomas that would be classified in category 171 and those that would be classified in the categories for the involved organs (Hardell and

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Sandström, 1979; Eriksson et al., 1981; Hardell and Eriksson, 1988; Eriksson et al., 1990; Hardell, 1981).

In the New Zealand Study, investigators divided their soft tissue sarcoma research into two studies with very similar, but not identical, methods. The first study (Smith et al., 1982a, 1983, 1984) consisted of patients and controls with cancer registrations in the years 1976-1980. The second study (Smith and Pearce, 1986) extended case-finding through 1982 and was the subject of an extremely abbreviated report. The controls in the second study consisted of 315 of the 338 cancer controls from the non-Hodgkin's lymphoma study (Pearce et al., 1985) whose cancer registrations were during the period 1977-1981. (The results for the additional 23 controls, who were interviewed near the end of the non-Hodgkin's lymphoma study, evidently were unavailable at the time the analyses for the second soft tissue sarcoma study were conducted.)

The first sarcoma study (Smith et al., 1982a, 1983, 1984) reported very similar results for phenoxy acids and chlorophenols when all subjects were included in the analyses, with relative risk estimates of 1.3 for any "potential" exposure and 1.6 for exposures ("definite or probable" for phenoxy acids, "potential" for chlorophenols) lasting more than 1 day and occurring more than 5 years prior to diagnosis (Table 7-12). For phenoxy acid exposures classified by the latter definition, sufficient data were presented to permit an analysis restricted to farmers. Thirty of the 82 cases, 13 of the 17 exposed cases, 44 of the controls, and 9 of the 13 exposed controls were farmers. Thus, the estimated relative risk is 3.0 (95% CI=1.1-8.3) among farmers. Controlling for farming by ("indirect") standardization yields an estimated relative risk of 1.9 (95% CI=0.8-4.5). Thus, as in some studies previously reviewed, accounting for the farmer/nonfarmer distinction has a material impact on the results from this study.

Very few details were presented for the second sarcoma study (Smith and Pearce, 1986). In comparison with a relative risk of 1.6 in the first study, the second study reported a relative risk of 0.8 for the principal measure of phenoxy acid exposure (Table 7-12, homogeneity-test p-value = 0.2 contrasting the two studies). The exposure prevalences in

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Table 7-12. Relative Risks of Soft Tissue Sarcomas and Non-Hodgkin's Lymphomas in Relation to Potential Exposure to Phenoxy Acids and Chlorophenols in Case-Control Studies in New Zealand

Measure	Soft tissue sarcomas		Non-Hodgkin's lymphomas (1978-1981) (Pearce et al., 1987)
	First study (1976-1980) (Smith et al., 1983, 1984)	Second study (1981-1982) (Smith and Pearce, 1986)	
Cases	82	51	183
Controls	92	315	338
<u>Phenoxy acids</u>			
Any potential exposure			
Cases	21	NR	44
Controls	19	NR	72
Relative risk (95% CI)	1.3 (0.7 - 2.7)	NR	1.2 (0.8 - 1.8)
Probable or definite exposure > 1 day, > 5 years before cancer registration			
Cases	17	6	29
Controls	13	46	50
Relative risk (95% CI)	1.6 (0.7 - 3.5) ^a	0.8 (0.3 - 1.9)	1.1 (0.7 - 1.8)
<u>Chlorophenols</u>			
Any potential exposure			
Cases	8	NR	21
Controls	7	NR	27
Relative risk (95% CI)	1.3 (0.5 - 3.8)	NR	1.5 (0.8 - 2.7)
Potential exposure > 1 day, > 5 years before cancer registration			
Cases	8	NR	20
Controls	6	NR	27
Relative risk (95% CI)	1.6 (0.5 - 4.7)	NR	1.4 (0.8 - 2.6)

^aAmong farmers, 3.0 (1.1-8.3); controlling for farming by standardization, 1.9 (0.8-4.5).
CI, confidence interval; NR, not reported.

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the two control groups were virtually identical (14.1% in the first study and 14.6% in the second), but the prevalences in the two case groups differed (exposure odds ratio = 2.0, 95% CI=0.7-5.4). Because of this difference, and because a relative risk estimate restricted to farmers cannot be computed with the data available from the second study, aggregation of the results would not be warranted.

The non-Hodgkin's lymphoma study (Pearce et al., 1986, 1987) reported little or no association with phenoxy acids and a somewhat stronger association with chlorophenols (Table 7-12). The latter association did not increase when the more restrictive measure of exposure was used. The various activities involving exposure to chlorophenols include the treatment of fence posts as well as treating pelts in meat works tanneries. Data that would permit an analysis restricted to farmers were not reported.

The authors continue to maintain that herbicide spraying is a full-time occupation in New Zealand and that none of the soft tissue sarcoma or malignant lymphoma cases had been commercial sprayers. Smith et al. (1984) estimated the prevalence of current and former commercial sprayers at approximately 1,500, which would be 0.17% of the male population of New Zealand in the early 1970s (Waterhouse et al., 1982). On the null hypothesis, therefore, only about 0.1 commercial sprayers would be expected among the cases in each of the two soft tissue sarcoma studies and about 0.3 commercial sprayers would be expected among the non-Hodgkin's lymphoma cases. Thus, soft tissue sarcoma risk could have been increased manyfold and non-Hodgkin's lymphoma risk could have been increased about 3-fold before even one commercial sprayer would be expected in any of the case groups. As a consequence, the absence of commercial sprayers in any of the case groups is not strong evidence against an effect.

In a letter to the editor, Pearce (1989) produced tabular data from his earlier case-control study by duration of use and by frequency of use. Although he maintained that his data exhibited little evidence of an association with non-Hodgkin's lymphoma, a nonsignificant increase in the risk was seen in the category 10-19 days of use per year

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(OR=2.2, 95% CI=0.4-12.6) before dropping back to 1.1 in the category greater than 19 years.

In a study of nine selected applicators in New Zealand who had sprayed herbicides for a minimum of 180 months (and hence 2,4,5-T), Smith et al. (1992) found a high correlation between tissue levels of TCDD and months sprayed. This is analogous to Fingerhut's finding that tissue levels of TCDD correlate well with duration of employment in the herbicide manufacturing industry. TCDD serum levels ranged from 131.0 ppt in a sprayer with 31 years of spraying to a low of 3.0 ppt in a sprayer who sprayed for only 7 years. The average was 53 ppt for the nine sprayers who sprayed an average of 16 years. Actually, the mean average TCDD serum level in Fingerhut's lowest exposure group who worked less than 1 year was higher (69 ppt) than the mean average of sprayers in the Smith et al. study. Smith's conclusions were based on his analysis that brief exposures to TCDD probably do not contribute to the increased cancer risks seen in studies in other countries. Although it is an interesting inference, this conclusion may be somewhat overstated without some information regarding what the tissue levels of TCDD were in the individual cases and controls of those other studies, information that only recently is becoming available and not for all studies. Unfortunately, the hypothesis suggested by several occupational accidents, such as in Seveso, Italy, and Nitro, West Virginia, that one-time large doses of exposure to 2,3,7,8-TCDD could, in fact, lead to residual high tissue levels of serum 2,3,7,8-TCDD years later, could not be tested in the studies of Swedes. Hardell and colleagues have provided little information regarding tissue levels of TCDD, past or present, in individuals who were participants in their studies. One study by Nygren et al. (1986) is cited frequently as evidence that Swedish subjects who were involved in spraying phenoxy herbicides have low levels of TCDD in adipose tissue samples. Thirty-one patients from the Regional Hospital in Umea were each relieved of a sample of adipose tissue for analysis of dioxin content. After "careful interviewing," it was determined that 13 of these patients had "sprayed" herbicide at some time during their past. Adipose tissue measurements indicated a mean of 2 ppt of 2,3,7,8-TCDD. The remaining 18 nonsprayers revealed a mean of 3 ppt.

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However, in correspondence with C. Rappe (1987) regarding this study, it was determined that of the only three that were soft tissue sarcomas, adipose tissue measurements of 2,3,7,8-TCDD are reported to be 2, 2, and 9 ppt. The one STS with the highest 2,3,7,8-TCDD level (9 ppt) of any of the 31 subjects is stated by the authors to have had only 10 days of "knapsack spraying" some 25 to 29 years earlier. What is striking about these 13 "cases" is that the total levels of *all* chlorinated dioxins are considerably greater, i.e., from 168 ppt to as much as 936 ppt per patient, and that TCDD levels are a mere fraction of the total. The vast majority consist of the higher-chlorinated PCDDs. It is not clear that spraying herbicides is or ever was a major occupational endeavor of this group of 13 or that any of these patients was exposed to 2,3,7,8-TCDD in large quantities. Based on recent correspondence with Hardell (1993), none of the patients reported by Nygren were members of any of his case-control studies. The total PCDD levels in the three STSs ranged from 674 ppt to 792 ppt (Rappe, 1987). These were nearly all highly chlorinated. Although the cases with tissue samples came from Hardell's clinic, they did not come from any case-control studies.

This analysis provides little information on tissue levels in Swedish applicators with STS. Furthermore, there may be some differences in applicator practices between New Zealand and other countries such as Sweden. Professional applicators in New Zealand are registered with the New Zealand Agricultural Chemicals Board (Smith et al., 1982b, 1992). And although it might appear that they could be expected to receive a great deal of exposure to TCDD, more than half of the applicators show serum TCDD levels below 50 ppt (Smith et al., 1992). Considering that they were spraying 2,4,5-T for 7 to 31 years until just recently, it seems remarkable that the distribution of serum TCDD levels is as low as it is. The authors report that professional pesticide applicators in New Zealand are "perhaps the group most heavily exposed to agricultural use of 2,4,5-T in the world." In other studies, shorter exposures to large quantities of TCDD-containing herbicides have occurred to a few personnel such as in the Ranch Hands Cohort, Seveso, Italy, and the Nitro, West Virginia,

accident. In Fingerhut's study, employees with less than 1 year of exposure to phenoxyacetic acids had mean serum levels averaging 69 ppt 2,3,7,8-TCDD.

7.6.4. Italy

Vineis and colleagues conducted a case-control study of soft tissue sarcomas in three provinces in northern Italy (Vineis et al., 1986). Phenoxy acid exposure classifications were based on job information provided on interviews or questionnaires. The assessments were made by "[t]wo experts with experience in chemical aspects of agriculture." Cases and controls were classified into three categories: "certainly unexposed," "exposure could not be ruled out" (abbreviated below as "possibly exposed"), and "certainly exposed." The authors implied that phenoxy acid herbicides of all types (2,4-D, 2,4,5-T, and MCPA) were used in the area during the periods of interest, but were able to document only the use of 2,4-D and MCPA (MCPA and MCPP like 2,4-D do not contain dioxin and dibenzofuran impurities). Thus, this study may have limited relevance to an interest in TCDD exposures.

The study indicated an inverse association between possible or certain phenoxy acid exposure and soft tissue sarcoma risk among men and a positive association among women (Table 7-13). This latter association was restricted to women who were alive at the time the exposure information was collected. (In the other studies in this review, in which the results were stratified by vital status at the time of the interview, no appreciable differences were found.) As shown in Table 7-13, when the analysis is restricted to persons who had ever worked in agriculture ("farmers"), the relative risk among all women is reduced from 1.9 to 1.1. Sufficient data are not available for an analysis that is both restricted to farming women and stratified by vital status.

The authors offered overmatching by location of residence as an explanation for the lack of association among deceased subjects. It would be extraordinary for overmatching or nondifferential misclassification (the latter being the usual explanation when reduced relative risks are obtained with exposure information from proxy respondents) to be so strong as to bias a relative risk of 2.4 all the way down to 0.8.

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Table 7-13. Relative Risks of Soft Tissue Sarcomas in Relation to Phenoxy Acid Exposure in Case-Control Study in Northern Italy, 1981-1983

Category	Measure	Men		Women	
		Unexposed	Possibly or certainly exposed	Unexposed	Possibly or certainly exposed
Living	Cases	21	2	16	5
	Controls	54	8	53	7
	Relative risk	1.0	0.6	1.0	2.4
	(95 % confidence interval)	NA	(0.1 - 3.3)	NA	(0.7 - 8.5)
Deceased	Cases	13	1	6	4
	Controls	17	6	7	6
	Relative risk	1.0	0.2	1.0	0.8
	(95 % confidence interval)	NA	(0.0 - 2.0)	NA	(0.1 - 4.1)
Total	Cases	34	3	22	9
	Controls	71	14	60	13
	Relative risk	1.0	0.4	1.0	1.9
	(95 % confidence interval)	NA	(0.1 - 1.7)	NA	(0.7 - 5.0)
Total, farmers only	Cases	12	3	5	9
	Controls	12	14	8	13
	Relative risk	1.0	0.2	1.0	1.1
	(95 % confidence interval)	NA	(0.0 - 0.9)	NA	(0.3 - 4.5)

NA, not applicable.

Source: Vineis et al., 1986.

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Rice is the principal agricultural crop in the study area and rice weeding was historically a predominantly female occupation. (Of 29 rice weeders in the study, all but two were women.) Rice weeding during the period 1950-1955 was manual and contact with the phenoxy herbicides was mainly through the skin.

Among all women in this study, rice weeding during the early 1950s is associated with a relative risk of 2.3 (95% CI=0.7-7.7). When the analysis is restricted to women who were farmers, however, the relative risk drops to 1.4 (95% CI=0.3-6.5).

7.6.5. Summary

From the standpoint of exposures to TCDD, the most important results from general-population case-control studies come from those studies conducted in northern Sweden (Hardell and Sandström, 1979; Hardell and Eriksson, 1988; Hardell et al., 1981), central Sweden (Eriksson et al., 1990), and New Zealand (Smith et al., 1982a, 1983, 1984; Smith and Pearce, 1986; Pearce et al., 1986, 1987). These studies were conducted in areas in which high proportions of phenoxy acid exposures involved 2,4,5-T. The exposure-assessment methods in these studies included the posing of specific questions about particular chemicals and herbicide preparations. Moreover, for all but the non-Hodgkin's lymphoma study in New Zealand (Pearce et al., 1986, 1987), available data permit analyses restricted to farmers and the other occupational categories within which the relevant exposures predominantly occur.

For soft tissue sarcomas, the Swedish studies are perhaps best represented by a relative risk of 2.3 (95% CI=1.0-5.4) for phenoxy acids among workers in agriculture, horticulture, and forestry in the study in central Sweden (Table 7-7) (Eriksson et al., 1990). This is justified by the following factors: the proportion of exposures to 2,4,5-T was high in this study, the methods of assessment were better, and there were analyses within relevant occupational categories. The authors in their current studies have redesigned their methods to accommodate readers' criticisms of their earlier studies and have made an effort to present risk estimates that are adjusted to reflect these criticisms.

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The relative risk estimate of 3.0 (95% CI=1.1-8.3) for phenoxy acid exposure among farmers in the first soft tissue sarcoma study in New Zealand (Smith et al., 1982a, 1983, 1984) seems to indicate that farming may be a confounder in this study. Indirect standardization for farming produces a relative risk of 1.9 (95% CI=0.8-4.5).

For malignant lymphomas, the case-control studies provide less evidence of a positive association. The relative risk estimates from the study by Hardell and colleagues (Hardell et al., 1981) were very high, even among persons employed in the special occupational groups, but this study was conducted before the researchers had improved their data collection methods. The studies in New Zealand (Pearce et al., 1986, 1987), Kansas (Hoar et al., 1986), eastern Nebraska (Zahm et al., 1990), and Iowa and Minnesota (Cantor et al., 1992) are more consistent with a much smaller increase in risk, or no increase at all, from exposures to TCDD.

The remaining case-control studies (Eriksson et al., 1981; Olsson and Brandt, 1988; Persson et al., 1989; Wingren et al., 1990; Woods et al., 1987) offer mixed results, some suggesting increases in the risk of soft tissue sarcoma or malignant lymphoma and others suggesting little or no increase. The informativeness of each of these studies, however, is limited by one or more of the following important drawbacks: study areas in which most phenoxy acid exposures did not involve 2,4,5-T, a lack of information on specific chemicals and preparations to which cases and controls were exposed, and an inability with available data to conduct analyses restricted to farmers and the other occupational groups in which the exposures of interest primarily occur. Apparently, farming as an occupation appears to affect risk estimates based on the findings from several studies where occupation is considered and should be considered as a potential confounder.

Vineis et al. (1992) presents the hypothesis that the excess risk of non-Hodgkin's lymphoma seen among farmers exposed to phenoxy herbicides may be caused by viruses. Such viruses induce proliferation and immortalization of B-cells, followed by T-cell impairment leading to cell-mediated immunity. Increased risks of NHL have been observed

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in immunologically deficient individuals. Hypothetically, the same effect could be the result of exposure to TCDD as suggested in some mouse studies (see Chapter 4, Immunotoxicity).

7.7. STUDIES OF PULP AND PAPER MILL WORKERS

Table 7-14 summarizes results for cancers of interest from three follow-up studies of pulp and paper mill workers. These studies are important because of the potential for exposure to PCDDs in this line of work. The study by Robinson et al. (1986) was of 3,572 persons who had worked for at least 1 year between 1945 and 1955 at any of five mills in the states of California, Oregon, or Washington. The study by Jäppinen et al. (1987) was of 3,454 workers in the Finnish pulp and paper industry who had worked continuously for at least 1 year between 1945 and 1961. The study by Henneberger et al. (1989) was of 883 persons who had worked for at least 1 year at a mill in New Hampshire. Jäppinen et al. (1987) studied cancer incidence. The other two studies were mortality studies.

Individually and in the aggregate, these studies give little indication of appreciable increases in the risk of non-Hodgkin's lymphomas, lung cancer, or stomach cancer among pulp and paper mill workers. Overall, the rate of all cancers combined was somewhat lower than expected. None of the studies examined connective and soft tissue cancers specifically. Analyses of specific cancers by work location, duration of employment, and latency were only occasionally conducted in these studies. No consistent results were found that would alter substantially the impression given by the results for the total cohorts. These studies do not specifically mention exposure to the PCDDs and are not designed to evaluate the risk of cancer to PCDDs.

Other studies of cancer among paper and pulp mill workers have been restricted to information on deaths, using either proportional mortality ratios (Milham, 1976; Milham and Demers, 1984; Schwartz, 1988; Solet et al., 1989) or mortality odds ratios (Wingren et al., 1991) as measures of relative risk. These studies are not highly informative because they usually rely on minimal information in death records and because they are subject to an upward bias due to the "healthy-worker effect" (i.e., a tendency for employed groups to have

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Table 7-14. Relative Risks for Selected Cancers from Follow-up Studies of Paper and Pulp Mill Workers

Cancer	Study	Observed deaths	Expected deaths	Relative risk	95% confidence interval
Non-Hodgkin's lymphomas	Robinson (1986)	12	8.9	1.3	0.7 - 2.3
	Jäppinen (1987)	2	3.5	0.6	0.1 - 1.9
	<u>Henneberger (1989)</u>	<u>4</u>	<u>3.8</u>	<u>1.1</u>	<u>0.3 - 2.5</u>
	Total	18	16.2	1.1	0.7 - 1.7
Lung cancer	Robinson (1986)	50	62.1	0.8	0.6 - 1.1
	Jäppinen (1987)	78	62.6	1.2	1.0 - 1.5
	<u>Henneberger (1989)</u>	<u>25</u>	<u>28.0</u>	<u>0.9</u>	<u>0.6 - 1.3</u>
	Total	153	152.7	1.0	0.8 - 1.2
Stomach cancer	Robinson (1986)	17	13.8	1.2	0.7 - 1.9
	Jäppinen (1987)	24	28.8	0.8	0.5 - 1.2
	<u>Henneberger (1989)</u>	<u>5</u>	<u>4.2</u>	<u>1.2</u>	<u>0.4 - 2.6</u>
	Total	46	46.8	1.0	0.7 - 1.3
All cancers combined	Robinson (1986)	160	211.5	0.8	0.6 - 0.9
	Jäppinen (1987)	196	203.8	1.0	0.8 - 1.1
	<u>Henneberger (1989)</u>	<u>97</u>	<u>87.9</u>	<u>1.1</u>	<u>0.9 - 1.3</u>
	Total	453	503.2	0.9	0.8 - 1.0

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favorable total mortality experience and causes of death other than cancers, when compared with the general population). The degree of bias in such studies varies, but it can be appreciable. For instance, in the cohort studied by Robinson et al. (1986), 915 deaths from all causes were observed and 1,150.3 were expected. If the relative risk estimates for stomach cancer and non-Hodgkin's lymphomas had been computed as proportional mortality ratios or mortality odds ratios, they would have been 1.5 and 1.7, respectively, instead of the values of 1.2 and 1.3 that were obtained from the authors' more valid comparisons of mortality rates (Table 7-14).

7.8. OTHER STUDIES

Studies of pesticide applicators are not informative because they contain little information on specific compounds and preparations to which individual persons were exposed and as such there is no evidence of exposure to TCDD. Studies with no information of this type include studies of licensed pesticide applicators by Wang and MacMahon (1979), Barthel (1981), Blair et al. (1983), Wiklund et al. (1987), Corrao et al. (1989), and a study of gardeners by Hansen et al. (1992). These studies contribute little or nothing to the discussion of TCDD or compounds like TCDD.

Axelsson and Sundell assembled a cohort of 348 Swedish railroad workers who had applied amitrol, 2,4-D, and 2,4,5-T (Axelsson and Sundell, 1974). In the most recent report (Axelsson et al., 1980), 17 deaths from tumors were observed (11.85 expected, relative risk 1.43, $p=.09$). The relative risk estimate for lung cancer was 1.4 (three observed deaths, $p=.37$) and the estimate for stomach cancer was 2.2 (three observed deaths, $p=.15$). Again as in most studies, no actual measurements of TCDD are available from this paper. Only potential exposure to the herbicides 2,4-D and 2,4,5-T are mentioned without any effort to quantify the exposure.

Riihimäki et al. (1982, 1983) followed a cohort of 1,971 Finnish men who had applied 2,4-D and 2,4,5-T. With allowance for a 10-year latency period, 20 cancer deaths were observed (24.3 expected, $RR=0.8$, 95% $CI=0.5-1.2$). The relative risk for lung

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cancer was 1.1 (12 deaths observed, 95% CI=0.6-1.8). The author points out that because of limitations in the study materials, only powerful carcinogenic effects are likely to be seen.

7.8.1. Vietnam Veterans

Distributions of TCDD levels in serum and adipose tissue are typically indistinguishable between Vietnam veterans and comparison populations unless the Vietnam veterans group has been carefully defined on the basis of military records to have engaged in activities known to have involved herbicide exposure (Centers for Disease Control Veterans Health Studies, 1988; Devine et al., 1990; Gross et al., 1984; Kahn et al., 1988; Kang et al., 1991; Pirkle et al., 1989; Schecter et al., 1989). Thus, the mere designation, "Vietnam veteran," is insufficient as an indicator of exposure to 2,4,5-T or TCDD exposure. This conclusion is also supported by Stellman and Stellman's (1986) review of military records for the purpose of developing an Agent Orange exposure index. Stellman and Stellman drew the further conclusion that "it is impossible to give any credence to any health effects study in which assignment of herbicide exposure levels to individual veterans is based solely on self-reports" (Stellman and Stellman, 1986). It is also insufficient to base an exposure index among Vietnam veterans on such crude information as military branch (Army, Marine, etc.), corps, or region of duty within Vietnam. Thus, a large number of studies of cancer experience among Vietnam veterans are uninformative from the standpoint of hypothetical effects of TCDD. These include studies by Breslin et al. (1988), the Centers for Disease Control (1987), Dalager et al. (1991), Fett et al. (1987), Greenwald et al. (1984), Kang et al. (1986), Kogan and Clapp (1988), Lawrence et al. (1985), and O'Brien et al. (1991).

One Vietnam veteran study by Kang et al. (1987) that further examined mortality in a subgroup of veterans who had ventured into the areas at the time when Agent Orange was being sprayed reported a nonsignificant odds ratio of 8.64 (CI=0.77-111.84) for soft tissue sarcoma. The number of cases was not provided. Presumably, these would be ground troops with a high likelihood of exposure.

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The only study of cancer among Vietnam veterans at present with information on activities involving TCDD exposure is a small mortality study by Michalek et al. (1990) of 1,261 Air Force veterans of Operation Ranch Hand. These persons were responsible for the aerial herbicide spraying missions in Vietnam. The researchers compared the Ranch Hand group with a group of 19,101 other Air Force veterans who were mainly involved in cargo missions in Southeast Asia and who did not have herbicide exposure. A total of 12 cancer deaths were observed in the Ranch Hand cohort (17.0 expected, $RR=0.7$, 95% $CI=0.3-1.1$). Calculated death rates of all specific cancers of interest in this review were equal to or less than the rates in the comparison group, with the exception of bone, connective tissue, skin, breast, and genitourinary organs. These numbers are too small for any meaningful comparisons.

Serum TCDD measurements were taken on 888 Ranch Hands (total). A few of the Ranch Hands, who were enlisted ground crew, exhibited tissue levels above 200 ppt of TCDD, but the median serum level was 12.4 ppt (range 0 to 618 ppm) for the entire group. The median serum level in the controls averaged 4.2 ppt (Wolfe et al., 1990). The majority of the Ranch Hands probably received little exposure. The subgroups of Ranch Hands that appear to have had the greatest exposure are nonflying enlisted personnel. The median serum 2,3,7,8-TCDD levels in 407 of them was 23.6 ppt. The next highest levels were in flying enlisted personnel with a median of 17.2 ppt. The remaining Ranch Hands exhibited levels that were not much elevated (under 10 ppt) from background (flying officers, both pilots and navigators, as well as nonflying officers). Not a great deal of cancer mortality is to be expected to date in this relatively youthful group, which has not quite reached the 20-year latency milestone. It would be of greater interest to reevaluate mortality and continue follow-up in the enlisted Ranch Hands only. They appear to be the subgroup with the greatest exposure. Further follow-up of the officers will probably not reveal any useful information that could be attributed to exposure to 2,3,7,8-TCDD. A more appropriate group in which to observe effects are members of the South Vietnamese Army who did the mainstay of the spraying around the perimeters of the military bases in Vietnam.

7.8.2. Residents of Seveso, Italy

Residents of Seveso, Italy, were exposed to 2,3,7,8-TCDD in a chemical accident in 1976. This group is important because of the high exposures, with approximately 200 cases of chloracne reported (Caramaschi et al., 1981). Children (Bertazzi et al., 1992) and adults (Bertazzi et al., 1989a, b) at the time of the accident are being studied separately. The group residing in the zone of highest estimated exposure consists of 556 adults and 306 children. The group residing in a zone of intermediate estimated exposure is larger, with 3,920 adults and 2,727 children. The group with lowest estimated exposure is larger still, with 26,227 adults and 16,604 children. The accuracy of the three estimated exposure zones has been questioned (Caramaschi et al., 1981; Merlo et al., 1986; Ratti et al., 1987; Merlo and Puntoni, 1986), especially because the ranking does not seem to correspond to the occurrence of chloracne in the area.

There is no question that at least some of the residents of the most heavily exposed area (zone A) received massive exposure to 2,3,7,8-T (Mocarelli et al., 1991). The 1990 analysis, based on tissue specimens taken in 1976, found that the highest detected levels were recorded just after the accident. Six children at the time who subsequently developed severe chloracne had serum 2,3,7,8-T levels ranging from 12,100 ppt to 56,000 ppt. Four other persons with slightly less severe chloracne exhibited levels ranging from 828 ppt to 17,300 ppt. These were similar to those of nine other residents of zone A who did not develop chloracne whose serum 2,3,5,8-TCDD levels ranged from 1,770 to 10,400 ppt (Mocarelli et al., 1991). None of the latter group of nine were reported to be ill at the time of sampling.

Thus far, the population in zones A, B, and R around Seveso has been followed for 10 years (Bertazzi et al., 1992, 1989a, b). Ten cancer deaths, too few to support a meaningful analysis of specific cancers, have been observed among children (Bertazzi et al., 1992). Results for the cancers of interest among adults are summarized in Table 7-15 (Bertazzi et al., 1989b). No excesses of mortality from lung cancer, stomach cancer, or all cancers combined are apparent. A moderate and statistically imprecise elevation in the death rate from a subset of the cancers that make up the non-Hodgkin's lymphomas is evident in

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Table 7-15. Relative Risks for Selected Cancers Among Adults Exposed to TCDD in Seveso, Italy

Cancer	Gender	Calendar period			
		1976-1981 RR (95% CI)	1982-1986 RR (95% CI)	No. deaths	1976-1986 RR (95% CI)
Connective and soft tissues	Male	(NA)	2.8 (0.3 - 31.1)	2	5.4 (0.8 - 38.6)
	Female	(NA)	3.0 (0.3 - 33.0)	1	2.0 (0.2 - 19.0)
"Other" lymphatic tissue ^a	Male	(NA)	1.3 (0.4 - 4.5)	3	0.9 (0.3 - 2.9)
	Female	1.0 (0.1 - 8.6)	1.6 (0.4 - 5.7)	4	1.4 (0.5 - 4.1)
Lung cancer	Male	0.7 (0.5 - 1.0)	1.0 (0.8 - 1.4)	99	0.9 (0.7 - 1.1)
	Female	0.7 (0.3 - 1.7)	0.7 (0.3 - 1.9)	10	0.7 (0.4 - 1.3)
Stomach cancer	Male	0.8 (0.5 - 1.3)	0.9 (0.6 - 1.4)	40	0.8 (0.6 - 1.2)
	Female	0.9 (0.5 - 1.7)	1.0 (0.5 - 2.0)	22	1.0 (0.6 - 1.5)
All combined	Male	0.8 (0.7 - 1.0)	1.0 (0.8 - 1.1)	325	0.9 (0.8 - 1.0)
	Female	0.8 (0.7 - 1.0)	0.8 (0.7 - 1.1)	176	0.8 (0.7 - 1.0)

^aA subset of non-Hodgkin's lymphomas.

NA, not available.

Source: Bertazzi et al., 1989b.

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the second 5-year period of follow-up. An excess of greater relative magnitude, but even more imprecisely estimated, in mortality from cancers of connective and soft tissues appears to have occurred in the same time period. Because the exposed Seveso residents have been followed for only 10 years since the accident (Bertazzi et al., 1989a, b), highly informative results will not come until additional time has elapsed.

In a preliminary study of cancer incidence in the same Seveso population (Pesatori et al., 1992), the relative risk estimate of connective, subcutaneous soft tissue sarcoma of males living in zone R is reported to be significantly elevated at 2.81 based on 6 cases (CI=1.1-7.4). In zones A and B, none were observed but 0.4 were expected to occur in males and 0.2 in females. For females, the risk in zone R of STS is 1.43 based on two cases. Other cancer sites that are also elevated are certain hematologic neoplasms in males (lymphoreticulosarcoma) and hepatobiliary tract cancers in both males and females.

Bertazzi et al. (1993) refined this study to include a more complete vital status ascertainment without adding additional years of follow-up to the cancer incidence data in the contaminated areas surrounding the factory where the accident took place. Cancer occurrence ascertainment was confined chiefly to the Lombardy region of Italy (with a population of 9 million persons) because only there can be found an efficient hospital and discharge registration system, according to the authors. Lombardy hospitals routinely provide hospitalization data to the regional health department. These results can be found in Table 7-16.

Of note in this update is the information that only 14 cases of cancer were reported to have occurred in zone A. But this is not unexpected. Only 724 residents were reported to have lived there. This number was based on the assignment of addresses by the municipal vital statistics offices. But it is far too small to produce any meaningful results. However, in the more populated region B, with 4,824 residents, and region R, with 31,647 residents, several findings were noted (Table 7-16). In zone B, hepatobiliary cancer in females (RR=3.3, 95% CI=1.3-8.1), lymphoreticulosarcoma in men (RR=5.7, 95% CI=1.7-19.0), and multiple myeloma in women (RR=5.3, 95% CI=1.2-22.6) were significantly elevated.

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Table 7-16. Relative Risks for Selected Cancers Among Adults Exposed to TCDD in Seveso, Italy, in Contaminated Areas B and R

Cancer	OBS	Males RR	95% CI	OBS	Females RR	95% CI
Region B						
All Malignancies	76	1.1	0.9 - 1.4	36	0.8	0.6 - 1.1
Trachea, Bronchus, Lung	18	1.1	0.7 - 1.8	0	—	—
Hepatobiliary	5	1.8	0.7 - 4.4	5	3.3	1.3 - 8.1
Liver	4	2.1	0.8 - 5.8	0	—	—
Hematopoietic System	8	2.1	1.0 - 4.3	6	1.9	0.8 - 4.4
Non-Hodgkin's Lymphoma	3	2.3	0.7 - 7.4	1	0.9	0.1 - 6.4
Lymphoreticulosarcoma	3	5.7	1.7 - 19.0	1	2.3	0.3 - 16.9
Hodgkin's Disease	1	1.7	0.2 - 12.8	1	2.1	0.3 - 15.7
Multiple Myeloma	2	3.2	0.8 - 13.3	2	5.3	1.2 - 22.6
Leukemia	2	1.6	0.4 - 6.5	2	1.8	0.4 - 7.3
Myeloid Leukemia	1	2.0	0.2 - 14.6	2	3.7	0.9 - 15.7
Region R						
All Malignancies	447	0.9	0.9 - 1.0	318	0.9	0.8 - 1.1
Trachea, Bronchus, Lung	96	0.8	0.7 - 1.0	16	1.5	0.8 - 2.5
Hepatobiliary	11	0.5	0.3 - 1.0	12	0.9	0.5 - 1.7
Liver	3	0.2	0.1 - 0.7	2	0.5	0.1 - 2.1
Connective & Soft	6	2.8	1.0 - 7.3	2	1.6	0.3 - 7.4
Non-Hodgkin's Lymphoma	12	1.3	0.7 - 2.5	10	1.2	0.6 - 2.3
Lymphoreticulosarcoma	4	1.1	0.4 - 3.2	6	1.7	0.7 - 4.2
Multiple Myeloma	1	0.2	0.0 - 1.6	2	0.6	0.2 - 2.8
Myeloid Leukemia	5	1.4	0.5 - 3.8	2	0.5	0.1 - 2.1
Genitourinary Organs	75	1.0	0.8 - 1.3	106	1.1	0.9 - 1.3
Breast	1	1.2	0.1 - 10.2			

Source: Bertazzi et al., 1993.

7-73

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In zone R, soft tissue sarcomas in men ($RR=2.8$, 95% $CI=1.0-7.3$) were the only site-specific cancers that were significantly elevated. Cancer of the genitourinary system was significantly depressed ($RR=0.8$, 95% $CI=0.6-1.0$). This is consistent with the animal data that suggest estrogen-induced protective effects in female rats.

The authors explain that the absence of cancer among chloracne victims is not unexpected at this time because of the relatively young age of the group and the small number of individuals affected.

Much has been made of the knowledge that these cancers have appeared within a relatively short period of time in the Seveso population for latent effects to have played any major role in the development of cancer; that is, they occurred too soon after the accident. And, indeed, it would appear that such is the case. But it should be remembered that a production plant making 2,4,5-T existed in that area many years prior to the date of the accident. It is possible that earlier exposure to dioxin from the preexisting plant could have been the initiating event for the cancers including the STSs. That would ensure enough time for latent effects to manifest themselves. The relatively intense exposures received by these victims may also have contributed to a shortening of the latent period. Furthermore, hematopoietic tumors have a shorter latency than most carcinomas.

7.8.3. Rice Oil Poisonings in Taiwan and Japan Involving Compounds Structurally Related to Dioxin

This section discusses two similar incidents involving ingestion of rice cooked with oils accidentally poisoned with PCBs and PCDFs. PCBs and PCDFs are structurally similar to the polychlorinated dioxins, and some of these are considered to be dioxin-like in their activity. The dioxin-like effects of these compounds are felt to be mediated through a cytosolic receptor (Poland, 1984; Poland et al., 1987; Goldstein and Safe, 1989). The dioxin-like polychlorinated biphenyl congeners, chlorinated dibenzofurans, and dioxins that have a high affinity to bind the Ah receptor induce similar effects in both animals and humans but appear to differ quantitatively in toxicity (McConnell, 1989; Ahlborg, Chapter 3,

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this document; Schechter, 1991). They appear to harm growth and reproduction, they may damage the immune system, and they also appear to cause cancer. These same effects have been observed in a number of different species including humans.

Two accidents involving ingestion of food contaminated with PCBs and dibenzofurans, in the Yusho (Japan) and Yu-Cheng (Taiwan) incidents, have been reported. The Yusho incident involved 1,900 people who in 1968 accidentally consumed up to 2 grams each of PCBs that had leaked into the rice oil at the facility where the rice oil was canned. The PCBs were primarily Kanechlor 400 that had been used as a heat exchange medium thousands of times. Commercial preparation Kanechlor 400 had a concentration that was 49% chlorinated. The use of this medium for exchange of heat resulted in an increase in the dibenzofuran contamination approximately 250 times. The final mixture that was actually present in the rice oil had a ratio of one molecule of dibenzofuran to every 200 molecules of PCBs.

These victims suffered many ill effects from their massive exposure that lasted only a few months. Tissue studies by the Japanese of the victims indicated that some of the PCBs and PCDFs were retained for many years after the initial exposure. The PCDFs are eliminated at a slower rate than PCBs. Concentrations measured several years following the Yusho accident indicated that the ratio of PCDFs to PCBs remaining in the adipose tissue of the victims was about 1 to 4 (Kuratsune et al., 1975). Japanese researchers have attributed most of the noncancer toxic effects to the presence of the PCDFs although these effects are consistent with PCB exposure. These toxic effects include comedo formation, acneform eruptions, hyperpigmentation, and hyperkeratosis. In addition, ocular lesions such as swollen meibomian glands filled with yellow infarct-like material and pigmentation of the conjunctiva were seen, similar to those effects of TCDD. For further discussion of these and other effects, see Part B of Chapter 7, which addresses noncancer health effects in humans.

Kuratsune et al. (1988) reported a significantly increased risk of liver cancer in male victims (9 observed vs. 1.6 expected; SMR=559, $p < .01$) and a nonsignificantly increased risk in female victims (2 observed vs. 0.66 expected; SMR=304), as well as a significantly

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increased risk of lung cancer in male victims (8 observed vs. 2.45 expected; SMR=326, $p < .01$). Some 1,761 patients (887 males and 874 females) were followed from date of registration to the end of 1983, 15 years after the accident in October of 1968. Thirty-three male cancer deaths had occurred by this time versus 15.51 expected. In female victims, 8 cancer deaths had occurred while 10.55 were expected. Comparisons were with the age-, sex-, and cause-specific death rates of Japan and, separately, of Nagasaki and Fukuoka prefectures in 1970, 1975, and 1980. The author reports that the risk of liver cancer remained elevated even after the influence of latency, alcohol consumption, and liver disease had been evaluated. Kuratsune said that because there was an uneven distribution of deaths in the provinces where most of the victims lived, it was too early to draw any conclusions. Apparently, most of the liver cancers occurred in Fukuoka prefecture. A statistically significant excess mortality was still present in males of Fukuoka even when liver cancer deaths occurring less than 9 years after the accident were eliminated. The author stated, "Such a markedly uneven geographical distribution of deaths can hardly be explained by exposure to the toxic rice oil alone." However, he cautioned that his findings suggest that the poisoning might have caused liver cancer at least in male patients. He concludes, "Our findings should not be disregarded, however, because the hepatocarcinogenicity of PCBs in animals has been well documented." Deaths from chronic liver diseases and cirrhosis are also elevated but not significantly.

An outbreak of illness similar to Yusho was reported among some 2,000 persons in the Taichung and Changhwa provinces of Taiwan in March 1979. The illness consisted of chloracne, hyperpigmentation, and meibomian gland dilatation. In October 1979, the illness was found to be the result of the ingestion of cooking oil contaminated with PCBs and PCDFs. Chen et al. (1980) reported on the blood PCB levels of 66 victims for which gas chromatograms had been prepared. Basically, blood concentration residues ranged from 11 ppb to 720 ppb in these patients. The mean value was 49 ppb; most values were under 100 ppb. In only two instances were the concentrations greater, at 120 ppb and 720 ppb. The authors reported that the higher value of 720 ppb occurred in a patient who had difficulty

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metabolizing and excreting PCB components. They also maintain that blood PCB levels of these patients are "much higher" than those of 72 Japanese Yusho patients (Koda and Masuda, 1975). Koda and Masuda reported the mean PCB value in Yusho patients was 5.9 ppb with a standard deviation of 4.5 ppb in 1973 and 1974. Chen et al. (1980) maintained that this difference is due to a lengthy time lapse from the exposure to PCB in Yusho patients before measurements were taken compared with a much shorter time lapse in Yu-Cheng patients. Furthermore, the patients of Yu-Cheng consumed a larger proportion of higher-chlorinated PCBs compared with those of Yusho and, as a result, the substance will be retained longer in the body, according to the authors. Ten years have now passed. Researchers should take a close look at this cohort now that the latent period has almost been achieved for liver cancer in order to confirm or deny that an excess liver cancer risk is present in these patients.

7.9. CONCLUSIONS

Of all the cancers examined in both the case-control and follow-up studies, STS provides the greater evidence of an association with TCDD relative to other sites. The original reports by Hardell, Sandstrom, and Eriksson of an association between STS and exposures involving TCDD-contaminated phenoxy herbicides have stood up to extensive criticism and a great deal of subsequent research. The degree of risk, as estimated in later studies by Hardell's research group, does not appear to be as great as originally suggested, but an association with TCDD exposure is strengthened by tissue measurements of 2,3,7,8-TCDD in potentially exposed groups.

It is also possible that the herbicides, phenoxyacetic acid, and/or chlorophenols may exert a confounding effect. The results of the recent Lynge (1993) study suggest that TCDD-free phenoxyherbicides and/or chlorophenols may by themselves increase the risk of STS. Eriksson et al. (1990) further suggested that the higher-chlorinated dioxin isomers may also be carcinogenic.

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Not every study that has looked for an association between TCDD exposure and soft tissue sarcoma risk has found one, but several studies of sound design and adequate size have done so to a greater or lesser extent. The results from the important cohort study by Fingerhut et al. (1991) of 5,000 chemical production and processing workers exposed to TCDD are corroborative, as are those from the second 5 years of follow-up of the persons exposed to TCDD in Seveso (Bertazzi et al., 1989a, b). The large IARC Registry cohort study also suggested an association between STS and phenoxy herbicide exposure, but the TCDD exposure component was less certain. The first New Zealand sarcoma study (Smith et al., 1983, 1984) also appeared to produce positive results when the analysis, presented above, was restricted to farmers to minimize bias. These results were produced by independent investigators using substantially different research methods and studying populations exposed under conditions much different from those in the studies by Hardell and colleagues, with TCDD exposure being the common link.

Moreover, no persuasive case has been made that the entirety of the association in these studies is real and not due to selection bias, differential exposure misclassification, confounding, or chance.

The evidence on malignant lymphomas in connection with TCDD exposure has not been substantiated, but recent evidence suggests an association between NHL and exposure to the herbicide 2,4-D (Zahm and Blair, 1992), which may contain dioxins other than 2,3,7,8-TCDD. The evidence from two large industrial cohort studies (Fingerhut et al., 1991; Saracci et al., 1991), and from the Seveso population suggest little if any evidence of increased risk. The limited evidence on TCDD exposure that can be extracted from the extensive case-control studies on NHL by the National Cancer Institute (Hoar et al., 1986; Zahm et al., 1990; Cantor et al., 1992) also does not indicate a consistent and pronounced increase in risk. It would be interesting to see results restricted to farmers from the New Zealand study of NHL. At the present time, however, the existing studies do not present even a minimally consistent picture of increased risks of malignant lymphoma among persons most probably exposed to TCDD.

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The evidence for lung cancer and TCDD exposure comes from the three recent cohort follow-up studies (Fingerhut et al., 1991; Manz et al., 1991; Zober et al., 1990), all of which provided good TCDD exposure surrogates and some actual TCDD serum level samples. All three studies showed increased risks of borderline statistical significance of about 40% to 100% in their highly exposed groups and low risks in their less exposed groups. In addition to the above studies, the report of a significantly increased lung cancer risk in male victims of the Japanese rice oil poisoning accident (Kuratsune, 1988) is also suggestive of a TCDD-like effect (see discussion of animal carcinogenicity in Chapter 6). While confounding or synergism by tobacco smoke cannot be excluded, the limited analyses conducted suggest that smoking cannot explain the entire increase and that the association is real.

It must be remembered that the confounding influence of other occupational chemicals and presence of asbestos in the workplace could have added to the observed lung cancer cases in these studies and helped to increase the lung cancer risk calculated for these studies.

The evidence for an association with stomach cancer is less than that for lung cancer. While the high exposure/long duration cohorts in both the Fingerhut and Manz studies suggest increased risks of at least 40%, the estimates are based on too few deaths for any conclusions to be made. There are no case-control studies relating to TCDD exposure and either lung or stomach cancer because the cancers are too common with too many potential causes. Further research is needed.

While males comprise all the case-control studies and the bulk of the cohort study analyses, animal and mechanism studies suggest that males and females might respond differently to TCDD, which reduces estrogen levels in reproductive tissues and reduces estrogen-receptor binding in rat and mouse liver. These antiestrogenic effects are thought to be responsible for decreased tumor incidences seen in the mammary gland, uterus, and pituitary of TCDD-treated female rats and may also be responsible for increased liver cancer seen in female but not male rats (see Sections 6.4.1 and 6.5.4) although in the Japanese rice oil poisonings, the reverse was the case. These female rat liver tumors may be ovary-

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dependent, while at the same time the ovaries appear to protect against TCDD-mediated tumor promotion in the rat lung (see Section 6.4.2). Thus, these complex mechanisms might very well affect human carcinogenicity in males and females differently. The only reported female cohort with good TCDD exposure surrogate information was that of Manz et al. (1991), which had a borderline statistically significant increase in breast cancer. While Saracci et al. (1991) did report reduced female breast and genital organ cancer mortality, this was based on few observed deaths and chlorophenoxy herbicide, rather than TCDD exposures. Bertazzi et al. (1993) reported a deficit of breast cancer and endometrial cancer in women living in geographical areas around Seveso contaminated by dioxin. Although Kogevinas et al. (1993) saw an increase in cancer incidence among female workers most likely exposed to TCDD, no increase in breast cancer was observed in her small cohort. In sum, TCDD cancer experience for women may differ from that of men but currently there are few results.

Other TCDD-related hormonal effects, including immune suppression, may result in multiorgan sensitivity and may contribute to the overall increased mortality from all malignancies combined seen in all four cohort production worker subcohorts with higher estimated TCDD exposures (Fingerhut et al., 1991; Manz et al., 1991; Zober et al., 1990; Saracci et al., 1991; Kogevinas et al., 1993). These increased relative risks, while not large (10% to 70%) are consistent and are either statistically significant or of borderline significance. While no one tissue site can account for this observed increase, lung cancer is also increased in four of these. Although these data suggest a hypothesis of "general carcinogenicity" consistent with a tumor promotor effect or immune suppression, it must be kept in mind that no single agent has ever been determined to cause an overall cancer response by the epidemiologic method. Although many carcinogens have been shown to increase the risk at several sites, the magnitude of the risk has differed with respect to each site. One would not expect to see the same magnitude of increase in risk at every site affected from exposure to a carcinogen.

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In conclusion, although there are uncertainties associated with the epidemiologic evidence that could have influenced risk estimates, the overall weight of evidence from the epidemiologic studies suggests that the generally increased risk of cancer is more than likely due to exposure to TCDD. The consistency of this finding in the four major cohort studies is corroborated by animal studies that show TCDD to be a multisite, multisex, and multispecies carcinogen.

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CHAPTER 7. EPIDEMIOLOGY/HUMAN DATA
PART B: EFFECTS OTHER THAN CANCER

CONTENTS

7.10. INTRODUCTION	7-94
7.11. CROSS-SECTIONAL STUDIES: USES AND LIMITATIONS	7-95
7.12. DESCRIPTION OF PRINCIPAL STUDIES	7-97
7.12.1. Occupational Studies	7-98
7.12.1.1. U.S. Chemical Workers: West Virginia	7-98
7.12.1.2. U.S. Chemical Workers: The NIOSH Study	7-99
7.12.1.3. BASF Accident Cohort	7-100
7.12.2. Studies of Community Residents	7-101
7.12.2.1. The Missouri Experience	7-101
7.12.2.2. Seveso, Italy	7-103
7.12.3. Studies of Vietnam Veterans	7-104
7.12.3.1. The Vietnam Experience Study	7-104
7.12.3.2. U.S. Air Force Ranch Hand Study	7-105
7.13. REVIEW OF EFFECTS ASSOCIATED WITH EXPOSURE TO 2,3,7,8-TCDD	7-107
7.13.1. Dermal Effects	7-107
7.13.1.1. Chloracne	7-107
7.13.1.2. Dermatologic Disorders Other Than Chloracne	7-111
7.13.1.3. Comments	7-113
7.13.2. Gastrointestinal Effects	7-114
7.13.2.1. Hepatic Effects	7-114
7.13.2.2. Liver Size	7-114
7.13.2.3. Enzyme Levels	7-115
7.13.2.4. GGT	7-116
7.13.2.5. AST and ALT	7-119
7.13.2.6. D-Glucaric Acid	7-123
7.13.2.7. Comment	7-125
7.13.2.8. Porphyrin Metabolism	7-126
7.13.2.9. Comment	7-128
7.13.2.10. Lipid Levels	7-129
7.13.2.11. Total Cholesterol	7-129
7.13.2.12. Triglycerides	7-135
7.13.2.13. Comment	7-136
7.13.3. Other Gastrointestinal Disorders	7-137

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CONTENTS (continued)

7.13.4.	Thyroid Function	7-137
7.13.4.1.	Comment	7-141
7.13.5.	Diabetes	7-144
7.13.5.1.	Comment	7-146
7.13.6.	Immunologic Effects	7-150
7.13.6.1.	Comment	7-165
7.13.7.	Neurologic Effects	7-166
7.13.7.1.	Neurobehavioral Assessments	7-174
7.13.7.2.	Neurologic Status	7-182
7.13.7.3.	Comment	7-185
7.13.8.	Circulatory System	7-185
7.13.8.1.	Comment	7-192
7.13.9.	Pulmonary Effects	7-194
7.13.9.1.	Comment	7-195
7.13.10.	Renal Effects	7-195
7.13.11.	Reproductive Effects	7-196
7.13.12.	Review of the Literature Prior to 1984	7-198
7.13.12.1.	Occupational Studies	7-198
7.13.12.2.	Environmental Studies	7-209
7.13.12.3.	The Seveso, Italy, Dioxin Accident of 1976	7-209
7.13.12.4.	Studies of Exposure to Agent Orange by Military Veterans and Vietnamese Civilians	7-211
7.13.13.	Review of the Literature From 1984 to 1992	7-214
7.13.14.	Environmental Studies	7-215
7.13.14.1.	The Times Beach, Missouri, 2,3,7,8-TCDD Episode	7-215
7.13.14.2.	Studies of Vietnam Experience in Ground Troops and Ranch Hands Published 1984-1992	7-218
7.13.15.	Study Results	7-221
7.13.15.1.	Atlanta Congenital Defects Program Study	7-221
7.13.15.2.	CDC Vietnam Experience Study	7-222
7.13.15.3.	American Legion Study	7-228
7.13.15.4.	Boston Hospital Study	7-228
7.13.15.5.	The Ranch Hand Study	7-229
7.13.15.6.	Reproductive Hormones	7-236
7.13.15.7.	Comment	7-237
7.14.	NONCANCER EFFECTS OF INGESTION OF RICE OIL CONTAMINATED WITH POLYCHLORINATED DIBENZOFURANS, QUATERPHENYLS, AND BIPHENYLS IN JAPAN (YUSHO) AND TAIWAN (YU-CHENG)	7-237

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CONTENTS (continued)

7.14.1.	Acute Effects in Adults and Children Directly Exposed to Contaminated Rice Oil	7-238
7.14.1.1.	Yusho	7-238
7.14.1.2.	Yu-Cheng	7-240
7.14.1.3.	Effects Observed in Offspring of Yu-Cheng Cases . . .	7-242
7.14.1.4.	Comment	7-244
7.15.	SUMMARY	7-245
7.15.1.	Effects Having a Positive Relationship With Exposure to 2,3,7,8-TCDD	7-250
7.15.1.1.	Chloracne	7-250
7.15.1.2.	Gamma Glutamyl Transferase (GGT) Levels	7-252
7.15.1.3.	Diabetes and Fasting Serum Glucose Levels	7-254
7.15.1.4.	Reproductive Hormones	7-256
7.15.2.	Possible Acute Effects of Exposure to 2,3,7,8-TCDD	7-257
7.15.2.1.	Dermatologic Conditions Other Than Chloracne	7-257
7.15.2.2.	Liver Enzymes Other Than GGT and Hepatomegaly . . .	7-258
7.15.2.3.	Pulmonary Disorders	7-259
7.15.2.4.	Neurologic Disorders	7-260
7.15.2.5.	Porphyrias	7-260
7.15.3.	Effects For Which Further Research Is Needed	7-261
7.15.3.1.	Diseases of the Circulatory System	7-261
7.15.3.2.	Reproductive Effects	7-263
7.15.3.3.	Specific Reproductive End Points	7-265
7.15.3.4.	Immunologic Effects	7-268
7.15.3.5.	Lipids	7-268
7.15.3.6.	Thyroid Function	7-269
	REFERENCES FOR CHAPTER 7, PART B	7-270

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PART B: EFFECTS OTHER THAN CANCER

7.10. INTRODUCTION

Human exposure to 2,3,7,8-TCDD has been associated with noncancer effects in most systems. The majority of effects have been reported among occupationally exposed groups, such as chemical production workers, pesticide users, and individuals who handled or were exposed to materials treated with 2,3,7,8-TCDD-contaminated pesticides, and among residents of communities contaminated with tainted waste oil (Missouri, USA) and industrial effluent (Seveso, Italy).

These effects represent a complex network of responses ranging from changes in hepatic enzyme levels, which, based on current evidence, do not appear to be related to clinical disease, to observable alterations in the character and physiology of the sebaceous gland, as in chloracne (Calvert et al., 1992; Taylor, 1979). This section of Chapter 7 describes by system the noncancer effects associated with exposure to 2,3,7,8-TCDD. The characterization of the effects by system provides a context within which to compare the results of the various studies. However, it is important to recognize that the observed effects are not independent events but rather may be one outcome in a series of interrelated outcomes, some of which we may be incapable of measuring with the present technology or which we currently do not recognize as an outcome of exposure to 2,3,7,8-TCDD. A summary of the effects observed in humans is outlined in Tables 7-17 to 7-43.

The information describing human effects attributed to exposure to 2,3,7,8-TCDD-contaminated materials is derived from a wide variety of sources, including clinical assessments (case reports) of exposed individuals and analytic epidemiologic studies using case-control, cross-sectional, and cohort designs. The case reports describe the acute outcomes of exposure to 2,3,7,8-TCDD and provide the basis for hypothesis generation for controlled epidemiologic studies; however, they are not suitable for testing causal relationships between exposure and related effects (Ashe and Suskind, 1950; Suskind et al., 1953; Bauer et al., 1961; Goldman, 1972).

As described in the previous section, cohort and case-control studies have been used to investigate hypothesized increases in malignancies among the various 2,3,7,8-TCDD-

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exposed populations (Fingerhut et al., 1991a, b; Manz et al., 1991; Eriksson et al., 1990). Cross-sectional studies have been conducted to evaluate the prevalence or extent of disease in living 2,3,7,8-TCDD-exposed groups (Suskind and Hertzberg, 1984; Moses et al., 1984; Lathrop et al., 1984, 1987; Roegner et al., 1991; Sweeney et al., 1989; Centers for Disease Control Vietnam Experience Study, 1988a; Webb et al., 1989). Many of the earliest studies were unable to define exposure-outcome relationships due to a variety of shortcomings, including small sample size, poor participation, short latency periods, selection of inappropriate controls, and the inability to quantify exposure to 2,3,7,8-TCDD or to identify confounding exposures. In more recent cross-sectional studies of U.S. chemical workers (Sweeney et al., 1989), U.S. Air Force Ranch Hand personnel (Roegner et al., 1991), and Missouri residents (Webb et al., 1989), serum or adipose tissue levels of 2,3,7,8-TCDD were measured to evaluate 2,3,7,8-TCDD-associated effects in exposed populations. The ability to measure tissue or serum levels of 2,3,7,8-TCDD for all or a large sample of the subjects confirmed exposure to 2,3,7,8-TCDD and permitted the investigators to test hypothesized dose-response relationships.

7.11. CROSS-SECTIONAL STUDIES: USES AND LIMITATIONS

Most of the studies that describe nonmalignant effects were designed as cross-sectional medical studies. These types of studies are useful for assessing the current status of the surviving study population; however, they are inherently limited by a number of factors, including survivor and participation biases, exposure and disease misclassification, recall bias, and interobserver variability. Survivor and participation biases may have occurred because the studies included only those who were living at the time of the study and did not or could not obtain similar information on those who died or were too ill to participate. Studies of groups exposed to agents that contribute to early deaths or cause severe illnesses may exclude the populations who were at highest risk. Exclusion of the sick and deceased whose condition was associated with 2,3,7,8-TCDD may erroneously cause the risk estimate to be closer to the null than the true risk.

Disease misclassification may be introduced in a variety of ways. Medical tests in many reviewed studies were most often performed once without follow-up. For some

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disorders, multiple testing is preferred to obviate normal variations in some test parameters, e.g., immunologic tests, hormone levels. In other situations, self-reported medical histories were collected and, by design, were not or could not be confirmed by medical records. When the exposed group incorrectly reports more disease than the unexposed group, recall and reporting biases may falsely raise the risk estimate.

Exposure misclassification, particularly in the early studies, was a major limitation. In the earlier studies of production workers or community residents, exposure to 2,3,7,8-TCDD was determined only by an individual's presence (residing or working) in an area that was contaminated with 2,3,7,8-TCDD (Suskind and Hertzberg, 1984; Moses et al., 1984; Hoffman et al., 1986; Poland et al., 1971; May, 1973, 1982; Martin, 1984; Bond et al., 1983, 1989; Filippini et al., 1981; Ideo et al., 1985; Mocarelli et al., 1986). The lack of a measurement to quantify exposure hindered the ability to confirm exposure and to assess the magnitude of an exposure-response relationship. If the misclassification is nondifferential, it tends to bias the measure of effect toward the null.

As described above, in later studies (Roegner et al., 1991; Sweeney et al., 1989; Centers for Disease Control Veterans Health Studies, 1988; Webb et al., 1989) researchers were able to confirm and quantify exposure to 2,3,7,8-TCDD in serum or adipose tissue. This breakthrough helped establish that certain previously exposed populations had 2,3,7,8-TCDD levels well above the background level of less than 20 picograms per gram of lipid (pg/g) (Patterson et al., 1989) and that the nonexposed comparison group was truly not exposed. Yet, because the occupational populations were last exposed to 2,3,7,8-TCDD-contaminated substances for as many as 40 years before being tested, levels attained at the time of exposure can only be estimated. It appears that for some populations with higher exposures, such as workers, the estimate reflects continuous exposure over an extended period. For example, despite the intervening period between last exposure to 2,3,7,8-TCDD and the determination of serum 2,3,7,8-TCDD levels in workers employed in the production of 2,4,5-TCP and 2,4,5-T (15 to 37 years after last occupational exposure), the duration of occupational exposure was highly correlated to serum levels of 2,3,7,8-TCDD obtained at the time of the study (1987-1988) (Pearson product moment correlation coefficient $[r]=0.7$) (Sweeney et al., 1989). These data suggest a strong relationship between length of

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occupational exposure and serum 2,3,7,8-TCDD regardless of the length of the intervening period.

The deposition, metabolism, and excretion of high doses of 2,3,7,8-TCDD in the human system have not been fully described. A study by Pirkle et al. (1989) suggests that 2,3,7,8-TCDD decays by one-half in approximately 7.1 years, based on a one-compartment model and using a standard half-life equation. If this is true, exposures to trichlorophenol production workers may have been as high as 30,000 pg/g (Fingerhut et al., 1991a) and in excess of 50,000 pg/g in some residents of Seveso (Mocarelli et al., 1991). The data may be limited by the lack of complete information on the manner in which human metabolism handles 2,3,7,8-TCDD exposure.

This section of Chapter 7 is a selective review of studies that, to date, provide the most information on the relationship between nonmalignant outcomes and exposure to 2,3,7,8-TCDD-contaminated materials. Animal studies have been reviewed in other chapters of this document and will not be discussed in detail. Case reports will not be reviewed but will be used to provide support for the analytic studies. In the assessment of mortality from nonmalignant causes of death, only cohort studies in which standardized mortality ratios (SMR) or equivalent population-based risk ratios were calculated will be discussed.

Many of the reviewed studies describe a variety of outcomes that will be discussed by system throughout the text. Presented but not yet published works described by investigators from the U.S. Air Force and the National Institute for Occupational Safety and Health (NIOSH) will be included in the review. Before the results of any analysis are presented, each institution requires extensive external peer review of the findings. Other criteria for including studies in this review of noncancer end points are described in the beginning of Chapter 7.

7.12. DESCRIPTION OF PRINCIPAL STUDIES

In the following section we have provided a summary of the population description and methods of the studies that reported results for two or more systems or effects. Studies that are referenced only once will be described when cited. Results of these studies will be described in subsequent sections.

7.12.1. Occupational Studies

7.12.1.1. *U.S. Chemical Workers: West Virginia*

In March 1949, an explosion of a TCP reaction kettle in a chemical plant in Nitro, West Virginia, and the subsequent cleanup exposed approximately 450 workers to 2,3,7,8-TCDD-contaminated substances. Examination of the workers in 1949 revealed a number of acute symptoms "characterized by skin, eye and respiratory tract irritation, headache, dizziness and nausea" (Suskind and Hertzberg, 1984). The acute symptoms subsided but were followed within a week or two by "acneform eruption, severe muscle pain affecting the extremities, thorax and shoulders, fatigue, nervousness and irritability, dyspnea, complaint of decreased libido and intolerance to cold" (Suskind and Hertzberg, 1984). Thirty years later, two independent, cross-sectional medical studies were conducted to evaluate the long-term consequences of exposure to 2,3,7,8-TCDD-contaminated substances among the surviving workers (Suskind and Hertzberg, 1984; Moses et al., 1984).

In a study by Suskind and Hertzberg (1984), a group of 204 (of a total of 419) active and retired white male workers exposed between 1948 and 1969 to the 2,4,5-T production process and to the reactor release were included in a clinical examination program. The control group consisted of 163 (46% participation) current or former employees of the same plant but who had no self-reported exposure to 2,4,5-T production or maintenance of the facility. The study collected demographic and medical histories and performed clinical chemistries, urinalysis, pulmonary function tests, dermatologic examination, and conduction velocities of the sural sensory and peroneal motor. Multiple linear regression analysis was used to compare the exposed and nonexposed groups.

For participation in a separate study of workers at the Nitro plant, Moses et al. (1984) invited all workers documented in union records to have worked in 2,4,5-T production and a systematic random sample of workers with no known 2,4,5-T production exposure. Fifty-five percent (N=226) of the persons invited participated in the study. Lifetime occupational and medical histories were collected and clinical chemistries, urinalysis, and dermatologic examinations were conducted. Exposure to 2,3,7,8-TCDD could not be discerned due to irreconcilable inconsistencies in self-reported work histories and the lack of good company records to estimate and confirm the likelihood of exposure. Although the authors recognized

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that absence of chloracne did not preclude exposure, nevertheless, they compared the results of the group with chloracne with those without chloracne. Thus, the study design was revised to explore the differences in health status in individuals with and without chloracne. Because exposed workers may have been included in the group diagnosed without chloracne, the usefulness of the data to quantify exposure-disease relationships is limited.

Two studies of this cohort also examined cancer and noncancer mortality of subsets of workers from this plant (Zack and Suskind, 1980; Collins et al., 1993).

7.12.1.2. *U.S. Chemical Workers: The NIOSH Study*

The study conducted by the National Institute for Occupational Safety and Health is a cross-sectional medical study of living workers who were previously employed for at least one day in one of two plants located in Newark, New Jersey, and Verona, Missouri. From 1951 to 1969, 490 workers employed at the New Jersey plant produced sodium 2,4,5-trichlorophenolate (NaTCP), 2,4,5-trichlorophenoxy acetic acid (2,4,5-T), and 2,4-dichlorophenoxy acetic acid (2,4-D). A high proportion of chloracne and other dermatologic abnormalities and cases of porphyria and hypomania were reported among the workers at the New Jersey facility (Poland et al., 1971; Bleiberg et al., 1964), which produced some of the most heavily 2,3,7,8-TCDD-contaminated NaTCP and 2,4,5-T among production facilities whose products were surveyed (Fee et al., 1975). At the Missouri plant, NaTCP and 2,4,5-T were produced intermittently for 4 months in 1968, and NaTCP and hexachlorophene were produced continuously for 22 months between April 1970 and January 1972. Prior to the NIOSH cross-sectional study, the health of the 96 Missouri production workers had not been previously studied.

For comparison, unexposed neighborhood referents were recruited using a random sampling procedure described by Sweeney et al. (1989). Referents were selected if they reported no prior history of occupational exposure to 2,3,7,8-TCDD and matched the worker by age (within 5 years), race, and gender. A total of 586 workers were eligible for inclusion in the study, of which 400 (68.3%) were living, 142 (24.2%) were deceased, and 44 (7.5%) could not be located. All 400 living workers were invited to participate in the study; 281 (70%) were examined. A description of the study population is included in the results.

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Worker and referent health and exposure status was assessed in 1987-1988 through an interviewer-administered medical and occupational history and comprehensive physical and psychological examinations (Sweeney et al., 1989). A lifetime medical history was obtained from each examined participant by interviewers who were blind to the exposure status of the respondent. Results of the pulmonary, hepatic, gastrointestinal, porphyria, mood dysfunction, and neurologic examinations have been published or accepted for publication (Calvert et al., 1991, 1992, 1993; Sweeney et al., 1993). Findings for diabetes and serum glucose level, reproductive endocrine function, and p450 metabolism have been presented in public meetings (Sweeney et al., 1992; Egeland et al., 1994; Halperin et al., 1992).

As a surrogate for cumulative exposure, serum 2,3,7,8-TCDD levels were measured in 237 workers and a random sample of 79 referents. Procedures for sample collection, preparation, adjustment for lipids, and statistical analysis were described in earlier reports (Fingerhut et al., 1989; Patterson et al., 1986a; Sweeney et al., 1990). The mean lipid-adjusted serum 2,3,7,8-TCDD level for workers was 220 pg/g, median 80 pg/g, ranging to 3,400 pg/g. The mean level was statistically significantly greater than that for referents (7 pg/g) ($p < 0.001$). Analyses of other congeners of dioxins and dibenzofurans were also conducted; only the 2,3,7,8-TCDD levels were different in the two exposure groups (Piacitelli et al., 1992).

7.12.1.3. *BASF Accident Cohort*

"On 17 November 1953, an uncontrolled decomposition reaction occurred during the production of 2,4,5-trichlorophenol at a BASF AG facility in Ludwigshafen, Germany" (Ott et al., 1993a). The reactor contents, which contained 2,3,7,8-TCDD, contaminated the building in which the TCP autoclave was housed. A series of studies have documented the effects and mortality experience of the workers present at the time of the decomposition reaction and exposed during the initial cleanup and equipment maintenance (May 1954 medical department list) (Cohort C1, N=69), individuals present during subsequent clean-demolition activities between 1954 and 1969 (Cohort C2, N=84), and a mixed group of workers identified as of December 1987 through interviews that include individuals who worked in the laboratory as safety inspectors and others who participated in the 1968-1969

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demolition activities (Cohort C3, N=101) (Zober et al., 1990; Ott et al., 1993b). Two hundred forty-seven study subjects were included in a mortality study that found a significantly elevated standardized mortality ratio (SMR) for all malignant neoplasms among workers with chloracne and 20 or more years since first exposure to 2,3,7,8-TCDD-contaminated chemicals (Zober et al., 1990).

Among 79% of the living subjects, lipid-adjusted serum 2,3,7,8-TCDD levels were measured in 138 (54%) of 254 study subjects during the period 1988-1992 (Ott et al., 1993a). The geometric mean of the 2,3,7,8-TCDD levels in the entire group was 15.4 ppt (ranging from <1 to 553.0 ppt) (Ott et al., 1993a) or 43 pg/g of lipid (M.G. Ott, personal communication, 1993). Geometric means for the cohorts are as follows: C1=1,009.5 pg/g lipid; C2=48.8 pg/g of lipid; and C3=83.7 pg/g of lipid. Background levels were determined in separate analyses of 102 unexposed individuals from Germany (Päpke et al., 1992). The geometric mean for 2,3,7,8-TCDD of the external referent group was 3.0, ranging from 0.6 to 9.1 pg/g of lipid. Based on regression analyses, serum 2,3,7,8-TCDD levels were highly correlated to duration of exposure and location of exposure. Chloracne severity was positively and significantly related to 2,3,7,8-TCDD concentrations.

Comprehensive batteries of clinical chemistry measurements were also measured for the 138 subjects between 1988 and 1993 (Ott et al., 1993b). Referents were selected from among BASF employees between the ages of 50 and 69 who participated in routine occupational medical examinations from 1989 to 1991. For some tests, there were as many as 6,000 referent values. For the immunologic parameters, the referent values were obtained from a group of 42 unexposed BASF employees who participated in a separate study that examined the immunologic function of 21 extruder personnel exposed to 2,3,7,8-tetrabrominated dibenzo-dioxins (2,3,7,8-TBDD) and -furans (2,3,7,8-TBDF) (Zober et al., 1992).

7.12.2. Studies of Community Residents

7.12.2.1. *The Missouri Experience*

During 1971, 2,3,7,8-TCDD-contaminated stillbottoms were removed from a hexachlorophene production facility and mixed with waste oil. This mixture was deposited

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on 45 residential, recreational, and industrial sites in southeastern Missouri in 1971 and 1972 (Daryl Roberts, personal communication). Waste oil mists were commonly used for summer dust control on roadways, horse arenas, truck depots, and other unpaved surfaces. Estimated contamination of the areas ranges from 1 to 2,200 ppb (Hoffman and Stehr-Green, 1989). A listing of potentially exposed persons was created (volunteers), and a survey was conducted to obtain baseline information to identify persons at high risk of exposure. Beginning in 1984, a series of studies were conducted to evaluate potential effects (Hoffman et al., 1986; Evans et al., 1988; Webb et al., 1989), including reproductive events, of residential exposure to 2,3,7,8-TCDD (Stockbauer et al., 1988).

The study by Hoffman et al. (1986) was conducted on 154 individuals (74% of total eligible) who were residents of the Quail Run Mobile Home Park between 1971 and 1983 because soil concentrations around the site were 2,200 ppb 2,3,7,8-TCDD. The comparison group of 155 (77% of total eligible) individuals was recruited from residents of a nearby mobile home park. The examination included tests for delayed hypersensitivity (the multitest Cornell Medical Index [CMI]; Merieux Institute, Miami, FL) and neurobehavioral effects, blood chemistries, urinalysis, height, weight, vital signs, and examination of the skin, peripheral pulses, lymph nodes, abdomen, and peripheral nervous system. The results of this study were plagued by the exclusion of skin test results of 150 of 294 participants due to high reader error. Furthermore, information on subject exposure to 2,3,7,8-TCDD was limited because it was based on a minimum residence of 6 months in areas with contaminated soil. Actual contact with contaminated soil was not assessed.

In the follow-up study by Evans et al. (1988), 50 persons from the initial study who did not respond to the delayed hypersensitivity skin tests were retested. These subjects were thought to have impaired immune function. The multitest CMI was reapplied to all test subjects.

Webb and colleagues (1989) examined 41 of 51 persons with various histories of exposure to 2,3,7,8-TCDD (residential, recreational, and occupational exposure) and for whom adipose tissue levels of 2,3,7,8-TCDD were measured. Of the 41 participants, 16 had adipose 2,3,7,8-TCDD levels less than 20 pg/g (within background range), 13 had levels between ≥ 20 and 60 pg/g, and 12 subjects had levels above 60 pg/g. Standard medical

examinations were conducted, and complete blood count with differential, a panel of automated chemistry tests, serum immunoglobulins, tests for porphyrins, and the multitest CMI were performed.

7.12.2.2. *Seveso, Italy*

In 1976, an explosion of a trichlorophenol reactor in a 2,4,5-T production facility in Medina, Italy, caused the contamination by 2,3,7,8-TCDD of the neighboring city of Seveso, Italy. The contaminated area was subdivided into three zones (A, B, and R) of decreasing mean soil levels of 2,3,7,8-TCDD (Mocarelli et al., 1988). The mean 2,3,7,8-TCDD concentration in Zone A was 230 $\mu\text{g}/\text{m}^2$; in Zone B, 3.0 $\mu\text{g}/\text{m}^2$; and in Zone R, 0.9 $\mu\text{g}/\text{m}^2$.

In 1979, Pocchiari et al. (1979) reported on initial efforts to screen residents in Zones A, B, and R for 2,3,7,8-TCDD-related effects. Since then, a series of cross-sectional medical studies have reported the final results of the screening (Caramaschi et al., 1981; Ideo et al., 1985; Mocarelli et al., 1986; Assennato et al., 1989). Within 1 year of the reactor release, 193 cases of chloracne were identified among residents of Zones A, B, and R, most of which resolved with time (Assennato et al., 1989). For Seveso residents, a standard diagnosis of chloracne was developed, in which all cases were stratified by severity: 0, no lesions; 1, a few comedones (up to 10, minimum stage); 2, numerous comedones and cysts (light stage); 3, comedones and cysts in specific regions (medium stage); and 4, comedones and cysts spreading from the face to other regions of the body (serious stage) (Caramaschi et al., 1981). Four studies investigated possible biochemical changes, particularly liver enzyme induction and lipid levels, among the 170 children diagnosed with chloracne and control groups (Caramaschi et al., 1981; Ideo et al., 1985; Mocarelli et al., 1986; Assennato et al., 1989).

In addition to chloracne, several other studies also evaluated peripheral neuropathy. In an early report by Pocchiari et al. (1979), tests for presence of peripheral nerve dysfunction were conducted for Seveso residents and for workers at the Icmesa production facility. Assennato et al. (1989) and Filippini et al. (1981) assessed the prevalence of peripheral neuropathy, comparing residents with and without chloracne (Assennato et al.,

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1989) or comparing residents having chloracne or abnormal serum hepatic enzyme levels with residents with no manifestations of 2,3,7,8-TCDD exposure (Filippini et al., 1981).

Two mortality studies, one of children ages 1-19 years and another of adults 20 years and older, examined death rates in residents of Zones A, B, and R 10 years after the explosion (1976-1986) (Bertazzi et al., 1989, 1992). The comparison population was composed of approximately 100,000 inhabitants of uncontaminated areas surrounding Seveso. Follow-up for both the young and older cohorts was 99%.

To date, the general limitation of the studies conducted in Seveso residents is the classification of exposure of subjects by residence in Zones A, B, or R, which is based on mean soil concentration of 2,3,7,8-TCDD. Although serum levels of some individuals exposed in Zone A at the time of the reactor release were in excess of 10,000 pg/g, levels of residents living in the other areas were not measured (Mocarelli et al., 1991). The weaknesses of using soil 2,3,7,8-TCDD levels to classify extent of exposure to 2,3,7,8-TCDD were aptly described by Bertazzi et al. (1989): "This (use of soil contamination) is a rather poor surrogate of exposure, and by no means an indicator of intake, since it does not take into consideration all the possible sources and ignores interindividual variability." This is the same problem encountered by some researchers investigating 2,3,7,8-TCDD-related effects among Missouri residents. One might also expand this limitation to each of the studies where environmental rather than personal levels of 2,3,7,8-TCDD contamination were used for exposure classification.

7.12.3. Studies of Vietnam Veterans

7.12.3.1. *The Vietnam Experience Study*

The Vietnam Experience Study is described by its authors as a "multidimensional assessment of the health of Vietnam veterans" (Centers for Disease Control Vietnam Experience Study, 1988a, b, c, d). This study was designed to examine effects among men who served in Vietnam.

The study population was composed of a random sample of men who enlisted in the U.S. Army from 1965 through 1971, whose military occupational status was other than "duty soldier," who enlisted for a single term with a minimum of 16 weeks active duty and who

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were discharged at pay grades of E-1 to E-5. The controls were selected from among veterans enlisting during the same period but whose duty station was the United States, Germany, or Korea. Participation involved completion of a telephone survey of current and past health status by 7,924 veterans who served in Vietnam and 7,364 veterans who served outside of Vietnam. A random subsample of 2,940 Vietnam and 1,972 non-Vietnam veterans participated in the health evaluation component.

In a separate study, serum 2,3,7,8-TCDD levels were measured in a subset of the examined population: 646 Vietnam veterans who served in Vietnam during 1967 and 1968 and 97 non-Vietnam veterans (Centers for Disease Control Veterans Health Studies, 1988). The mean serum 2,3,7,8-TCDD level was not different between Vietnam (mean = 4.1 pg/g lipid [standard deviation (SD) $\pm$ 2.3]) and non-Vietnam (4.2 pg/g, SD $\pm$ 2.6) veterans. Two Vietnam veterans had levels above the background level of 20 pg/g: 25 pg/g and 45 pg/g.

The overall strengths of this study are that it is a large study, with good power to detect many common disorders; participation in the questionnaire part of the study was good (87% for Vietnam veterans; 84% for non-Vietnam veterans); there was good comparability between the two cohorts in demographic characteristics, although there were differential participation rates in the examination; and validation of selected self-reported effects was conducted. This study is limited primarily by the differential participation rates in the examination (75% for Vietnam veterans; 63% for non-Vietnam veterans). The low level of 2,3,7,8-TCDD in the sample of veterans made it impossible to conduct dose-response analyses.

7.12.3.2. U.S. Air Force Ranch Hand Study

One of the largest epidemiologic studies of U.S. military personnel stationed in Vietnam is being conducted by the U.S. Air Force. The study population consists of Air Force personnel who served in Operation Ranch Hand units in Vietnam from 1962 to 1971 and who were employed in the dissemination of Agent Orange through aerial spraying. Comparisons included Air Force personnel who flew or maintained C-130 aircraft in Southeast Asia during the same time period.

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The study design includes a series of cross-sectional medical studies conducted at 5-year intervals beginning with the baseline study in 1982 (N=1,045 exposed, 1,224 unexposed). Two follow-up evaluations were conducted in 1985 (N=1,016 exposed, 1,293 unexposed) and 1987 (N=995 exposed, 1,299 unexposed). Each cross-sectional study included comprehensive physical and psychological evaluations. In the 1982 baseline and 1985 and 1987 follow-up studies, exposure was based on the comparison of the Ranch Hand group versus the comparison group. An additional analysis approximated exposure (low, medium, high) for the Ranch Hand group by using historical military data and herbicide procurement and usage records. The results of these analyses were prepared by Lathrop and colleagues (1984 and 1987). In 1988, serum 2,3,7,8-TCDD levels were measured for a sample of the 1987 Ranch Hand group (N=866) and the 1987 comparison group (N=804). The 1987 examination data were then reanalyzed using lipid-adjusted serum 2,3,7,8-TCDD levels as the relative measure of exposure. The median serum 2,3,7,8-TCDD level adjusted for lipids for the Ranch Hand group was 12.8 pg/g, ranging to 618 pg/g. For the comparison group, the median level was 4.2, ranging to 54.8 pg/g (Roegner et al., 1991).

The overall strengths of this study are that it is a large study, with good power to detect many common disorders; follow-up was very good as is continued participation of Ranch Hand and comparison populations. The physical and psychological examinations are extensive, planned to evaluate most, if not all, outcomes hypothetically associated with 2,3,7,8-TCDD. Continued reevaluation of the subjects (every 5 years) permits investigators to monitor the development of chronic diseases and to test for additional outcomes as new biochemical and toxicologic data become available. Finally, the determination of serum 2,3,7,8-TCDD levels permitted validation of the exposure matrix based on historical records and the subsequent development of disease-specific dose-response models. Repeated measures of serum 2,3,7,8-TCDD over time will also provide valuable information on its half-life in humans.

Noteworthy caveats in the study include the fact that the majority of the population currently has serum levels under the background level of 20 pg/g (median = 12.8 pg/g, range to 600 pg/g). These data suggest that, although there are some Ranch Hands who were exposed to very high levels of 2,3,7,8-TCDD, most of the study group had lower

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exposures. In addition, the serum 2,3,7,8-TCDD levels indicated that the exposure matrix used in the analysis of the baseline and 1984 studies did not appropriately describe the potential for exposure. Therefore, data described in this chapter will refer only to the baseline and 1984 results where Ranch Hands as a group were compared to the comparisons and, most often, to the reanalysis of the 1987 data using serum levels.

Many analyses were performed for this study. For ease of discussion in this report, only the adjusted odds ratios for the three categories of serum 2,3,7,8-TCDD selected by Roegner and colleagues (1991) are presented and discussed. The categories are: ≤ 10 pg/g 2,3,7,8-TCDD; $15\text{--}33.3$ pg/g 2,3,7,8-TCDD; and > 33.3 pg/g 2,3,7,8-TCDD.

A consequence of conducting a comprehensive study in which a large number of statistical tests are performed is an increased possibility of spurious findings. The reader should be cognizant of this limitation, looking for consistencies with the results of other studies and in the toxicologic literature rather than statistical significance.

7.13. REVIEW OF EFFECTS ASSOCIATED WITH EXPOSURE TO 2,3,7,8-TCDD

The following section contains a review of the case reports and epidemiologic studies that describe effects associated with exposure to materials contaminated with 2,3,7,8-TCDD.

7.13.1. Dermal Effects

7.13.1.1. *Chloracne*

The most widely recognized dermal effect of exposure to 2,3,7,8-TCDD-contaminated substances is chloracne. Chloracne is a persistent acneiform condition characterized by comedones, keratin cysts, and inflamed papules with hyperpigmentation and a unique anatomic distribution, occurring subsequent to acute and chronic exposure to a variety of chlorinated aromatic compounds (Crow et al., 1978; Moses and Prioleau, 1985). This acne-like condition is reported to have occurred with and without other effects in at least a few workers after all reported accidents at TCP production facilities (Ashe and Suskind, 1950; Suskind et al., 1953; Goldman, 1972; May, 1973; Zober et al., 1990), among individuals involved in daily production of 2,3,7,8-TCDD-contaminated products (Bleiberg et al., 1964; Poland et al., 1971; Pazderova-Vejlupkova et al., 1981; Moses et al.,

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1984; Moses and Prioleau, 1985; Suskind and Hertzberg, 1984; Bond et al., 1989), among three laboratory workers exposed to pure 2,3,7,8-TCDD (Oliver, 1975), and among at least 193 (0.6%) Seveso residents, mostly children (Reggiani, 1978; Caramaschi et al., 1981; Ideo et al., 1985; Mocarelli et al., 1986; Assennato et al., 1989). Chloracne was not found among Missouri residents (Hoffman et al., 1986; Webb et al., 1989) examined 10 years after exposure or among Ranch Hand personnel (Lathrop et al., 1984; Roegner et al., 1991). In U.S. Army Vietnam veterans, chloracne-like skin lesions were rarely observed on examination (0.9% in Vietnam veterans versus 0.8% in non-Vietnam veterans, $OR=1.4$, 95% $CI=0.7-2.9$) (Centers for Disease Control Vietnam Experience Study, 1988a).

Based on reports from Seveso and studies of chemical workers, chloracne appeared shortly after exposure to 2,3,7,8-TCDD-contaminated chemicals (Caramaschi et al., 1981; Zober et al., 1990). The eruption of blackheads, usually accompanied by cysts, was observed between 2 weeks and 2 months after the reactor release (Reggiani, 1980). In Seveso, within 6 months of the explosion, 34 cases of chloracne were identified among children, whereupon a more intensive search was undertaken among school children (Caramaschi et al., 1981). In chemical workers involved in the TCP reactor release at BASF Ludwigshafen, Germany, most cases of chloracne (that were also diagnosed with cancer) developed within 2 days after first exposure (Ott et al., 1993a; Zober et al., 1990). One case of chloracne did not develop for 2 years, but the authors suggest that the etiology of this case is unclear.

For many affected individuals, the condition disappeared after discontinuation of exposure (Assennato et al., 1989) despite high serum 2,3,7,8-TCDD levels (Mocarelli et al., 1991). But for a few, the chloracne remained for many years (Suskind and Hertzberg, 1984; Moses and Prioleau, 1985). Of the 204 exposed workers in the study by Suskind and Hertzberg (1984), 52% had persistent chloracne for at least 10 years after the TCP and 2,4,5-T processes ceased, 34% reported a history of chloracne, and 14% reported no history of chloracne. Moses et al. (1984) reported that the mean duration for persistent chloracne was 26.1 ± 5.9 years.

There are very few human data from which to determine definitively the threshold level of 2,3,7,8-TCDD at which chloracne occurs or who is at greatest risk to develop

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chloracne. Data from analyses of chloracne cases among chemical workers and its relationship to serum and adipose tissue levels of 2,3,7,8-TCDD and hexachlorinated (HxCDD) dioxins provide some basic yet useful information on the characteristics of chloracne cases, particularly the interindividual susceptibility to chloracne (Bond et al., 1989; Ott et al., 1987; Beck et al., 1989; Mocarelli et al., 1991). Bond et al. (1989) described 325 cases (15%) of chloracne among 2,192 workers exposed to 2,3,7,8-TCDD and HxCDDs or octachlorinated (OCDD) dioxins between 1938 and 1982 during chemical production activities (not as a result of a reactor accident). Cases were identified through company-maintained medical records; age- and calendar year-specific incidence rates were estimated based on age- and calendar year-specific person-years of employment contributed by the cohort; and risk factors were adjusted for by logistic regression. The analysis found that risk of chloracne was highest among workers who were exposed at younger ages, among those who had the longest length of exposure to 2,4,5-trichlorophenol or pentachlorophenol production operations, and among jobs rated at the highest intensity of exposure (Ott et al., 1987). These characteristics of chloracne cases in Michigan workers are consistent with those observed in chloracne cases from the BASF accident cohort (Ott et al., 1993a). Although this study may underestimate the incidence rate of chloracne due to possible underreporting or misdiagnosis of cases, and misclassification of exposure may have occurred, this study was the first of its kind to explore analytically the risk factors associated with occupationally acquired chloracne. It is unfortunate that these exposure estimates have not been validated by serum or adipose tissue 2,3,7,8-TCDD, HxCDD, and OCDD levels.

Serum levels of 2,3,7,8-TCDD and HxCDD have been measured in chloracne cases of Seveso residents (Mocarelli et al., 1991) and German chemical workers (Beck et al., 1989; Ott et al., 1993a). Mocarelli et al. (1991) described chloracne in persons from Zone A who had very high serum 2,3,7,8-TCDD levels ranging from 820 to 56,000 pg/g measured within 1 year of the reactor release (Table 7-17). The study also included other individuals from Zone A, but without chloracne, who had serum 2,3,7,8-TCDD levels that ranged from 1,770 to 10,400 pg/g. With the exception of one person with chloracne who was 16 years old at the time of the accident, all of the cases were in children under age 11. Those without chloracne, for the most part, were over age 30. It is not clear whether the

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Table 7-17. Serum 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) Levels in Seveso Residents with Chloracne and Adipose Tissue Levels of 2,3,7,8-TCDD and Hexachlorinated (HxCDD) Dioxins in German Chemical Workers

Author	Population	2,3,7,8-TCDD level (pg/g) ^a	HxCDD level (pg/g) ^a	Year of chloracne diagnosis	Half-life extrapolated 2,3,7,8-TCDD ^b	Half-life extrapolated HxCDD ^b
Beck et al., 1989	Chemical workers ^c	174	247	1955	750	10,000
		99	166	1955	4,010	6,720
		147	5,101	1963	2,350	81,620
		61	172	1957(?)	2,470	6,970
		50	517	1969	380	3,940
		16	58	1955	650	2,360
		1,280	1,019	1978	3,380	2,690
		49	3,442	1974(?)	210	14,760
		50	9,613	1972(?)	260	50,740
		2,252	3,087	1984	2,850	3,910
		158	1,191	1977(?)	460	3,490
		6	283	1970	40	1,970
Mocarelli et al., 1991	Seveso residents	56,000 ^d	—	1976-1977		
		27,800	—	1976-1977		
		26,400	—	1976-1977		
		15,900	—	1976-1977		
		12,100	—	1976-1977		
		17,300	—	1976-1977		
		7,420	—	1976-1977		
		1,690	—	1976-1977		
		828	—	1976-1977		

^apg/g of lipid (parts per trillion).

^bHalf-life extrapolation calculated by authors (Beck et al., 1989) using the formula $C_o = C_t \times 2^n$ where C_o = original concentration of 2,3,7,8-TCDD or HxCDD, C_t = concentration at time t, n = number of half-life periods, and t = half-life period of 5.8 years. Exposures occurred between 1949 and 1986.

^cMeasured in 1984.

^dMeasured in 1976.

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children were more susceptible to the chloranogenic effects or whether they had greater exposure to 2,3,7,8-TCDD-contaminated soil or airborne effluent.

As documented by others, adult TCP production workers also developed chloracne (Beck et al., 1989; Suskind and Hertzberg, 1984; Bond et al., 1989). Adipose tissue levels of 2,3,7,8-TCDD and HxCDD measured in adult chloracne cases of German chemical production workers suggest that these cases may have been a function of the combined exposure, making it difficult to isolate the contribution of the different chlorinated compounds. All cases had estimated adipose levels of greater than 200 pg/g 2,3,7,8-TCDD and in excess of 2,000 pg/g lipid HxCDD at the time of diagnosis. Estimated levels were based on the half-life extrapolation of the adipose tissue level measured in 1986 to the date of last occupational exposure, which may have occurred between 1949 and 1984. Similarly, Ott (1993a) found that 80% of the severe chloracne cases had estimated (back-calculated) levels of 250 pg/g. Yet, 26% of nonchloracne cases had estimated 2,3,7,8-TCDD concentrations of 250 pg/g.

Data from studies of Seveso residents conducted from 1982 to 1985 indicate that, despite high serum 2,3,7,8-TCDD levels, the chloracne resolved in all but one person by 1983 (Assennato et al., 1989). The fact that the cases of chloracne in Seveso residents resolved within 10 years may explain why no chloracne was observed in the Ranch Hand group although some serum 2,3,7,8-TCDD levels exceeded 600 pg/g in 1988 (Roegner et al., 1991) and may have been as high as 2,400 pg/g at the time of last occupational exposure, assuming 21 years since last exposure and a 7-year half-life. Nevertheless, residual chloracne was observed 30 years after first exposure among workers from Nitro, West Virginia, which may suggest that chronic high exposure to 2,3,7,8-TCDD or exposures higher than experienced by the Ranch Hands may account for long-term persistence of chloracne.

7.13.1.2. *Dermatologic Disorders Other Than Chloracne*

Dermal effects other than chloracne attributed to 2,3,7,8-TCDD exposure include a variety of symptoms and conditions that occurred less frequently than chloracne but appeared in several groups subsequent to acute and continuous exposure to 2,3,7,8-TCDD-

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contaminated TCP and 2,4,5-T. Two reports indicated that after acute episodes of exposure, e.g., accidents, individuals complained of red and irritated eyes, conjunctivitis, and blepharitis (inflammation of the eyelids) (Ashe and Suskind, 1950; Baader and Bauer, 1951). Other investigators also found cases of eyelid cysts several months after acute exposure (Suskind et al., 1953; Kimmig and Schulz, 1957a, b; Poland et al., 1971; Reggiani, 1980) and up to 25 years after exposure (Moses et al., 1984; Suskind and Hertzberg, 1984).

Hyperpigmentation and hirsutism (also known as hypertrichosis or abnormal distribution of hair) were diagnosed among chemical workers in the United States (West Virginia and New Jersey) (Ashe and Suskind, 1950; Suskind et al., 1953; Bleiberg et al., 1964; Poland et al., 1971), Germany (Bauer et al., 1961; Goldman, 1972), and Czechoslovakia (Jirasek et al., 1974) who were exposed to 2,3,7,8-TCDD-contaminated TCP during manufacturing processes or industrial accidents and among laboratory workers in England exposed while synthesizing pure 2,3,7,8-TCDD (Oliver, 1975). Upon reexamination 25 years later, hypertrichosis was observed in exposed workers (5.4% exposed vs. 1.8% unexposed) from the West Virginia plant, particularly among workers with persistent chloracne on clinical examination (10.3% with persistent chloracne vs. 0% with history of chloracne only) ($p < 0.001$) in one of two independent studies (Suskind and Hertzberg, 1984). A second study by Moses et al. (1984) found no evidence of hypertrichosis, although 31% of the exposed workers had evidence of residual chloracne (Moses et al., 1984). Studies of Vietnam veterans have reported no significant increase in the prevalence of either hyperpigmentation or hypertrichosis (Roegner et al., 1991; Centers for Disease Control Vietnam Experience Study, 1988a). Three cases of hypertrichosis but not hyperpigmentation were observed among Missouri residents, one with serum levels less than 20 pg/g and two with levels between 20 and 60 pg/g (Webb et al., 1989). Neither disorder was noted on examination among residents of the Quail Run Mobile Home Park (Hoffman et al., 1986).

Actinic or solar elastosis was found to be more prevalent among West Virginia workers diagnosed with active chloracne at the time of their examinations in 1979 (Suskind and Hertzberg, 1984) (exposed = 59.1% vs. unexposed = 30.1%, $p < 0.01$). No significant difference was observed in the age-adjusted prevalence of actinic elastosis in workers with or

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without chloracne in the study by Moses et al. (1984). Actinic elastosis is known to be directly related to sun exposure; however, the amount of sun exposure, skin type, or other factors contributing to the sensitivity of the skin to sunlight were not assessed in the report. No other studies of TCP production workers, the Ranch Hands, or U.S. Army Vietnam veterans have found an increase in the prevalence of actinic elastosis.

Among the group of workers studied by Suskind and Hertzberg (1984), three cases of Peyronie's disease were noted. Peyronie's disease is a rare condition characterized by progressive scarring of the penile membrane. No explanation for this finding was expressed nor has the condition been noted (or perhaps looked for) in other studies (Bond et al., 1989; Moses et al., 1984; Roegner et al., 1991).

In 1984, a statistically significant excess of nonmelanotic skin cancer was reported among Ranch Hand personnel involved in the aerial spraying of herbicides over Vietnam compared with a matched comparison group (Lathrop et al., 1984). The comparison group was composed of Air Force personnel assigned to cargo missions outside the sprayed areas of Vietnam. A follow-up study of the same cohorts in 1987 confirmed the excess of basal cell carcinoma and attributed the increase to sunlight exposure (Lathrop et al., 1987). However, in the reanalysis of the 1987 examination data, skin neoplasms of any kind were not related to serum 2,3,7,8-TCDD level (Roegner et al., 1991).

7.13.1.3. *Comments*

From an epidemiologic perspective, chloracne is a common consequence of exposure to chemicals contaminated with 2,3,7,8-TCDD and some other polyhalogenated hydrocarbons. Available data on serum or adipose tissue levels of 2,3,7,8-TCDD have not determined the threshold at which a case of chloracne occurs. Evidence from Bond et al. (1989) suggests that for chemical production workers the risk of chloracne may be related to the part of the process in which the workers were engaged, the amount of time spent in the contaminated region, and the intensity of the exposure while in the area. Chloracne is also related to short-term high-intensity exposures as observed in Seveso residents (Reggiani, 1980) and occupational cohorts (Ott et al., 1993a). Few studies have been successful in evaluating the relationship between history of chloracne and long-term nonmalignant effects.

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However, Zober et al. (1990) noted that the SMR for all malignant neoplasms for workers with chloracne and who had at least 20 years of latency (time since first exposure) was statistically significantly elevated (SMR 201; 90% CI=122, 135). Future studies that are able to evaluate the association between history of chloracne and effects would provide useful information in this regard.

Other conditions, such as hyperpigmentation and hypertrichosis, may be more acute effects of 2,3,7,8-TCDD exposure that resolve over time, because they were not observed in studies where the cohorts were examined years after the cessation of exposure. Actinic keratosis, Peyronie's disease, and basal cell carcinoma may not be due to 2,3,7,8-TCDD because actinic keratosis and Peyronie's disease have been observed in a single cohort. Likewise, the excess basal cell carcinoma was noted only in one study group, and the results could not be replicated when a better indicator of personal exposure to 2,3,7,8-TCDD, serum 2,3,7,8-TCDD, was used as a surrogate for exposure in the statistical models.

7.13.2. Gastrointestinal Effects

7.13.2.1. *Hepatic Effects*

Changes in liver function and structure after exposure to 2,3,7,8-TCDD are commonly observed in experimental animals (Greig et al., 1973; Vos et al., 1974; Jones and Greig, 1975; McConnell et al., 1978a, b; Jones et al., 1981; Zinkl et al., 1973; Kociba et al., 1976, 1978; Gasiewicz et al., 1980; DeCaprio et al., 1986). The changes are not always consistent from one species to another, but they have prompted examination of hepatic effects among exposed human populations. As with animals, there is wide variation in the type and degree of hepatic effects reported in humans after exposure to 2,3,7,8-TCDD-contaminated materials. This section describes selected hepatic effects associated with 2,3,7,8-TCDD exposure observed in humans, including hepatomegaly and hepatic enzyme changes.

7.13.2.2. *Liver Size*

Increased liver size is consistently reported in treated animals after exposure to 2,3,7,8-TCDD (Vos et al., 1974; Allen et al., 1977; McConnell et al., 1978a, b; Kociba et al., 1978; Gasiewicz et al., 1980). Among exposed human populations, four case reports in

three populations, but not controlled epidemiologic studies, described evidence of enlarged livers or hepatomegaly. Liver size was reportedly increased among two TCP production workers in West Virginia within a few months after a TCP reactor explosion (Ashe and Suskind, 1950; Suskind et al., 1953) and among "several" production workers in Czechoslovakia exposed to TCP, the butyl ester of 2,4,5-T, and sodium pentachlorophenol (Jirasek et al., 1974). Temporary liver enlargement was observed in 5 of 22 Seveso residents who had severe chloracne (Reggiani, 1980). The hepatomegaly lasted "several" months without concomitant elevation in hepatic enzymes. Fortunately, the effect appeared to be transient. Cross-sectional medical studies of TCP production workers (Bond et al., 1983; Suskind and Hertzberg, 1984; Moses et al., 1984; Calvert et al., 1992), Vietnam veterans (Centers for Disease Control Vietnam Experience Study, 1988a; Roegner et al., 1991), and Missouri residents (Webb, 1989; Hoffman et al., 1986) have found little evidence of excess hepatomegaly in the exposed populations. Additionally, no dose-response relationship was observed between serum levels of 2,3,7,8-TCDD and physical findings of an enlarged liver for either the study of Ranch Hands (≤ 10 pg/g 2,3,7,8-TCDD, RR=0.39, 95% CI=0.11-1.33; $15 \leq 33.3$ pg/g 2,3,7,8-TCDD, RR=1.47, 95% CI=0.57-3.79; > 33.3 pg/g 2,3,7,8-TCDD, RR=1.6.9, 95% CI=0.60-4.75) (Roegner et al., 1991) or the NIOSH study of TCP productions workers (two workers; four referents; OR=0.46, 95% CI=0.09, 2.43) (Calvert et al., 1992).

One Missouri resident was found to have hepatomegaly, but he also was suffering from diabetes mellitus. His adipose tissue 2,3,7,8-TCDD level was 430 pg/g (Webb et al., 1989). The differences in findings between the case reports and the controlled epidemiologic studies suggest that hepatomegaly may be a resolvable, acute effect as a result of exposure to high levels of 2,3,7,8-TCDD.

7.13.2.3. Enzyme Levels

Laboratory studies have demonstrated changes in hepatic enzyme levels after 2,3,7,8-TCDD exposure, although there is considerable interspecies variation in the observed effect (Zinkl et al., 1973; Kociba et al., 1976, 1978; Gasiewicz et al., 1980; Olson et al., 1980). Epidemiologic studies and case reports describe elevated liver enzymes among exposed TCP

production workers and among Seveso residents (Mocarelli et al., 1986; May, 1982; Martin, 1984; Moses et al., 1984; Calvert et al., 1992; Ott et al., 1993b).

7.13.2.4. *GGT*

Increased levels of gamma glutamyl transferase (GGT) may suggest activity such as cholestasis, liver regeneration, or drug or xenobiotic metabolism (Table 7-18). The studies of Seveso children demonstrate an increase in GGT levels occurring shortly after the explosion and then a gradual decline to near normal levels within 5 years. In one of the earliest studies of Seveso children with (N=141) and without chloracne (N=138), 2.8% of the children with chloracne had out-of-range GGT levels, but none of the children without chloracne had an out-of-range level ($p < 0.001$) (Caramaschi et al., 1981). These results were echoed in a study of children from Zones A, B, and R of Seveso, in which enzyme levels were measured yearly between June 1977 and June 1982 (Mocarelli et al., 1986). GGT levels were elevated in children of Zone A, particularly in boys, during the first 2 years after the explosion (1977: exposed = 9.73 U/L, unexposed = 7.28 U/L, $p < 0.01$; 1978: exposed = 9.88 U/L, unexposed = 8.26 U/L, $p < 0.05$) (Mocarelli et al., 1986). Levels in girls during the same years were elevated but did not achieve statistical significance. For the next 4 years of the study, GGT levels remained elevated in boys and girls from Zone A compared to unexposed children, but the values declined with time.

GGT was found to be elevated among TCP production workers from one plant in Great Britain and in workers from West Virginia, Missouri, and New Jersey up to 30 years after last occupational exposure to 2,3,7,8-TCDD-contaminated chemicals (May, 1982; Martin, 1984; Moses et al., 1984; Calvert et al., 1992). The findings of two studies of British workers were similar. Mean GGT levels were increased, but not statistically elevated, in workers with chloracne compared to unexposed controls tested 10 years after exposure to 2,3,7,8-TCDD-contaminated chemicals as a result of a TCP reactor explosion (chloracne, GGT=39 U/L; controls, 27.7 U/L [May, 1982]) (chloracne, GGT=32 U/L; controls, 32 U/L [Martin, 1984]). Similarly, a statistically significant excess in the proportion of individuals with abnormally high GGT levels was found among West Virginia workers with chloracne who were examined as many as 30 years after exposure (Moses et

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Table 7-18. Mean Serum Levels of Gamma Glutamyl Transferase (GGT) Among Seveso and Missouri Residents, TCP Production Workers, BASF Accident Cohort, and Vietnam Veterans

Author	Population	Exposed			Unexposed		
		N	Mean level ^a	(SD)	N	Mean level ^a	(SD)
Mocarelli et al., 1986	<u>Seveso residents</u>						
	(1977) (Boys)	52 ^b	9.73	(4.72-20.04)	42 ^b	7.28	(3.71-14.26 ^c)
	(Girls)	32 ^b	9.10	(3.62-22.85)	43 ^b	8.05	(2.99-21.68)
	(1982) (Boys)	106 ^b	9.70	(4.29-21.93)	138 ^b	8.99	(4.45-18.20)
	(Girls)	117 ^b	9.04	(4.25-19.23)	140 ^b	8.59	(3.85-19.14)
Assennato et al., 1989	1976	193 ^d	11.94	(16.80)	— ^e	—	—
	1982	152 ^d	11.67	(7.94)	123 ^f	11.23	(7.41)
	1984	142 ^d	10.53	(7.07)	196 ^f	11.19	(7.05)
	1985	141 ^d	10.57	(7.38)	167 ^f	10.94	(4.68)
Webb et al., 1989	<u>Missouri residents</u>						
	<20 ^g	16	24.0	(15.5)	—	—	—
	20-60	13	17.7	(7.23)	—	—	—
	>60	12	32.8	(23.90)	—	—	—
Hoffman et al., 1986	Missouri residents in Quail Run Mobile Home Park	140	30.0 ^h	(88.3)	141	20.1	(23.4)
May, 1982	TCP production workers in Great Britain	41	39.0	—	31	27.7 ^c	—
Martin, 1984	TCP production workers in Great Britain	41	40.0	(14-91)	120	32.0	(11-90)
Moses et al., 1984	TCP and 2,4,5-T production workers in West Virginia	22 ^d	26.3 ^c	(27.0)	9 ^f	17.4	(11.0)
Calvert et al., 1992	TCP and 2,4,5-T production workers in Missouri and New Jersey	280	58.5 ^{c,i}	(73.7)	259	47.4	(41.1)

^aUnits = U/L.

^bNumber of samples.

^cp < 0.05.

^dChloracne.

^eNo data for controls in 1976.

^fNo chloracne.

^gAdipose tissue level of 2,3,7,8-TCDD in pg/g of lipid.

^h% Abnormal: exposed = 3.6; unexposed = 3.6.

ⁱ% Abnormal: exposed = 10.7; unexposed = 5.0; OR=227 (95% CI=1.17, 4.39).

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Table 7-18. (continued)

Author	Population	Exposed			Unexposed		
		N	Mean level ^a	(SD)	N	Mean level ^a	(SD)
Ott et al., 1993b	BASF accident cohort	133	30.5	(58.4)	6,708	29.9	(43.5)
Centers for Disease Control Vietnam Experience Study, 1988a	U.S. Army ground troops	2,490	43.2 ^j		1,972	41.1 ^k	—
Roegner et al., 1991	U.S. Air Force Ranch Hand personnel						
	Unknown $\leq 10^{l,m}$	338	31.49	—	777	34.64	—
	Low 15- $\leq 33.3^{l,m}$	191	38.28	—			
	High $> 33.3^{l,m}$	182	40.82	—			

^aUnits = U/L.

^jGeometric mean.

^k% Abnormal (Vietnam veterans, 5.5%; non-Vietnam veterans, 4.4; OR=1.3, 95% CI=1.0-1.8).

^lSerum 2,3,7,8-TCDD level in pg/g of lipid.

^mContrasted to unexposed comparison population.

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al., 1984) (chloracne, mean GGT=26.3 U/L, 23% abnormal; no chloracne, mean GGT=17.4 U/L, 9% abnormal; $p < 0.003$). Yet, compared to controls, GGT was not elevated in another study of West Virginia workers (Suskind and Hertzberg, 1984).

In a study by Calvert et al. (1992), the mean GGT level and the proportion of workers with out-of-range levels were statistically significantly elevated among TCP workers from New Jersey and Missouri (workers, mean GGT=58.5 U/L; unexposed referents, mean GGT=47.4 U/L, $p < 0.03$; workers, 11% abnormal; referents, 5% abnormal, OR=2.27, 95% CI=1.17-4.39). Based on the logistic regression model in Table 7-19, the increases in GGT were limited to workers with high serum 2,3,7,8-TCDD levels (> 100 pg/g) and extremely high lifetime alcohol consumption (> 30 alcohol years) (alcohol year = 1 alcoholic beverage/day for 1 year). The contribution of other potentially confounding exposures that may have affected GGT levels was not explored in this study. Other studies of TCP production workers in Michigan and West Virginia or the BASF accident cohort did not report elevations in GGT levels (Bond et al., 1983; Suskind and Hertzberg, 1984; Ott et al., 1993b).

Both the Vietnam Experience Study and the U.S. Air Force Ranch Hand Study found statistically significant elevations in GGT levels (Centers for Disease Control Vietnam Experience Study, 1988a; Roegner et al., 1991). In Army Vietnam veterans, mean GGT levels were 43.2 U/L compared with 41.1 U/L in non-Vietnam veterans (OR for out-of-range value = 1.3, 95% CI=1.0-1.8) (Centers for Disease Control Vietnam Experience Study, 1988a). In the 1987 follow-up study, the comparison of the adjusted mean GGT level in the comparison group and in each of the three Ranch Hand groups defined by 2,3,7,8-TCDD level found statistically significant increases in the Ranch Hand population (≤ 10 pg/g 2,3,7,8-TCDD, $p < 0.017$; $15 \leq 33.3$ pg/g 2,3,7,8-TCDD, $p < 0.043$; > 33.3 pg/g 2,3,7,8-TCDD, $p < 0.001$) (Roegner et al., 1991).

7.13.2.5. *AST and ALT*

Abnormal levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) may indicate liver cell damage from a number of causes, including hepatic necrosis, metastatic carcinoma, or obstructive jaundice (AST and ALT) or infectious or toxic hepatitis

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Table 7-19. Logistic Regression Model for an Out-of-Range Serum Gamma-Glutamyltransferase^a (GGT) Level Using the Categorical TCDD^b Exposure Measure^c

Variable	Beta	Standard error of the estimate	X ²	P
Intercept	-4.12	0.50	67.01	<0.001
Exposure (worker = 1, referent = 0)	0.37	0.43	0.74	0.195
Per alcohol-year	-2.4 x 10 ⁻⁶	2.6 x 10 ⁻³	0.00	0.999
Current alcohol drinker (yes = 1, no = 0) ^d	0.90	0.42	4.70	0.030
Alcohol-years/exposure interaction ^{e,f}	7.2 x 10 ⁻³	3.5 x 10 ⁻³	4.24	0.039
Triglyceride level	3.8 x 10 ⁻³	1.1 x 10 ⁻³	11.94	<0.001

^aReference value: 96 IU/L; a level was considered out of range if it exceeded the reference value. Reference values were defined as the 95th percentile for the referent cohort.

^bTCDD = 2,3,7,8-tetrachlorodibenzo-*para*-dioxin.

^cN=536 observations.

^dThe results from this logistic regression analysis change little when this term is dropped from the model.

^eInteraction between alcohol-years and exposure.

^fExposure odds ratios (ORs) for an abnormal γ -glutamyltransferase level among workers by selected alcohol-year levels, adjusting for all variables in the model, are as follows: OR=2.96 (95% confidence interval [CI]=1.34-6.54) for 100 alcohol-years; OR=1.79 (95% CI=0.81-3.97) for 30 alcohol-years; OR=1.45 (95% CI=0.63-3.36) for 1 alcohol-year; and OR=1.44 (95% CI=0.62-3.34) for 0 alcohol-years.

Source: Calvert et al., 1992b. Used with permission from the author.

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and cirrhosis (AST). Elevated levels of these enzymes may also be due to nonhepatic origins, such as myocardial infarction, acute pancreatitis (AST and ALT), or skeletal, cerebral, or renal necrosis (AST).

With respect to exposure to 2,3,7,8-TCDD, elevations in serum ALT and AST appear to be transient effects of acute exposure (Table 7-20). Case reports note that some populations have increased serum ALT levels shortly after exposure (Seveso children, British and Czechoslovakian TCP production workers) (May, 1973; Jirasek et al., 1974; Caramaschi et al., 1981; Mocarelli et al., 1986). Whereas epidemiologic studies conducted 10 to 30 years after last exposure reported no effects in exposed workers, Vietnam veterans and Missouri residents compared to unexposed control groups (Table 7-20) (Suskind and Hertzberg, 1984; May, 1982; Martin, 1984; Bond et al., 1983; Calvert et al., 1992; Centers for Disease Control Vietnam Experience Study, 1988a; Roegner et al., 1991; Hoffman et al., 1986; Webb et al., 1989; Ott et al., 1993b), or workers with and without chloracne (Moses et al., 1984). Furthermore, it appears from a single study that current exposure to 2,3,7,8-TCDD-contaminated substances must exceed some threshold to produce enzyme elevations (Poland et al., 1971). Normal levels of AST (AST=10.6 U/L) were found in workers who volunteered to participate in a medical study conducted concurrently with their employment in a New Jersey chemical facility producing TCP and 2,4,5,-T (Poland et al., 1971). This was the same plant included in a later cross-sectional medical study of workers that found, in 1988, a high mean serum 2,3,7,8-TCDD level for the group (220 pg/g) but no elevations in AST or ALT (Calvert et al., 1992; Fingerhut et al., 1991a). Similarly, no increases in AST were noted in the BASF accident cohort (Ott et al., 1993b).

Several reports illustrate the probable transiency of ALT and AST elevations after heavy 2,3,7,8-TCDD exposure. Both enzymes were reported to be elevated among TCP production workers employed in Czechoslovakia who were described as exhibiting symptoms of "chemical intoxication" (Jirasek et al., 1974). The authors reported that AST and ALT levels were "different" in 11 (20%) of the 55 examined workers. These workers were exposed to 2,3,7,8-TCDD-contaminated chemicals, pentachlorophenol, and its production by-products, which include hexa-, hepta-, and octachlorinated dioxins and dibenzofurans. The exposures occurred between 1965 and 1968, with the ensuing effects beginning shortly

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Table 7-20. Serum Alanine Aminotransferase (ALT) Among Seveso Children and Missouri Residents, TCP Production Workers, BASF Accident Cohort, and Vietnam Veterans

Author	Population	Exposed			Unexposed		
		N	Mean level ^a	(SD)	N	Mean level ^a	(SD)
Mocarelli et al., 1986	<u>Seveso children</u>						
	1977 Boys	46 ^b	12.25 ^c	(7.14-20.99)	45 ^b	9.33 ^c	(3.73-23.33)
	Girls	100	12.97	(6.68-25.18)	141	11.99	(5.51-26.12)
	1982 Boys	33	10.74	(5.09-22.65)	39	10.74	(5.09-22.65)
	Girls	119	12.27	(6.5-23.17)	136	12.19	(6.46-23.01)
Caramaschi et al., 1981	Seveso children	141 ^d	3.5 ^e		0 ^f	0 ^e	
Moses et al., 1984	TCP production workers; 10-30 years postexposure	105 ^d	15.9	(13.0)	101 ^f	15.7	(13.0)
Calvert et al., 1992	TCP production workers; 15-37 years postexposure	280 ^g	33.8	(22.6)	259	33.0	(21.2)
Ott, 1993a	BASF accident cohort	133	14.8	(8.4)	6,721	15.1	(10.0)
Hoffman et al., 1986	Missouri residents in Quail Run Mobile Home Park	134	— ^h		135	— ^h	
Webb et al., 1989	<u>Missouri residents</u>						
	<20 ⁱ	16	22.7	(2.76)			
	20-60	12	22.5	(2.39)			
	>60	12	23.3	(3.06)			
Centers for Disease Control Vietnam Experience Study, 1988a	U.S. Army ground troops	2,490	26.4 ^{j,k}		1,972	25.8	
Roegner et al., 1991	U.S. Air Force Ranch Hand personnel						
	Unknown ≤10 ^l	339	19.16	—	777	20.34	
	Low 15-≤33.3 ^l	191	20.83	—	—	—	
	High >33.3 ^l	182	20.09 ^c	—	—	—	

^aALT; units U/L.

^bNumber of samples.

^cp<0.05.

^dChloracne.

^e% Abnormal.

^fNo chloracne.

^g% Abnormal: workers 4.3; unexposed 5.0; OR=0.85 (95% CI=0.38, 1.89).

^h% Abnormal: exposed 6.0; unexposed 3.0.

ⁱAdipose 2,3,7,8-TCDD level in pg/g of lipid.

^jGeometric mean.

^k% Abnormal; Vietnam veterans, 5.3; non-Vietnam veterans, 4.4; OR=1.2, 95% CI=0.9-1.5.

^lSerum 2,3,7,8-TCDD in pg/g of lipid.

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thereafter. The observation period lasted from 1967 to 1973. In a follow-up report of the same population conducted in approximately 1977, Pazderova-Vejlupkova et al. (1981) did not report liver enzyme levels but suggested that the levels were not abnormal.

Similarly, ALT was increased in 5 of 14 TCP workers from Great Britain who were in the manufacturing building at the time of a TCP reactor explosion in 1968 (May, 1973). Levels for AST were not reported. In 1977, workers from the same facility were reevaluated. No elevations in AST or ALT were found in production and laboratory workers with chloracne (May, 1982).

Finally, during the first year after the TCP reactor explosion, Caramaschi et al. (1981) evaluated AST and ALT among Seveso children with and without chloracne. Only ALT was statistically significantly elevated in children with chloracne. In a larger study, Mocarelli et al. (1986) tested liver enzyme levels yearly from 1977 to 1982 in male and female children from Seveso and from the unexposed surrounding area. In the 1977, 1979, 1980, and 1981 test series ALT, but not AST, was statistically significantly ($p < 0.05$) elevated among male children in Seveso compared with unexposed comparisons. Female children had normal levels compared with controls for all years. In 1982, ALT levels in the exposed boys returned to normal.

None of the studies reporting elevations in ALT or AST identified clinical evidence of liver disease in the study populations. Therefore, in the absence of reports of hepatic or nonhepatic diseases related to changes in ALT or AST levels among exposed individuals, it is possible that the increases in ALT and AST are related to high-level, acute exposure to 2,3,7,8-TCDD-contaminated chemicals and that, barring additional exposure, the enzyme levels decrease with time.

7.13.2.6. *D-Glucaric Acid*

In five studies of Seveso residents, TCP production workers, and Vietnam veterans, urinary excretion of D-glucaric acid was measured to determine if exposure to 2,3,7,8-TCDD induced hepatic microsomal activity (Table 7-21). D-glucaric acid excretion is an indirect but valid indicator of enzyme induction.

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Table 7-21. Mean D-Glucaric Acid Levels Among Seveso Residents, TCP Production Workers, and Vietnam Veterans

Author	Population	Exposed			Unexposed		
		N	Mean	(SD)	N	Mean	(SD)
Ideo et al., 1985	Seveso adults Levels measured in 1978 (Zone B)	117	27.1 ^{a,b,c}	—	127	19.8 ^{a,b,d}	—
	Seveso children Zone A, 1976	14 ^e	39 ^{a,b,c}	—	17 ^f	20.5 ^b	—
	Zone B 1979	26.8	— ^g	—	—	—	—
	1980	17.0	—	—	—	—	—
May, 1982	TCP production workers in Great Britain	41	2.07 ^h	—	31	1.52 ^h	—
Martin, 1984	TCP production workers in Great Britain	39	2.09 ^c	(0.7-7.9)	126	1.59	(0.8-8.3)
Calvert et al., 1992	TCP and 2,4,5-T production workers in Missouri and New Jersey	273	14.1 ⁱ	(11.1)	256	13.2 ⁱ	(7.9)
Roegner et al., 1991	U.S. Air Force Ranch Hand personnel						
	Unknown $\leq 10^j$	317	14.11 ^{k,l}	—	727	14.11 ^k	—
	Low 15- ≤ 33.3	180	14.43 ^l	—	—	—	—
	High > 33.3	173	15.22 ^l	—	—	—	—

^aUnits: $\mu\text{mol/g}$ of creatinine.

^bMedian level.

^c $p < 0.05$.

^dResidents of unexposed community.

^eSkin lesions.

^fNo skin lesions.

^gd-Glucaric acid levels measured in 1979 were significantly higher than levels measured in 1980 ($p < 0.05$), no data presented.

^hd-Glucaric acid/creatinine ratio.

ⁱUnits = $\mu\text{g/g}$ of creatinine.

^jSerum 2,3,7,8-TCDD levels in pg/g of lipid.

^kUnits = μM .

^lContrasted to unexposed comparison population.

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Ideo and colleagues (1985) measured urinary D-glucaric acid in adults and children from all zones of Seveso and from nearby uncontaminated towns. Of adults tested in 1978, D-glucaric acid excretion was significantly elevated in adults residing in Seveso, Italy, at the time of the reactor explosion compared with residents of unexposed communities (Seveso = $27.1 \mu\text{mol/g}$ of creatinine vs. unexposed = $19.8 \mu\text{mol/g}$ of creatinine, $p < 0.05$). No further studies of adults have been published. A series of studies evaluated D-glucaric acid excretion in Seveso children (Ideo et al., 1985) (Table 7-21). In 1976, the levels in children from Zone A with chloracne ($39 \mu\text{mol/g}$ of creatinine) were significantly greater than in children without chloracne ($20.5 \mu\text{mol/g}$ of creatinine). Additional studies, conducted until 1981, found significant yearly decreases in urinary D-glucaric acid excretion. By 1981, levels were within normal range.

D-glucaric acid-creatinine ratios were used to assess urinary excretion in TCP production workers tested within a year of suspension of TCP production and 10 years after a TCP reactor explosion. For exposed workers with or without chloracne at the time of the study, the D-glucaric acid-creatinine ratio was significantly higher than that of the unexposed controls (exposed = 2.09; unexposed controls = 1.59; $p < 0.05$) (Martin, 1984).

Other studies of Air Force Ranch Hands or TCP production workers that examined D-glucaric acid excretion did not find increases in exposed populations 10 to 37 years after last exposure to 2,3,7,8-TCDD-contaminated chemicals (Roegner et al., 1991; Calvert et al., 1992).

7.13.2.7. *Comment*

The evidence presented by the large number of case reports and epidemiologic studies of groups exposed to 2,3,7,8-TCDD-contaminated chemicals suggests that hepatic enzyme induction occurred in some populations within a short time after high-level 2,3,7,8-TCDD exposure. In most cases, enzyme levels decreased as the time from exposure increased. However, even after more than 15 years since last exposure, levels of GGT continue to be significantly elevated in relation to serum 2,3,7,8-TCDD in TCP production workers with above-average alcohol consumption (Calvert et al., 1992) and in Air Force Ranch Hands (Roegner et al., 1991). Other Vietnam veterans, U.S. Army ground troops, also have

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significantly increased GGT levels compared with non-Vietnam veterans, but this increase in the Army veterans is probably not due to exposure to high levels of 2,3,7,8-TCDD. In the population of Army Vietnam veterans studied, the mean serum 2,3,7,8-TCDD was approximately 4 pg/g (Centers for Disease Control Veterans Health Studies, 1988), compared to a mean of 220 pg/g (range to 3,400 pg/g) in the production workers and a median of 12 pg/g (range to 600 pg/g) in the Ranch Hands.

The finding of continued elevation of GGT may be a spurious result or it may reflect activity related to the continued presence of above-background levels of 2,3,7,8-TCDD in exposed individuals.

7.13.2.8. *Porphyrin Metabolism*

In rats and mice, exposure to 2,3,7,8-TCDD has been clearly shown to produce alterations in porphyrin metabolism (Goldstein et al., 1973, 1982; Smith et al., 1982; Jones et al., 1981; DeVerneuil et al., 1983; Cantoni et al., 1981). Whether 2,3,7,8-TCDD is associated with porphyrin changes in humans, particularly porphyria cutanea tarda (PCT), is a subject of some debate. PCT is a form of acquired or inherited porphyria caused by a deficiency of the enzyme uroporphyrinogen decarboxylase and the resulting overproduction and excretion of uroporphyrin (Sweeney, 1986). The predominant characteristics of PCT include skin fragility, blistering upon sun exposure, dark pigmentation, excess hair growth, hepatomegaly, reddish-colored urine, and urinary excretion of uro- and heptacarboxylicoporphyrins (Strik, 1979). PCT has been associated with excessive alcohol intake, oral estrogens, iron overload, hepatomas, and exposure to polyhalogenated hydrocarbons (Strik, 1979). A particularly large outbreak of PCT occurred after consumption of grain treated with hexachlorobenzene (HCB) (Cam and Nigogosyan, 1963). Cases of PCT were described in two populations of TCP production workers (Bleiberg et al., 1964; Jirasek et al., 1974) and among members of a family with inherited uroporphyrin decarboxylase deficiency who were living in Seveso at the time of the reactor explosion (Strik, 1979).

In 1964, Bleiberg reported that based on the Watson-Schwartz test, 11 of 29 New Jersey TCP production workers with chloracne had porphyria cutanea tarda as a result of increased urinary uroporphyrins, coproporphyrins, and urobilinogen. In a later study of 73

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workers from the same plant in New Jersey, including four of the individuals that Bleiberg et al. (1964) found to have elevated urinary porphyrins, Poland et al. (1971) identified one individual with uroporphyrinuria. The report did not explain if this individual was one of the four described by Bleiberg et al. (1964). In the NIOSH study that examined workers from the same plant in New Jersey, the pattern of urinary porphyrin excretion for each participant was assessed to determine the presence of PCT (Calvert et al., 1993). No difference was found between workers and an unexposed control group in the prevalence of PCT (OR=0.93, 95% CI=0.19, 4.54). Furthermore, there were no differences in the risk between workers and the control group for an out-of-range uroporphyrin concentration or an out-of-range coproporphyrin concentration. Because this study was conducted at least 15 years after last occupational exposure to TCDD, it was not possible to determine whether porphyrinuria occurred during the years more proximal to occupational 2,3,7,8-TCDD exposure.

There is some question as to the appropriateness of the clinical test Bleiberg et al. (1964) used to measure porphyrin levels. The Watson-Schwartz test was capable of measuring only the presence of porphobilinogen. The test was rarely positive in cases of exposure to hepatotoxins. Bleiberg's findings suggest that either other unspecified tests were used to measure uro- and coproporphyrin levels or the authors misinterpreted the function of the Watson-Schwartz test.

Jirasek et al. (1974) found 11 of 55 Czechoslovakian TCP production workers to have elevated urinary uroporphyrins that decreased during the observation period; the authors did not describe the test used to measure urinary uroporphyrins or coproporphyrins. Ten years later in a follow-up study, Pazderova-Vejlupkova et al. (1981) found no evidence of increased excretion of uroporphyrins or dermatological indications of PCT in the same group of workers.

There is some question that the porphyria noted in the New Jersey (Bleiberg et al., 1964) and Czechoslovakian workers was due to 2,3,7,8-TCDD exposure. Jones and Chelsky suggest that the observed cases of PCT in both plants may be due to exposure to HCB or a combination of both 2,3,7,8-TCDD and HCB (Jones and Chelsky, 1986). HCB was manufactured at the New Jersey plant from 1951 until 1960 and was produced at the facility

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in Czechoslovakia during the production of pentachlorophenol and TCP (Jirasek et al., 1973). Evidence of porphyria in other studies of individuals exposed to 2,3,7,8-TCDD-contaminated substances is minimal. Although Suskind and Hertzberg (1984) sampled urine for porphyrins in the examination of West Virginia TCP workers, the authors report that the data are not valid. However, there was no dermatologic evidence of porphyria identified among the exposed workers. Moses et al. (1984) found no difference in porphyrin levels when comparing TCP workers with and without chloracne. Finally, no PCT was reported among three laboratory workers exposed to pure 2,3,7,8-TCDD (Oliver, 1975), the only humans known to have documented unintentional exposure to uncontaminated 2,3,7,8-TCDD.

In 1977, 60 Seveso residents were tested for elevated porphyrins, exclusive of the family with inherited deficiency of uroporphyrinogen decarboxylase. None of the 60 residents developed PCT; however, 13 (22%) exhibited secondary coproporphyrinuria, 5 of whom showed a slight increase of urocarboxyporphyrins, heptacarboxyporphyrins, and coproporphyrins classified as a "transition constellation to CHP type A" (Doss et al., 1984). Porphyrin levels were retested in 1980. Porphyrin levels returned to normal in 12 individuals. In three of those with transition CHP, porphyrin levels were higher than those in 1977 and were attributed to liver damage and alcohol consumption. Doss et al. (1984) suggested that in the Seveso family with uroporphyrinogen decarboxylase deficiency, the exposure to 2,3,7,8-TCDD-contaminated effluent caused an exacerbation of a preexisting enzyme deficiency.

7.13.2.9. *Comment*

It is possible that the PCT and elevated urinary porphyrins observed in the New Jersey and Czechoslovakian workers were a direct result of exposure to hexachlorobenzene. In the follow-up studies, urinary porphyrin levels in workers were not elevated (Pazderova-Vejlupkova et al., 1981; Poland et al., 1971) or did not differ from levels in the control group (Calvert et al., 1992). The transient elevations in coproporphyrins among 22 Seveso residents described by Doss et al. (1984) may be a direct result of acute exposure to 2,3,7,8-TCDD.

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The association is not clear. However, 2,3,7,8-TCDD is a potent porphyrigen in rats and mice and, therefore, high acute exposures may have contributed to the observed changes in porphyrin levels in these populations.

7.13.2.10. *Lipid Levels*

Animal studies provide conflicting evidence on the relationship between exposure to 2,3,7,8-TCDD and serum lipid levels. Some studies suggest that short-term high exposure to 2,3,7,8-TCDD increases serum cholesterol (Greig et al., 1973; Zinkl et al., 1973; Poli et al., 1980; Gasiewicz et al., 1980; Schiller et al., 1986; Gasiewicz and Neal, 1979; Olson et al., 1980) and triglyceride fractions (Schiller et al., 1986; McConnell et al., 1978a, b; Gasiewicz and Neal, 1979), whereas other studies suggest a decrease (Gasiewicz et al., 1980; Olson et al., 1980) or no change in triglyceride levels (Poli et al., 1980). The human data appear to be similarly confusing. A number of case reports and epidemiologic studies have described increases in the levels of serum lipid fractions, particularly total cholesterol and triglycerides, in TCP production workers, laboratory workers, Seveso and Missouri residents, and Vietnam veterans. Others report no differences between subject and reference levels. A summary of the reported levels is included in Tables 7-22 and 7-23.

7.13.2.11. *Total Cholesterol*

Two case reports of workers with presumably high exposures to 2,3,7,8-TCDD-contaminated chemicals described elevations in total cholesterol. In 50% of 55 Czechoslovakian TCP production workers examined between 1968 and 1969 who exhibited signs of "chemical intoxication," total cholesterol was noted as elevated (Jirasek et al., 1974). In a follow-up study 10 years later, lipid levels among workers removed from exposure were not significantly different from referent levels, but total cholesterol levels remained significantly increased (Pazderova-Vejlupkova et al., 1981). In a separate report, three laboratory workers who were exposed during the synthesis of pure 2,3,7,8-TCDD developed serum cholesterol levels in excess of 7.7 mmol/L (Oliver, 1975). No information on pre-exposure cholesterol levels was provided in either report.

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Table 7-22. Mean Total Cholesterol Levels Among Seveso and Missouri Residents, TCP Production Workers, BASF Accident Cohort, and Vietnam Veterans

Author	Population	Exposed			Unexposed		
		N	Mean ^a level	(SD)	N	Mean ^a level	(SD)
Mocarelli et al., 1986	Seveso children 1977	16 ^b	4.62	3.26-5.98	28 ^b	4.45	(3.12-5.77)
	1982	182 ^b	4.48	2.47-5.99	250 ^b	4.41	(2.99-5.83)
Caramaschi et al., 1981	Seveso children	138	15.2 ^{c,d}	—	120	12.5 ^{c,e}	—
Assennato et al., 1989	Seveso residents 1976	193 ^d	4.78	(0.99)	— ^f	—	—
	1982	152 ^d	4.06	(0.80)	123 ^g	4.14	(0.77)
	1984	142 ^d	4.09	(0.88)	196 ^g	4.12	(0.86)
	1985	141 ^d	4.14	(0.91)	167 ^g	4.13	(0.78)
May, 1982	TCP production workers in Great Britain	41	5.97	—	31	6.6	—
Martin, 1984	TCP production workers in Great Britain	39	6.02 ^h	—	126	5.6	—
Poland et al., 1971	TCP production workers in New Jersey	71	6.12	(0.82)	—	—	—
Moses et al., 1984	TCP production workers in West Virginia	105 ^d	5.38	(0.88)	101 ^g	5.37	(0.85)

^aUnits = mmol/L.

^bNumber of samples.

^c% abnormal.

^dChloracne.

^eNo chloracne.

^fNo data for controls in 1976.

^gp < 0.05.

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Table 7-22. (continued)

Author	Population	Exposed			Unexposed		
		N	Mean ^a level	(SD)	N	Mean ^a level	(SD)
Suskind and Hertzberg, 1984	TCP production workers in West Virginia	200	5.46	(0.07)	163	5.28	(0.08)
	TCP production workers: Chloracne vs. never chloracne	105 ^d	5.44	(0.08)	28 ^a	5.30	(0.18)
Ott et al., 1993b	BASF accident cohort	135	6.14 ^m	(1.01)	6,581	6.37 ^m	(1.17)
Calvert et al., 1993	TCP and 2,4,5-T production workers in Missouri and New Jersey	278	5.7 ⁿ	—	259	5.6 ⁿ	—
Hoffman et al., 1986	Missouri residents in Quail Run Mobile Home Park	142	4.97 ^k	(0.96)	148	5.2	(1.09)
Webb et al., 1989	Missouri residents <20 ^b	16	5.88	(1.10)	—	—	—
	20-60 ^b	12	6.60	(0.93)	—	—	—
	>60 ^b	12	6.76	(0.97)	—	—	—
Centers for Disease Control Vietnam Experience Study, 1988a	U.S. Army Vietnam veterans	2,490	5.43 ^{ij}	—	1,972	5.36	—
Roegner et al., 1991	Air Force Ranch Hand personnel						
	Unknown ≤10 ^k	338 ^l	5.53	—	777	5.51	—
	Low 15-≤33.3 ^k	191	5.55	—	—	—	—
	High >33.3 ^k	182	5.68 ^k	—	—	—	—

^aAdipose tissue levels of 2,3,7,8-TCDD in pg/g of lipid.

^bGeometric mean.

^j% abnormal: Vietnam veterans, 5.1; non-Vietnam veterans, 4.7; OR=1.1, 95% CI=0.8-1.5.

^kSerum 2,3,7,8-TCDD levels in pg/g of lipid.

^lContrasted to unexposed comparisons.

^mAdjusted for age, body mass index, smoking history.

ⁿAdjusted for age, body mass index, smoking, gender.

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Table 7-23. Mean Triglyceride Levels Among Seveso and Missouri Residents, TCP Production Workers, BASF Accident Cohort, and Vietnam Veterans

Author	Population	Exposed			Unexposed		
		N	Mean ^a	(SD)	N	Mean ^a	(SD)
Mocarelli et al., 1986	Seveso children 1977 1982	38 ^b	0.97	(0.6-1.50)	36 ^b	0.95	(0.63-1.51)
		207 ^b	0.91	(0.52-1.60)	257 ^b	0.86	(0.47-1.56)
Assennato et al., 1989	Seveso residents 1976	193	0.99	(0.43)	—	—	—
	1982	152	0.87	(0.40)	123	0.85	(0.37)
	1984	142	0.94	(0.59)	196	0.88	(0.46)
	1985	141	0.84	(0.44)	167	0.87	(0.55)
May, 1982	TCP production workers in Great Britain	41 ^d	2.03	—	31 ^e	1.83	—
Martin, 1984	TCP production workers in Great Britain	39 ^d	1.97 ^f	(0.4-4.0)	126 ^e	1.41	(0.3-3.2)
Moses et al., 1984	TCP production workers in West Virginia	93 ^d	1.69 ^g	(1.26)	93 ^e	1.46	(0.73)
Suskind and Hertzberg, 1984	TCP production workers in West Virginia	200	1.65	(0.08)	163	1.76	(0.08)
Ott et al., 1993b	BASF accident cohort	135	1.91 ^h	(1.19)	4,471	1.97 ^h	(1.65)
Calvert et al., 1993	TCP and 2,4,5-T production workers in Missouri and New Jersey	273	1.20	(—)	259	1.15 ⁱ	(—)

^aUnits = mmol/L.

^bNumber of samples.

^cAdipose tissue 2,3,7,8-TCDD levels in pg/g of lipid.

^dChloracne.

^eNo chloracne.

^fp<0.01.

^gp=0.056.

^hAdjusted for age, body mass index, smoking history.

ⁱAdjusted for age, body mass index, smoking, gender.

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Table 7-23. (continued)

Author	Population	Exposed		Unexposed	
		N	Mean ^a (SD)	N	Mean ^a (SD)
Hoffman et al., 1986	Missouri residents in Quail Run Mobile Home Park	141	1.07 (0.73)	146	1.19 (1.07)
Webb et al., 1989	Missouri residents <20 ^c	16	2.17 (2.08)	---	---
	20-60 ^c	12	1.81 (1.19)	---	---
	>60 ^c	12	2.69 (1.06)	---	---
Centers for Disease Control Vietnam Experience Study, 1988a	U.S. Army Vietnam veterans	2,490	1.06 ^{j,k} ---	1972	1.05 ---
Roegner et al., 1991	U.S. Air Force Ranch Hand personnel				
	Unknown ≤10 ^l	338	1.02 ^{f,m} ---	777	1.16 ---
	Low 15-≤33.3 ^l	191	1.37 ^{f,m} ---		
	High >33.3 ^l	182	1.35 ^{f,m} ---		

^j% Abnormal: Vietnam veterans, 4.7; non-Vietnam veterans, 5.3; OR=0.9, 95% CI=0.7-1.2.

^kGeometric mean.

^lSerum 2,3,7,8-TCDD levels in pg/g of lipid.

^mContrasted to unexposed comparison population.

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The results of epidemiologic studies conflict. Among British TCP production workers whose last exposure to 2,3,7,8-TCDD-contaminated chemicals was less than 1 year at the time of the study, total cholesterol levels in exposed workers with (6.02 mmol/L) and without (6.14 mmol/L) chloracne were significantly elevated compared to unexposed controls (5.6 mmol/L) (Martin, 1984) (Table 7-22), whereas May (1982) found unexposed workers (6.6 mmol/L) to have cholesterol levels higher than those of exposed workers with chloracne (5.97 mmol/L). Martin (1984) also found reduced, but not significantly, HDL cholesterol among exposed workers with chloracne (1.19 mmol/L) compared to unexposed controls (1.25 mmol/L). The differences in the results may be due to differences in the control groups and inclusion of different workers in the exposed groups.

Cholesterol levels in West Virginia TCP production workers were compared with unexposed workers from the same plant (Suskind and Hertzberg, 1984). No difference was identified in mean cholesterol levels between workers and controls. However, when lipid fractions were examined, there was a larger but nonsignificant percentage of exposed workers with elevated LDL cholesterol (7.7%) compared to unexposed controls (6.3%). A comparison of workers with persistent chloracne, no chloracne, or a history of chloracne found a significant association ($p < 0.05$) between the proportion of out-of-range LDL cholesterol values and persistent chloracne. An out-of-range LDL was defined as above the 90th percentile of the total range of values. In a second study that compared West Virginia workers with and without chloracne, no difference was found in mean cholesterol levels (Moses et al., 1984). In the NIOSH study, there was little difference between the adjusted mean total cholesterol levels for workers (5.7 mmol/L) and referents (5.6 mmol/L) and no relation to increasing serum 2,3,7,8-TCDD levels (Calvert et al., 1993). The mean levels were adjusted for age, body mass index, age, and gender.

Mean cholesterol levels were no different between workers in the BASF accident cohort (6.14 mmol/L) and the referent population (6.37 mmol/L) and were not related to current or log TCDD back-calculated levels (Ott et al., 1993b). In addition, no significant differences were noted between the exposed and unexposed populations for HDL and LDL levels.

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In general, cholesterol levels among exposed community residents were not increased. Despite their high exposure to 2,3,7,8-TCDD-contaminated TCP, neither children nor adults from Seveso were found to have elevated serum cholesterol levels compared to controls (Mocarelli et al., 1986; Assennato et al., 1989). Evaluated from 1976 through 1985, cholesterol levels in this population remained constant throughout the study period (Table 7-22). Similarly, among Missouri residents, serum cholesterol was not related to residence in the Quail Run Mobile Home Park (Hoffman et al., 1986) or to adipose tissue 2,3,7,8-TCDD levels (Webb et al., 1989).

Among U.S. Army veterans, there was no difference in total cholesterol levels between groups serving in Vietnam or other arenas (Centers for Disease Control Vietnam Experience Study, 1988a). In contrast, there was a statistically significant positive relationship between Ranch Hands with serum 2,3,7,8-TCDD levels above 33.3 pg/g and total cholesterol in Air Force Ranch Hands (Roegner et al., 1991). The total cholesterol-HDL ratio was also highest in this serum 2,3,7,8-TCDD category.

7.13.2.12. *Triglycerides*

Elevated triglyceride levels were reported only in three of the studied populations. Among British TCP workers, triglycerides were significantly higher in exposed workers with (1.97 mmol/L) and without (1.90 mmol/L) chloracne compared to unexposed controls (1.41 mmol/L) (Martin, 1984). TCP production workers from West Virginia who had chloracne had statistically nonsignificant increase in mean triglyceride levels (chloracne = 1.69 mmol/L; without chloracne = 1.46 mmol/L) (Moses et al., 1984). In addition, compared to the unexposed background population, triglyceride levels in Air Force Ranch Hands were significantly elevated for all serum 2,3,7,8-TCDD categories (Table 7-23). Among workers in the NIOSH study there appeared to be a small rise in triglyceride levels with increasing serum 2,3,7,8-TCDD (Calvert et al., 1993). The mean adjusted triglyceride levels and the percent of abnormal triglyceride values increase with increasing serum 2,3,7,8-TCDD level (<158 femtograms/liter [fg/L], mean = 1.04 mmol/L, % abnormal = 5.7; 158-520 fg/L, mean = 1.26 mmol/L, % abnormal = 6.1; 521-1,515 fg/L, mean = 1.23 mmol/L, % abnormal = 6.1; 1,516-19,717 fg/L, 1.35 mmol/L, % abnormal = 1.7, $p < 0.05$ compared

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to referents [1.15 mmol/L]). Odds ratios and 95% confidence intervals for the quartiles are OR=0.7 (95% CI=0.5, 12.0), OR=1.1 (95% CI=0.4, 3.2), OR=0.9 (95% CI=0.3, 2.9), and OR=1.7 (95% CI=0.6, 4.6), respectively. The authors suggest that despite this small rise with 2,3,7,8-TCDD level, the influence of factors such as gender, body mass index, use of beta-blocker medication, and smoking had far greater effects on lipid concentration than did 2,3,7,8-TCDD level. Similarly, triglyceride levels in the BASF accident cohort were similar to those in the referent cohort and not related to 2,3,7,8-TCDD level (Ott et al., 1993b).

Triglyceride levels were not elevated in Missouri (Hoffman et al., 1986; Webb et al., 1989) or Seveso residents (Mocarelli et al., 1986; Assennato et al., 1989) or in U.S. Army Vietnam veterans (Centers for Disease Control Vietnam Experience Study, 1988a).

7.13.2.13. *Comment*

The effect of exposure to 2,3,7,8-TCDD-contaminated chemicals on cholesterol or triglyceride levels is not consistently well defined in the available studies. It is possible the transient elevations in total cholesterol and triglyceride levels may have occurred after high 2,3,7,8-TCDD exposure, as in the experience of the British and Czechoslovakian TCP workers and British laboratory workers. However, this scenario does not concur with the evidence from Seveso or among Ranch Hands. Despite their very high exposure to 2,3,7,8-TCDD-contaminated chemicals, neither adults nor children from Seveso had lipid levels above the referent level. In contrast, Ranch Hands continue to have elevated lipid levels despite the extended length of time between exposure and testing. Other factors, such as dietary fat intake, familial hypercholesterolemia, alcohol consumption, and exercise, which also affect cholesterol and other lipid levels, may be factors that were not considered in many of these studies.

In the 1992 phase of the Ranch Hand study, additional parameters that may affect lipid levels were examined. It is hoped that this new information will help clarify the increased levels observed in the Ranch Hand population.

7.13.3. Other Gastrointestinal Disorders

A variety of gastrointestinal disorders other than liver conditions were reported among TCDD-exposed groups. After heavy, acute, or chronic exposure, chemical workers in West Virginia (Ashe and Suskind, 1950), West Germany (Baader and Bauer, 1951; Bauer et al., 1961), and Czechoslovakia (Jirasek et al., 1974) consistently reported transient episodes of right upper quadrant pain, loss of appetite, and nausea. None of the reports suggest an etiology for these symptoms nor were the symptoms reported in later follow-up studies of any cohorts (Suskind and Hertzberg, 1984; Moses et al., 1984; Pazderova-Vejlupkova et al., 1981).

Three investigations of TCP production workers reported an increased prevalence of a history of upper gastrointestinal tract ulcer across all age strata of West Virginia workers (exposed = 20.7% vs. unexposed = 5.5%) (Suskind and Hertzberg, 1984) and all digestive system diseases (type not specified) among workers employed in a plant in Midland, Michigan (prevalence: exposed = 1.5% vs. unexposed = 0.5%) (Bond et al., 1983). The factors contributing to these conditions have not been examined fully. Neither the Ranch Hand study (Roegner et al., 1991) nor the NIOSH study (Calvert et al., 1992) found increased risk of upper gastrointestinal tract ulcers with increasing serum TCDD level.

7.13.4. Thyroid Function

The thyroid plays an essential role in the maintenance of metabolic rate, food intake, and differentiation and maturation of various cell types. Because many of the toxic effects of 2,3,7,8-TCDD noted in animals resemble the signs of thyroid dysfunction, researchers considered the role of the thyroid in 2,3,7,8-TCDD toxicity (Neal et al., 1979). Some studies found a single high dose of 2,3,7,8-TCDD resulted in decreased levels of serum thyroxine (T4), indicating hypothyroidism, but no consistent findings were reported for alterations in 3,5,3'-triiodothyronine (T3); researchers report decreases, no change, and increases in levels of T3 (Bastomsky, 1977; Potter et al., 1983; Pazdernik and Kozman, 1985; Potter et al., 1986; Henry and Gasiewicz, 1987; Roth et al., 1988; Muzi et al., 1989). Furthermore, hypothyroidism induced in rats was protective against 2,3,7,8-TCDD-induced weight loss, immunotoxicity, and mortality (Rozman et al., 1984; Pazdernik and Kozman,

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1985). This protective effect was reversed when T4 supplements were given to these animals (Rozman et al., 1985). A recent study by Henry and Gasiewicz (1987), however, found that in hamsters serum T3 and T4 levels increased after 2,3,7,8-TCDD administration, putting in question the role of the thyroid in 2,3,7,8-TCDD-induced toxicity.

These animal findings suggest that if the thyroid plays a role in human toxicity, hypothyroidism would be manifested as a reduction in serum T4; in extreme cases of 2,3,7,8-TCDD toxicity, however, one may experience hyperthyroidism. Only two studies of production workers examined this issue (Suskind and Hertzberg, 1984; Ott et al., 1993b). Suskind and Hertzberg (1984) performed T4 radioimmunoassay and thyroxine-binding globulin (TBG) tests and found no significant differences between exposed and unexposed workers. Quantitative results were not presented. Similarly, TSH, T4, and TGB levels were within normal range in the BASF accident workers, although TGB levels were positively related to 2,3,7,8-TCDD levels (Ott et al., 1993b). The Ranch Hand study indicated a reduction of T3% uptake (Table 7-24) and an increase in the mean level of thyroid-stimulating hormone (TSH) (Table 7-25) with increasing serum 2,3,7,8-TCDD level; these results, however, did not reach statistical significance. Among Army Vietnam veterans, mean TSH levels, but not mean free thyroxine index (FTI) levels, were statistically significantly higher than among non-Vietnam veterans, after adjustment for the six entry characteristics of age and year of enlistment, race, enlistment status, general technical test score, and primary military occupation (Table 7-24) (Centers for Disease Control Vietnam Experience Study, 1988a). However, the percent of values that were out-of-reference range did not differ significantly for TSH (Vietnam veterans, 1.0%; non-Vietnam veterans, 0.6%, OR=2.0, 95% CI=0.9-4.3) and FTI (Vietnam veterans, 5.4%; non-Vietnam veterans, 4.6%, OR=1.2, 95% CI=0.9-1.5). The exposure levels were low, based on the sample for which 2,3,7,8-TCDD was measured.

The most recent study was conducted among infants in The Netherlands (Pluim et al., 1992). In a letter to the editor, Pluim and colleagues (1992) examined thyroid function among term breast-fed infants in relation to the total toxic equivalents per kg of breast milk fat (TEQ/kg) of dioxins (congeners were not specified). The authors measured T4, TBG, and TSH levels sequentially in infants at birth, at 1 week of age, and at 11 weeks of age (Tables

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Table 7-24. Levels of Triiodothyronine Percent (T3%) Uptake or Free Thyroxine Index in Vietnam Veterans

Outcome	Author	Population	Exposed			Unexposed	
			N	Mean level	Adjusted RR	N	Mean level
T3% uptake	Roegner et al., 1991	U.S. Air Force Ranch Hand personnel					
		Unknown $\leq 10^a$	338	30.67	1.14	772	30.66
		Low 15- $\leq 33.3^a$	194	30.36	0.93		
		High $> 33.3^a$	181 ^b	29.99	0.45		
Free thyroxine index							
	Centers for Disease Control Vietnam Experience Study, 1988a	U.S. Army Vietnam veterans	2,490	2.2 ^c	Adjusted OR (95% CI) 1.2 (0.9,1.5)	1,972	2.2 ^c

^aSerum 2,3,7,8-TCDD in pg/g of lipid.

^bp<0.05 comparison of veterans at background level with ≥ 33.3 pg/g TCDD.

^cGeometric mean.

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Table 7-25. Levels of Thyroid-Stimulating Hormone (TSH) in Vietnam Veterans, Nursing Infants, and BASF Accident Cohort

Author	Population	Exposed			Unexposed		
		N	Mean level ^a		N	Mean level ^a	SD
Roegner et al., 1991	U.S. Air Force Ranch Hand personnel			Adjusted RR (95 % CI)			
	Unknown $\leq 10^b$	338	0.948	1.45 (0.62,3.40)	616	0.920	---
	Low 15- $\leq 33.3^b$	194	0.978	0.88 (0.24,3.02)			
	High $> 33.3^b$	181	1.026 ^c	2.15 (0.80,5.79)			
Centers for Disease Control Vietnam Experience Study, 1988a	U.S. Army Vietnam veterans	2,490	1.6 ^d	Adjusted OR (95 % CI) 2.0 (0.9-4.3)	1,972	1.6 ^d	---
Pluim et al., 1992	Neonates; The Netherlands						
	At birth	11 ^e	11.9	1.9 ^f	14 ^g	10.4	1.3 ^f
	1 wk postnatal	11	2.56	0.41	15	2.93	0.41
	11 wks postnatal	12	2.50 ^c	0.26	18	1.81	0.19
Ott et al., 1993b	BASF chemical workers	130	1.19 (0.90) ^h	---	---	---	---

^aUnits: μ U/ml.

^bSerum 2,3,7,8-TCDD level in pg/g of lipid.

^cp < 0.05 compared to low-exposure group.

^dGeometric mean.

^eHigh-exposure group: 29.2-62.7 ng toxic equivalents/kg (toxic equivalencies [TEQ] per kg milk fat).

^fStandard error of the mean.

^gLow-exposure group: 8.7-28 ng TEQ/kg.

^hStandard deviation.

ⁱNo referent values.

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7-25 and 7-26). Infants were classified in the high-exposure group if the breast milk contained 29.2-62.7 ng TEQ/kg and in the low-exposed group if the breast milk contained less than 28.0 ng TEQ/kg. At 1 week and 11 weeks postnatal, T4 and T4/TGB ratios were significantly higher among infants in the "high-exposure group." At 11 weeks, TSH was also higher in infants ingesting milk with the higher concentration of dioxins. The authors suggest that exposure to high levels of dioxins either in utero or through breast milk modulates the hypothalamic-pituitary-thyroid regulatory system of the infant (Pluim et al., 1992).

In summary, experimental animal studies provide some evidence that thyroid may mediate, at least in part, 2,3,7,8-TCDD toxicity. These results were based on animals receiving a single high dose of 2,3,7,8-TCDD. In addition, studies were performed in only two species of animals, and the effects may be species specific. The applicability of these findings to humans, therefore, needs further evaluation.

7.13.4.1. *Comment*

Few human studies examined the relationship between 2,3,7,8-TCDD exposure and thyroid function, and the results of present research are equivocal. These studies examined individuals with relatively lower exposure to 2,3,7,8-TCDD than the animals, and exposure was chronic. Little or no information has been reported for the effects specifically in production workers or Seveso residents, two groups with documented high serum 2,3,7,8-TCDD levels (Fingerhut et al., 1991a; Mocarelli et al., 1991). The data from the study of Ranch Hands, which measured serum 2,3,7,8-TCDD levels, suggest that in adults there are few long-term effects. Although the study of nursing infants suggests that ingestion of breast milk with a higher total TEQ may alter thyroid function, the study is limited by small numbers and a short observation period (Pluim et al., 1992).

The variety of ways in which 2,3,7,8-TCDD may affect thyroid function in humans makes it difficult to predict the net outcomes in terms of individual laboratory tests. It is possible that different relationships may exist among thyroid function indicators, depending on the exposure level of the individual.

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Table 7-26. Levels of Thyroxine-Binding Globulin (TBG), Thyroxine (T4), or T4/TGB in Nursing Infants and BASF Accident Cohort

Outcome			Exposed			Unexposed		
			N	Mean level	Standard deviation	N	Mean level	Standard deviation
TBG (nmol/L)	Pluim et al., 1992	Neonates; The Netherlands						
		At birth	15	589.5	30.5 ^d	18	520.1	27.2 ^d
		1 wk postnatal	19	546.2	19.1	19	532.6	16.3
		11 wks postnatal	16	500.7	13.0	18	519.0	29.4
T4 (nmol/L)		Neonates; The Netherlands						
		At birth	15	134.3	4.8 ^d	18	122.5	4.1 ^d
		1 wk postnatal	19	178.7 ^c	5.5	19	154.5	6.3
		11 wks postnatal	16	122.2 ^c	3.0	18	111.1	4.0
T4/TBG	Pluim et al., 1992	Neonates; The Netherlands						
		At birth		0.232	0.008 ^d	18	0.240	0.007 ^d
		1 wk postnatal		0.332 ^c	0.011	19	0.291	0.009
		11 wks postnatal		0.247 ^c	0.009	18	0.220	0.008

^aHigh-exposure group: 29.2-62.7 ng toxic equivalents/kg (TEQ per kg milk fat).

^bLow-exposure group: 8.7-28 ng TEQ/kg.

^cp<0.05.

^dStandard error of the mean.

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Table 7-26. (continued)

Outcome			Exposed			Unexposed		
			N	Mean level	Standard deviation	N	Mean level	Standard deviation
TBG (nmol/L)	Author	Population						
TBG (mg/L)	Ott et al., 1993b	BASF accident cohort	145	12.7	3.2	42*	12.7	2.9
T4 (µg/dL)	Ott et al., 1993b	BASF accident cohort	144	7.8	1.9	42*	8.3	1.5
T4/TBG (mU/L)	Ott et al., 1993b	BASF accident cohort	131	6.3	1.3	42*	6.6	1.6

*Source: Zober et al., 1992.

7.13.5. Diabetes

Diabetes and fasting serum glucose levels were evaluated in cross-sectional medical studies due to the apparently high prevalence of diabetes and abnormal glucose tolerance tests in one case report of 55 TCP workers (Pazderova-Vejlupkova et al., 1981). In this group, evaluated 10 years after exposure ended, approximately 50% of the subjects had either confirmed cases of diabetes or abnormal glucose tolerance tests.

The results of later medical studies are mixed. Cross-sectional studies of workers from Nitro, West Virginia (Suskind and Hertzberg, 1984; Moses et al., 1984), found no difference in glucose levels between the exposed and control populations, although no quantitative values were presented in either study. Similarly, the adjusted odds ratio for out-of-range fasting glucose levels comparing Vietnam veterans to non-Vietnam veterans was not statistically significant (OR=1.0, 95% CI=0.4-2.2). But a comparison of the adjusted mean fasting glucose levels between the two groups was marginally significant (Vietnam veterans, 5.2 mmol/L; non-Vietnam veterans, 5.1 mmol/L, $p < 0.05$) (Centers for Disease Control Vietnam Experience Study, 1988a). Mean fasting glucose levels in the BASF accident cohort were marginally elevated compared to the referent population and associated with current levels of 2,3,7,8-TCDD ($p = 0.062$) but not the back-extrapolated level (Ott et al., 1993b). Results from the NIOSH (Sweeney et al., 1992) and Ranch Hand studies (Roegner et al., 1991) more strongly suggest that serum 2,3,7,8-TCDD levels may be positively and significantly related to diabetes and fasting serum glucose levels.

In the NIOSH study, the relationship between exposure to 2,3,7,8-TCDD and possible alterations in glucose metabolism was assessed using two outcome measures: case definition of diabetes and fasting serum glucose levels. Participants met the case definition of diabetes if their fasting serum glucose level was 7.8 mmol/L or greater on two consecutive occasions (National Diabetes Data Group, 1979) or if they reported a positive history of physician-diagnosed diabetes after the date of first employment at the plant. Based on logistic regression, the risk of diabetes was found to increase by approximately 12% for every 100 pg/g 2,3,7,8-TCDD ($p < 0.001$) (Table 7-27). Adjusted fasting serum glucose levels in workers and referents were also compared in a separate analysis using linear regression. Fasting serum glucose was 10% higher in workers with 1,000 pg/g 2,3,7,8-TCDD than in

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Table 7-27. Multiple Logistic Regression Model for Cases of Diabetes^a for TCP Production Workers

Variable	Beta	Standard error	Odds ratio ^b	p-Value ^c
Intercept ^d	-8.8913	1.7131	--	0.001
Serum TCDD	0.00114	0.0003	1.12	<0.001
Age	0.0776	0.0204	2.17	<0.001
Body mass index	0.0996	0.0370	1.65	0.007
Race 0=other, 1=white	-1.6659	0.4706	0.19	<0.001
Family history of diabetes (0=no, 1=yes)	0.6957	0.3641	2.01	0.056

^aHistory of physician-diagnosed diabetes (diagnosed after exposure to 2,3,7,8-TCDD-contaminated chemicals) or fasting serum glucose level of 7.8 mmol/L on 2 consecutive days.

^bOdds ratios for continuous variables are based on the difference of 100 pg per gram of lipid of TCDD, 10 years of age, and 5 kg/m² body mass index.

^cp-Values are based on Wald Chi-square statistic.

^dNumber of observations = 530.

Source: Sweeney et al., 1992.

workers and referents with 20 pg/g 2,3,7,8-TCDD ($p < 0.001$) (Table 7-28). Yet, based on the magnitude of the regression coefficients, age, and body mass index (kg/m^2), both known risk factors for diabetes appear to have greater influence on the increases in both the risk of diabetes and elevated fasting serum glucose levels than 2,3,7,8-TCDD level.

In the Ranch Hand study, diabetic status was assessed by measuring fasting serum glucose and 2-hour postprandial glucose and by using a case definition of diabetes. Diabetes was defined as having a verified history of diabetes or an oral glucose tolerance test of ≥ 11.1 mmol/L (200 mg/dL) (Roegner et al., 1991). The analyses of all three parameters suggest a consistent association between serum 2,3,7,8-TCDD levels above 33.3 pg/g and an increased risk of diabetes. Adjusted relative risks comparing Ranch Hands with serum 2,3,7,8-TCDD above 33.3 pg/g and the unexposed comparison group were statistically significantly elevated for fasting serum glucose levels and diabetes (glucose, $\text{RR} = 2.95$, $p < 0.001$; diabetes, $\text{RR} = 2.51$, $p < 0.001$) (Table 7-29) and for the 2-hour postprandial glucose test ($\text{RR} = 2.35$, $p < 0.035$). In addition, Ranch Hand personnel meeting the case definition for diabetes were also more likely to have earlier onset of diabetes than the unexposed comparisons (Wolfe et al., 1992a).

7.13.5.1. *Comment*

The limited information on the risk of diabetes or other alterations in glucose metabolism in humans in relation to 2,3,7,8-TCDD is not well addressed by the available animal literature, of which there is very little. The effects of 2,3,7,8-TCDD on glucose metabolism have been evaluated only in a few laboratory studies. Although these studies suggest that 2,3,7,8-TCDD may alter glucose metabolism, for the most part the animal studies do not corroborate the direction of the findings of two cross-sectional medical studies (Wolfe et al., 1992a; Sweeney et al., 1992). Animal studies appear to contradict the human data, suggesting that 2,3,7,8-TCDD lowers glucose levels. Several studies of rats and rhesus monkeys showed consistent decreases in serum glucose levels after daily doses administered over 30 days (Zinkl et al., 1973) or after a single dose of 2,3,7,8-TCDD (McConnell et al., 1978a; Gasiewicz et al., 1980; Schiller et al., 1986; Ebner et al., 1988) (Table 7-30). In one study glucose levels continued to drop up to 3 weeks postexposure (Gorski et al., 1990).

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Table 7-28. Multiple Linear Regression Model for Log Fasting Serum Glucose for TCP Production Workers and Unexposed Referents

Variable	Parameter estimate	Ratio	p-Value
Intercept*	4.2112	0.0548	0.0001
Serum TCDD	0.00008	0.00002	<0.001
Age	0.00288	0.00064	<0.001
Body mass index	0.0066	0.0014	<0.007

Adjusted R^2 = 0.10.

*Number of observations = 508.

Source: Sweeney et al., 1992.

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Table 7-29. Adjusted Relative Risk (RR) for Fasting Serum Glucose Levels, Cases of Diabetes, and Mean 2-Hour Postprandial Glucose Levels by Category of Lipid Adjusted Serum 2,3,7,8-TCDD in Ranch Hands

Category serum 2,3,7,8-TCDD (pg/g)	Fasting serum glucose (RR)	Diabetes (RR) ^a	2-Hour postprandial glucose level (RR) ^b
Unknown $\leq 10^c$	0.66	0.82	0.88
Low 15- $\leq 33.3^c$	1.18	1.01	0.60
High $> 33.3^c$	2.95 ^d	2.51 ^d	2.35 ^e

^aDefined as having a verified history of diabetes or 2-hour postprandial glucose level of ≥ 11.1 mmol/L (200 mg/dL).

^bComparison of diabetics and normals.

^cSerum 2,3,7,8-TCDD levels in pg/g of lipid.

^d $p < 0.001$.

^e $p = 0.035$.

Source: Roegner et al., 1991.

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Table 7-30. Effects of Exposure to 2,3,7,8-TCDD on Serum Glucose Levels in Nonhuman Mammalian Species

Author	Species	Route	Dose (μ /kg)	Duration	Percentage of serum glucose levels in control animals ^a
Zinkl et al., 1993	CD rat	oral	0.1 1.0 10.1	1x/day for 30 days	91 ^b 71 51
Gasiewicz et al., 1980	Rats	ip (TPN) ip (chowfed)	100 ^c	1 time	29 ^d 51 ^d
Schiller et al., 1986	Fischer rat	gavage	30 60 90 180 ^c 270 ^c 360 ^c	1 time	74 ^{d,e} 54 ^{d,e} 30 ^{d,e} 43 ^{d,e} 38 ^{d,e} 39 ^{d,e}
Gorski et al., 1990	Sprague-Dawley rat	ip	125 ^c	1 time	day 4 75 ^{b,d} day 8 67 ^{b,d} day 16 50 ^{b,d} day 21 31 ^{b,d}
McConnell et al., 1978a	Rhesus monkey	gavage	70 350 ^c	1 time	(decreased) ^{b,d}
DeCaprio et al., 1986	Hartley guinea pig	oral	0.01 0.06 0.44	90 days	NC ^{b,e,f} NC ^{b,e,f} NC ^{b,e,f}
Ebner et al., 1988	New Zealand rabbits	ip	1 50	1 time	NC 15 min NC 1 hour 125 ^{d,e} 2 day 87 10 day 80 ^{d,e}

^aRelative to control values.

^bFemale animals.

^cLethal dose.

^dSignificantly different from controls.

^eMale animals.

^fData not displayed.

ip = intraperitoneal.

TPN = total parenteral nutrition.

NC = no change.

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Dose-related decreases were also noted in CD rats fed 0.1, 1.0, or 10.0 $\mu\text{g/kg}$ daily for 30 days (Zinkl et al., 1973). In contrast, lower daily doses of approximately 0.004 $\mu\text{g/kg/day}$ administered to guinea pigs over a 90-day period produced no significant changes in either glucose or insulin levels (DeCaprio et al., 1986). The results of the animal studies suggest that glucose levels are altered, generally decreased, by short-term, high-dose exposure to 2,3,7,8-TCDD but that the response may be species specific. The diametrically opposed findings of both human and most animal studies may, hypothetically, be due to a number of factors, for example, the species studied, the length of the exposure and short observation periods, the rate of insulin metabolism after 2,3,7,8-TCDD exposure, possible differential effects of 2,3,7,8-TCDD on the various types of islet cells, and the high, usually single, 2,3,7,8-TCDD dose.

The outcome measures used in the NIOSH and the Ranch Hand studies, case of diabetes and fasting serum glucose, did not permit a determination of the type of diabetes involved. However, both studies show that highly 2,3,7,8-TCDD-exposed individuals may be at a slightly greater risk for developing diabetes than individuals with background levels of 2,3,7,8-TCDD. They also experience a greater prevalence of elevated fasting glucose levels, which may be a precursor to conversion to a diabetic state. However, in the NIOSH study, the traditional risk factors for diabetes--age, body mass index or weight, and family history of diabetes--appear substantially more influential than 2,3,7,8-TCDD in the development of diabetes.

7.13.6. Immunologic Effects

Animal toxicologic studies have demonstrated numerous immunologic effects after exposure to 2,3,7,8-TCDD (see Chapter 4 for a more comprehensive review). In humans, the information with which to assess the immunologic consequences of exposure is sparse. Six epidemiologic studies and one case report have described the immunologic function of populations exposed to 2,3,7,8-TCDD (Evans et al., 1988; Hoffman et al., 1986; Webb et al., 1989; Centers for Disease Control Vietnam Experience Study, 1988a; Roegner et al., 1991; Jennings et al., 1988; Reggiani, 1978; Ott et al., 1993b) and as has one study of extruder personnel exposed to brominated dioxins and furans (Zober et al., 1992).

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Evaluation of the immunologic status in exposed residential populations has not found a relationship between exposure and impaired status. Immunocompetence was tested twice in 44 children who were residents of the region of Seveso with the highest 2,3,7,8-TCDD contamination and in 43 age-matched children who did not reside in the contaminated area (Reggiani, 1978). Twenty of the exposed children had chloracne and 24 had no skin lesions. The tests included serum immunoglobulins, complement levels, lymphocyte subpopulations, and lymphocyte activity analysis. Although no data were presented, the authors reported that the various measures were within normal range and that there was no difference between the two groups.

Initial studies of Missouri residents who had the potential for exposure to 2,3,7,8-TCDD-contaminated soil suggested that 2,3,7,8-TCDD caused depression in cell-mediated immunity (delayed hypersensitivity), as demonstrated by a statistically significant increase in anergy (exposed vs. nonexposed: 11.8% vs. 1.1%) (Hoffman et al., 1986). This study, however, was limited by the exclusion from test results of 61% and 32% of the exposed and unexposed groups, respectively. A follow-up study confirmed the presence of substantial bias in the first study. Evans et al. (1988) retested 28 of the 50 exposed residents and 15 of the 27 unexposed residents who did not respond (anergic) or responded weakly (relatively weakly) to an antigen challenge in the first study. A follow-up study could not confirm the presence of anergy. Both studies found in the exposed residents increased frequencies in CD4/CD8 ratio of less than 1.0 (Table 7-31). No other abnormalities were noted by Hoffman et al. (1986) (Tables 7-32 to 7-37).

In a later study of Missouri residents, Webb et al. (1989) found no clinical evidence of immunosuppression in 40 individuals whose adipose 2,3,7,8-TCDD levels ranged from under 20 pg/g to over 430 pg/g (top of range not given). Tests included serum immunoglobulins, T-cell surface markers OKT3, OKT4, OKT8, OKT11, Leu11c, CD4/CD8 ratio, (CD4 + CD8)/CD3, and B1 and B2 cells. In logistic regression, significant ($p < 0.05$) relationships were noted for IgG, %CD3, %T11, %CD8, and %CD4 + LEU8 POS, controlling for age and sex (Tables 7-32 to 7-37).

The effect of past occupational exposure on immunologic function was examined in 18 British workers who were evaluated 17 years after accidental industrial exposure to

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Table 7-31. CD4/CD8 Ratios in Missouri Residents, Vietnam Veterans, and BASF Accident Cohort

Author	Population	Exposed			Unexposed		
		N	Mean level (SD)		N	Mean level	
Roegner et al., 1991	U.S. Air Force Ranch Hand personnel						
	Unknown $\leq 10^a$	126	1.72	---	301	1.89	---
	Low 15- $\leq 33.3^a$	72	1.91				
	High $> 33.3^a$	72	1.99				
Centers for Disease Control Vietnam Experience Study, 1988a	U.S. Army Vietnam veterans	2,490	1.8 ^b	OR < Reference Range 0.9 OR > Reference Range 1.1	1,972	1.8 ^b	---
Hoffman et al., 1986	Missouri residents	135	1.9 (0.8)	% Abnormal 8.2	142	1.9 (0.6)	% Abnormal 6.3
Webb et al., 1989	Missouri residents						
	<20 ^c	16	2.0 (0.7)	---	---	---	---
	20-60 ^c	12	2.1 (1.0)				
	>60 ^c	12	1.4 (0.7)				
Ott et al., 1993b	BASF accident cohort	132	1.6 (0.94)	---	42 ^d	1.5 (0.6)	---
Zober et al., 1992	BASF personnel exposed to TBDD and TBDF ^e	21	1.6 (0.5)	---	42	1.5 (0.6)	---

^aSerum 2,3,7,8-TCDD level in pg/g of lipid.

^bGeometric mean.

^cAdipose 2,3,7,8-TCDD level in pg/g of lipid.

^dFrom Zober et al., 1992.

^eTBDD = 2,3,7,8-tetrabrominated dibenzo-*p*-dioxins and TBDF = 2,3,7,8-tetrabrominated dibenzofurans.

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Table 7-32. Total Lymphocytes in 2,4,5-T Production Workers, Missouri Residents, Vietnam Veterans, and BASF Accident Cohort

Author	Population	Exposed			Unexposed		
		N	Mean level ^a (SD) ^b		N	Mean level (SD)	
Roegner et al., 1991	U.S. Air Force Ranch Hand personnel						
	Unknown $\leq 10^\circ$	127	1,954 --- ^d	---	301	1,972 ---	
	Low 15- $\leq 33.3^\circ$	73	2,011 ---				
	High $> 33.3^\circ$	74	2,032 ---				
Centers for Disease Control Vietnam Experience Study, 1988a	U.S. Army Vietnam veterans	2,490	1,973 ---	OR < Reference Range 1.0 OR > Reference Range 1.2	1,972	1,936 ---	
Hoffman et al., 1986	Missouri residents	135	2,465 (724)	---	142	2,311(634)	
Webb et al., 1989	Missouri residents			% Lymphocytes			
	<20°	16	2,200 (830)	32			
	20-60°	12	2,300 (600)	32	---	---	
	>60°	12	2,200 (720)	28			
Jennings et al., 1988	2,4,5-T production workers exposed 17 yrs prior to the study	18	1,980 (840)	---	15	2,020 (470)	

^aUnits: counts/mm³.

^bSD = standard deviation.

^cSerum 2,3,7,8-TCDD level in pg/g of lipid.

^d--- = data not presented.

^eAdipose 2,3,7,8-TCDD level in pg/g of lipid.

^fFrom Zober et al., 1992.

^gTBDD = 2,3,7,8-Tetrabrominated dibenzo-*p*-dioxins;

TBDF = 2,3,7,8-Tetrabrominated dibenzofurans.

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Table 7-32. (continued)

Author	Population	Exposed			Unexposed		
		N	Mean level ^a (SD) ^b		N	Mean level (SD)	
Ott et al., 1993b	BASF accident cohort	133	1,978.3 (805)	% Lymphocytes 33.4 (9.4)	42	2,267.6 (837.5)	% Lymphocytes 36 (12.4)
Zober et al., 1992	BASF personnel exposed to TBDD ^a and TBDF ^a	21	2,179.5 (678)	% Lymphocytes 33.4 (8.4)	42	2,267.6 (837.5)	% Lymphocytes 36 (12.4)

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Table 7-33. B1 Levels in Production Workers, 2,4,5-T Missouri Residents, Vietnam Veterans, BASF Accident Cohort, and Extruder Personnel

Author	Population	Exposed			Unexposed		
		N	Mean level ^a (SD)		N	Mean level ^a (SD)	
Roegner et al., 1991	U.S. Air Force Ranch Hand personnel						
	Unknown $\leq 10^b$	127	176 --		301	172 --	
	Low $15 \leq 33.3^b$	71	183 --				
	High $> 33.3^b$	73	191 --				
Centers for Disease Control Vietnam Experience Study, 1988a	U.S. Army Vietnam veterans	2,490	240 ^c	OR < Reference Range 1.1 OR > Reference Range 1.2	1,972	230 ^c --	
Webb et al., 1989	Missouri residents < 20 ^d	16	190 (865)	% B1 cells 9.1	--	--	
	20-60 ^d	12	189 (983)	8.3			
	> 60 ^d	12	171 (573)	7.8			
Jennings et al., 1988	2,4,5-T production workers exposed 17 yrs prior to the study	18	210 (110)	--	15	160 (80)	

^aUnits: cells/mm³.

^bSerum 2,3,7,8-TCDD level in pg/g of lipid.

^cGeometric mean.

^dAdipose 2,3,7,8-TCDD level in pg/g lipid.

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Table 7-33. (continued)

Author	Population	Exposed			Unexposed		
		N	Mean level ^a (SD)		N	Mean level ^a (SD)	
Ott et al., 1993b	BASF accident cohort	133	10.4 ^a (6.0)	---	42	12.3 ^{a,f} (5.1)	---
Zober et al., 1992	BASF personnel exposed to TBDD ^g and TBDF ^g	21	276.3 (156)	% B cells 12.5 (4.4)	42	286.4 (199.3)	12.3 (5.1)

^a% B1 cells.

^fFrom Zober et al., 1992.

^gTBDD = 2,3,7,8-tetrabrominated dibenzo-*p*-dioxin

TBDF = 2,3,7,8-tetrabrominated dibenzofuran.

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Table 7-34. CD4 Levels in Production Workers, Missouri Residents, Vietnam Veterans, and BASF Chemical Workers

Author	Population	Exposed			Unexposed		
		N	Mean level ^a (SD)		N	Mean level (SD)	
Roegner et al., 1991	U.S. Air Force Ranch Hand personnel						
	Unknown $\leq 10^b$	127	867 --		301	907 --	--
	Low $15 \leq 33.3^b$	72	945 --				
	High $> 33.3^b$	72	929 --				
Centers for Disease Control Vietnam Experience Study, 1988a	U.S. Army Vietnam veterans	2,490	1,020 ^c --	OR < Reference Range 1.0 OR > Reference Range 1.4	1,972	990 ^c --	--
Hoffman et al., 1986	Missouri residents	135	1,021 (353)	% Abnormal 0.7	142	1,033 (346)	% Abnormal 0.0
Webb et al., 1989	Missouri residents			% T4 cells			
	< 20 ^d	16	1,084 (485)	48	--	--	--
	20-60 ^d	12	1,198 (391)	51			
	> 60 ^d	12	963 (403)	42			

^aUnits: cells/mm³.

^bSerum 2,3,7,8-TCDD level in pg/g of lipid.

^cGeometric mean.

^dAdipose 2,3,7,8-TCDD level in pg/g of lipid.

Table 7-34. (continued)

Author	Population	Exposed			Unexposed		
		N	Mean level* (SD)		N	Mean level (SD)	
Jennings et al., 1988	2,4,5-T production workers exposed 17 yrs prior to the study	18	950 (340)	---	15	1,040 (290)	---
Ott et al., 1993b	BASF chemical workers	133	---	% T4 cells 42.5 (10.4)	42*	---	% T4 cells 45.1 (8.9)
Zober et al., 1992	BASF personnel exposed to TBDD ^f and TBDF ^f	21	973.3 (381.9)	% CD4 44.8 (6.1)	42	1,032 (444.6)	% CD4 45.1 (8.9)

*From Zober et al., 1992.

^fTBDD = 2,3,7,8-Tetrabrominated dibenzo-*p*-dioxins;

TBDF = 2,3,7,8-Tetrabrominated dibenzofurans.

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Table 7-35. CD8 Levels in 2,4,5-T Production Workers, Missouri Residents, Vietnam Veterans, and BASF Accident Cohort

Author	Population	Exposed			Unexposed		
		N	Mean level ^a (SD)		N	Mean level ^a (SD)	
Roegner et al., 1991	U.S. Air Force Ranch Hand personnel						
	Unknown $\leq 10^b$	126	485 ---		301	473 ---	
	Low 15- $\leq 33.3^b$	71	465 ---				
	High $> 33.3^b$	73	475 ---				
Centers for Disease Control Vietnam Experience Study, 1988a	U.S. Army Vietnam veterans	2,490	560 ^c ---	OR < Reference Range 1.0 OR > Reference Range 0.9	1,972	550 ---	
Hoffman et al., 1986	Missouri residents	135	592 (223)	% Abnormal 1.5	142	578 (198)	% Abnormal 0.0
Webb et al., 1989	Missouri residents			% T8 cells			
	< 20 ^d	16	562 (215) ^e	26			
	20-60 ^d	12	645 (225)	28			
	> 60 ^d	12	807 (381)	35			

^aUnits: cells/mm³.

^bSerum 2,3,7,8-TCDD level in pg/g of lipid.

^cGeometric mean.

^dAdipose tissue 2,3,7,8-TCDD level in pg/g of lipid.

^ep < 0.05.

Table 7-35. (continued)

Author	Population	Exposed			Unexposed		
		N	Mean level ^a (SD)		N	Mean level ^a (SD)	
Jennings et al., 1988	2,4,5-T production workers exposed 17 yrs prior to the study	18	630 (280)	---	15	590 (230)	---
Ott et al., 1993b	BASF accident cohort	132	---	% T8 cells 31.9 (10.4)	42 ^f	---	% T8 cells 32.0 (7.1)
Zober et al., 1992	BASF personnel exposed to TBDD ^g and TBDF ^g	21	665 (282.6)	% CD8 30.8 (7.9)	42	717.2 (282.4)	% CD8 32.0 (7.1)

^fFrom Zober et al., 1992.^gTBDD = 2,3,7,8-Tetrabrominated dibenzo-*p*-dioxins;

TBDF = 2,3,7,8-Tetrabrominated dibenzofurans.

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Table 7-36. IgG Levels in Missouri Residents, Vietnam Veterans, and BASF Accident Cohort

Author	Population	Exposed			Unexposed	
		N	Mean level ^a (SD)		N	Mean level ^a (SD)
Roegner et al., 1991	U.S. Air Force Ranch Hand personnel					
	Unknown $\leq 10^b$	335	1,087 ---	---	757	1,120
	Low 15- $\leq 33.3^b$	190	1,122 ---			
	High $> 33.3^b$	175	1,122 ---			
Centers for Disease Control Vietnam Experience Study, 1988a	U.S. Army Vietnam veterans	2,490	1,078 ^c	OR < Reference Range 1.0 OR > Reference Range 1.0	1,972	1,077 ^c
Webb et al., 1989	Missouri residents					
	< 20 ^d	16	1,064 ^e (273)	---	---	---
	20-60 ^d	12	1,146 (193)			
	> 60 ^d	12	1,151 (223)			
Ott et al., 1993b	BASF accident cohort	132	1,199 ^f (226)	---	194	1,182.6 (310.0)
Zober et al., 1992	BASF personnel exposed to TBDD ^g and TBDF ^g	21	1,057.7 (199.0)	---	42	1,102.9 (207.1)

^aUnits: mg/dl.

^bSerum 2,3,7,8-TCDD level in pg/g of lipid.

^cGeometric mean.

^dAdipose 2,3,7,8-TCDD level in pg/g of lipid.

^ep < 0.05 trend.

^fSignificant positive relation between IgG and current 2,3,7,8-TCDD level and back-extrapolated 2,3,7,8-TCDD level (p < 0.01).

^gTBDD = 2,3,7,8-Tetrabrominated dibenzo-*p*-dioxins;

TBDF = 2,3,7,8-Tetrabrominated dibenzofurans.

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Table 7-37. IgM Levels in Missouri Residents, Vietnam Veterans, BASF Accident Cohort, and Extruder Personnel

Author	Population	Exposed			Unexposed	
		N	Mean level ^a (SD)		N	Mean level ^a (SD)
Roegner et al., 1991	U.S. Air Force Ranch Hand personnel				757	103
	Unknown $\leq 10^b$	335	107 ---			
	Low $15 \leq 33.3^b$	190	96 ---			
	High $> 33.3^b$	175	106 ---			
Centers for Disease Control Vietnam Experience Study, 1988a	U.S. Army Vietnam veterans	2,490	121 ^c ---	OR < Reference Range 1.0 OR > Reference Range 1.0	1,972	121 ^c
Webb et al., 1989	Missouri residents					
	< 20 ^d	16	128 (89)	---	---	---
	20-60 ^d	12	157 (57)			
	> 60 ^d	12	114 (44)			
Ott et al., 1993b	BASF accident cohort	132	139.6 (65.1)	---	192	134.7 (70)
Zober et al., 1992	Personnel exposed to TBDD ^e and TBDF ^e	21	142 ^f (52.6)	---	42	114.7 ^f (46.5)

^aUnits: mg/dl.

^bSerum 2,3,7,8-TCDD level in pg/g of lipid.

^cGeometric mean.

^dAdipose 2,3,7,8-TCDD level in pg/g of lipid.

^eTBDD = 2,3,7,8-Tetrabrominated dibenzo-*p*-dioxins;

TBDF = 2,3,7,8-Tetrabrominated dibenzofurans.

^fp=0.04.

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chemicals contaminated with 2,3,7,8-TCDD (Jennings et al., 1988). It is not clear from the article when occupational exposure to 2,3,7,8-TCDD ended for these workers. Exposed workers and unexposed controls were matched for age, race, sex, smoking habit, alcohol consumption, and percent of ideal body weight. There were no significant differences in the levels of immunoglobulins, T and B lymphocytes, responsiveness to phytohemagglutinin A, and in the number of CD4 and CD8 counts. Three measures were found to be statistically significantly ($p < 0.05$) higher in workers than in controls: antinuclear antibodies (ANA) (8 workers vs. 0 controls, $p < 0.01$) (when Hep2 cells were used as substrate but not when rat liver cells were used), immune complexes (workers = 11 vs. 3 controls, $p < 0.05$), and natural killer cells (NK) (workers = $0.21 \times 10^6/L$ vs. controls = $0.59 \times 10^6/L$, $p < 0.002$) identified by the monoclonal antibody Leu-7 (Table 7-38). No other well-conducted studies of exposed individuals have measured these parameters. In the discussion, the authors could not explain the physiologic basis of their findings and suggested that further research was needed.

Among participants in the BASF accident study cohort, with the exception of natural killer cells and helper-inducer cells, the proportions of some lymphocyte populations (B cells, T cells, T helper cells, T suppressor cells) were lower among workers, but the distribution of cells in referents and workers was equivalent (Ott et al., 1993b). Levels for IgA, IgG, IgM, and complement C4 and C3 were slightly higher in workers than in the unexposed referent population. There also appeared to be slight dose-related increases in IgA, IgG, and complement C4 with 2,3,7,8-TCDD levels measured in October 1988 and February 1992. IgA and IgG were related to the half-life extrapolated 2,3,7,8-TCDD levels. It must be noted, however, that the statistically significant relationship between IgA and 2,3,7,8-TCDD is most likely due to a case of liver cirrhosis and the association with IgG due to a liver carcinoma.

Among the 21 extruder personnel exposed to both 2,3,7,8-TBDD and 2,3,7,8-TBDF, of the 16 parameters tested, only complement C4 was statistically significantly ($p < 0.01$) associated with concentrations of 2,3,7,8-TBDD and 2,3,7,8-TBDF (Zober et al., 1992). Borderline associations were noted between 2,3,7,8-TBDF and decreases in total lymphocyte count ($p = 0.056$), T-cell count ($p = 0.045$), T-helper cell count (CD4) ($p = 0.045$) and an

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Table 7-38. Levels of Natural Killer Cells in Missouri Residents, Vietnam Veterans, and Extruder Personnel

Author	Population	Exposed			Unexposed	
		N	Mean level		N	Mean level
Roegner et al., 1991	U.S. Air Force Ranch Hand personnel					
	Unknown $\leq 10^a$	126	455 ^{b,c}		291	414 ^b
	Low 15- $\leq 33.3^a$	70	378			
	High $> 33.3^a$	72	386			
Jennings et al., 1988	2,4,5-T production workers exposed 17 yrs prior to the study	18	400 ^e (210) ^d		15	590 ^e (230)
Zober et al., 1992	BASF personnel exposed to TBDD ^f and TBDF ^f	21	280.5 (175.2)		42	250.8 (149.9)

^aSerum 2,3,7,8-TCDD level in pg/g of lipid.

^bUnits: cpm.

^cNet response.

^d $p < 0.05$.

^eUnits: $10^3/\text{mm}^3$.

^fTBDD = 2,3,7,8-Tetrabrominated dibenzo-*p*-dioxins;

TBDF = 2,3,7,8-Tetrabrominated dibenzofurans.

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increase in complement C3 (0.054), and between 2,3,7,8-TBDD and a decreased percent lymphocyte count ($p=0.054$). However, with the exception of complement C3, the associations appear to be driven by a single individual with the highest 2,3,7,8-TBDD levels (478 pg/g of lipid) who, at the time of the study, exhibited no evidence of clinical immunodeficiency.

Two studies extensively evaluated immunologic function in Vietnam veterans. No significant differences were noted among U.S. Army ground troops and the comparison population in lymphocyte subset populations, T-cell populations, or serum immunoglobulins (Tables 7-31 to 7-37) (Centers for Disease Control Vietnam Experience Study, 1988a). Comprehensive immunologic profiles were developed for each participant of the U.S. Air Force Ranch Hand Study (Tables 7-31 to 7-38) (Roegner et al., 1991). Significant positive associations were found between IgA and serum 2,3,7,8-TCDD. The authors suggest that the rise in IgA is consistent with a subclinical inflammatory response, but the authors could not explain the source of the inflammatory response.

7.13.6.1. *Comment*

The information on immunologic function in humans, children or adults, relative to exposure to 2,3,7,8-TCDD is scarce. The available epidemiologic studies are restricted to adults and do not describe a consistent pattern of effects among the studies. Natural killer cells (NK) were increased among one population of 2,3,7,8-TCDD chemical workers examined 17 years after exposure ended (Jennings et al., 1988). These findings were not corroborated in Ranch Hands (Roegner et al., 1991), the BASF accident cohort (Ott et al., 1993b), or workers exposed to 2,3,7,8-TBDD and TBDF (Zober et al., 1992). Dose-related elevations in IgA were observed in Ranch Hands in relation to current levels and in the BASF accident cohort with respect to both current and half-life extrapolated 2,3,7,8-TCDD levels. Yet, IgA was not higher in adult Missouri residents with adipose 2,3,7,8-TCDD levels above background (Webb et al., 1989). IgG was also significantly related to 2,3,7,8-TCDD in the BASF accident cohort but not in Ranch Hands. While complement C3 and C4 were elevated in both the BASF accident cohort and the extruder personnel, no other study examined these end points.

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The effect of acute, high exposure to 2,3,7,8-TCDD among children from Seveso was reportedly negative within 2 years after exposure (Reggiani, 1978). Although no data have been published illustrating the values obtained from the tests of immunologic function in these children, the author indicates that the measured parameters were no different in the exposed and unexposed children after two series of tests.

More comprehensive evaluations of immunologic function with respect to 2,3,7,8-TCDD exposure are necessary to assess more definitively the relationships observed in nonhuman species. Longitudinal studies of the maturing human immunologic system may provide the greatest insight, particularly since animal studies have found many of their significant results in immature animals and breast milk is a source of 2,3,7,8-TCDD and other related compounds. Additional studies of highly exposed adults may also shed light on the effects of long-term chronic exposures. Therefore, there appears to be too little information to suggest definitively that 2,3,7,8-TCDD, at the levels observed, is a immunotoxin in humans.

7.13.7. Neurologic Effects

Although there are few studies reporting neurologic abnormalities related to 2,3,7,8-TCDD exposure in adult animal models, neurologic effects are reported to have occurred shortly after exposure in occupationally exposed individuals (Ashe and Suskind, 1950; Baader and Bauer, 1951; Bauer et al., 1961; Goldman, 1972; Jirasek et al., 1974; Oliver, 1975; Pocchiari et al., 1979) (Table 7-39) and in Seveso residents (Filippini et al., 1981) (Table 7-40). Previous case reports and studies found symptoms referable to the central (CNS) and peripheral (PNS) nervous system among workers and community residents exposed to 2,3,7,8-TCDD-contaminated materials. While human studies reveal a wide spectrum of effects due to 2,3,7,8-TCDD (Sweeney et al., 1989), very few toxicologic studies have focused on the nervous system. Singer et al. (1982) reviewed the following animal studies, which examined the relationship of CNS dysfunction and 2,3,7,8-TCDD exposure: Elovaara et al. (1977) found anomalous CNS function in some rats exposed to a single dose of 2,3,7,8-TCDD, and Creso et al. (1978) reported the CNS symptoms of irritability, restlessness, and increased aggression in rats administered 2,3,7,8-TCDD. No

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Table 7-39. Case Reports of Psychological and Neurologic Effects Among Individuals Exposed to 2,3,7,8-TCDD-Contaminated Materials

Author	Exposure	Population	Evaluation	Findings
Ashe and Suskind, 1950 West Virginia	Precursor and reaction products of TCP process TCP, ^a NaOH, ^b 2,4,5-T ^c	4 employees involved in cleanup after 1949 explosion or worked with equipment used prior to explosion; studied 6 mos and 1 yr after explosion	Clinical history and examination	Headaches Insomnia Social withdrawal Nervousness Fatigue Crying spells Numbness in feet Decreased libido, muscle strength, sensory ability, myelin sheath and fiber destruction of sural nerve
Suskind et al., 1953 West Virginia	TCP 2,4,5-T	36 workers with chloracne after TCP reactor explosion 25 persons exposed during production of TCP and 2,4,5-T 1948-1953	Clinical history and examination	Fatigue Nervousness Irritability Reduced libido Back and limb pain Vertigo Paresthesia
Baader and Bauer, 1951 Nordheim- Westfalan, West Germany	PCP ^d TCP	10 men in PCP production experimental TCP production	Record review case history	Self-reported: pain and weakness Paresthesia and pain in gluteal and femoral region

^aTCP = 2,4,5-trichlorophenol.

^bNaOH = sodium hydroxide.

^c2,4,5-T = 2,4,5-trichlorophenoxyacetic acid.

^dPCP = pentachlorophenol.

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Table 7-39. (continued)

Author	Exposure	Population	Evaluation	Findings
Bauer et al., 1961 Hamburg, West Germany	TCP 2,4,5-T	31 men in TCP and 2,4,5-T production with residual chloracne, continual "neuromuscular weakness," vasovagal disturbances, psychopathological disturbances" 5 yrs after first exam	Clinical history and examination Hamburg-Wechsler Test Rorschach Psychogram	Reduced libido Headaches, dizziness Decreased libido Irritability Depression Sleep disturbances Anorexia Paresthesia Tremor Muscle weakness Hamburg-Wechsler indicative of acquired decrease in mental efficiency Rorschach Psychogram indicative of decreased emotional reactivity, slowed thinking process for concentration
Goldman, 1972 Ludwigshafen, West Germany	TCP 2,4,5-T	42 BASF workers with chloracne after TCP reactor explosion in 1953	Clinical history and examination	Neurasthenia

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Table 7-39. (continued)

Author	Exposure	Population	Evaluation	Findings
Jirasek et al., 1974 Spolano, Czechoslovakia	TCP 2,4,5-T PCP, HCB*	55 workers in TCP and 2,4,5-T production Followed: 1969-1973	Clinical examination history and examination	35 patients with "neurasthenic or depressive syndrome"
Oliver, 1975 England	Pure 2,3,7,8-TCDD	3 scientists synthesis of 2,3,7,8-TCDD	Not described	Subject A: Fatigue and headache Subject B: Headaches Loss of vigor and fatigue Irritability and anger Poor concentration Subject C: Loss of concentration (apathy and fatigue) "Loss of energy and drive" Some difficulty sleeping

*HCB = hexachlorobenzene.

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Table 7-39. (continued)

Author	Exposure	Population	Evaluation	Findings
Pazderova-Vejlupkova et al., 1981 Spolano, Czechoslovakia	TCP 2,4,5-T PCP, HCB	4-year follow-up of 44 production workers	Clinical psychiatric examination	Psychiatric changes at start of "intoxication" 83 % with neurotic symptoms; neurasthenia syndromes with depressive component; depressive syndrome with endogenous component (N=55) 16 % with pseudoneurasthenia syndrome and CNS arteriosclerosis 3 % with no psychiatric signs or symptoms Psychiatric changes in exposed individuals at follow-up (1973-1979) 58 % with neurotic symptoms and no depressive or anxiety symptoms 18 % with severe pseudoneurasthenia and signs of dementia (usually in patients over 50 years old but dementia occurred in 30-year-old patient)

7-170

05/13/94

DRAFT--DO NOT QUOTE OR CITE

Table 7-39. (continued)

Author	Exposure	Population	Evaluation	Findings
Pazderova-Vejlupkova et al., 1981 (contd.)				24 % with no psychiatric signs or symptoms.
Kimmig and Schultz, 1957b Hamburg, Germany	TCP 2,4,5-T	31 production workers with chloracne (1953-1954)	Clinical history and examination	Tiredness (N=3) Headaches (N=5)
Poland et al., 1971 New Jersey	TCP 2,4,5-T 2,4-D ^f HCB TCP reactor explosion in 1960 Daily exposure 1951-1969	73 male workers in production, maintenance, office areas	Noted histories of smoking, alcohol, medications MMPI ^g given to 52 production and 17 unexposed administrative staff	Headaches (N=8) Severity of acne significantly correlated with high score on the mania scale of MMPI.

^f2,4-D = 2,4-dichlorophenoxyacetic acid.

^gMinnesota Multiphasic Personality Inventory.

7-171

05/13/94

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Table 7-40. Cross-Sectional Studies of Psychological and Neurologic Effects Among Residents of Missouri and Seveso Exposed to 2,3,7,8-TCDD-Contaminated Materials

Author	Exposure	Study population	Comparison population	Evaluation	Findings
Webb et al., 1989 Missouri	2,3,7,8-TCDD-contaminated waste oil 1971	68 volunteers in State Dioxin Registry; estimated exposure to 20-100 ppb TCDD for 2 yrs or 100 ppb for 6 months	36 volunteers in State Dioxin Registry with no history of exposure to 2,3,7,8-TCDD	Missouri Dioxin Health Studies. ^a Progress Report (1983)	No differences in neurologic exam or in feeling of pins-needles; loss of sensation in extremities, tingling in fingers and toes, diminished VIB 256, diminished VIB 64, diminished pin sensation, diminished thermal sensation (PRR=2%) ^a
Pocchiari et al., 1979 Seveso, Italy	TCP TCP reactor accident July 1976	446 residents of Seveso and Meda, Italy 200 workers from Icmesa plant	255 residents of nearby towns without contamination None	Not detailed but included clinical exam and NCV ^b Exam, EMG ^c , NCV	1977: neurologic damage in 6.7% of Seveso residents and in 1.2% unexposed residents 1978: neurologic damage in 11.7% of Seveso residents workers: 4% neuropathic clinical signs exam, EMG, NCV

^aPrevalence risk ratio.

^bNerve conduction velocity.

^cElectromyography.

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Table 7-40. (continued)

Author	Exposure	Study population	Comparison population	Evaluation	Findings
Filippini et al., 1981 Seveso, Italy	See description above	308 residents of Seveso, Italy	305 residents of nearby towns without contamination	Symptoms: pain, tingling, numbness Sensory review Muscle tone and strength NCV of ulnar and peroneal nerves	Elevated PRR for peripheral neuropathy in Seveso residents with indicators of exposure (high GGT, ALT, AST, or chloracne) (PRR=2.8, 95% CI=1.2-6.5) Seveso residents with predisposing factors (alcohol or inflammatory disease) (PRR=2.6, 95% CI=1.2-5.6)

7-173

05/13/94

DRAFT--DO NOT QUOTE OR CITE

animal studies have specifically evaluated peripheral or autonomic functions related to 2,3,7,8-TCDD exposure.

7.13.7.1. *Neurobehavioral Assessments*

Numerous case reports cite symptoms referable to the nervous system occurring after acute exposure among occupationally exposed individuals (Creso et al., 1978; Ashe and Suskind, 1950; Suskind et al., 1953; Goldman, 1972), as well as chronic exposure to 2,3,7,8-TCDD-contaminated materials (Oliver, 1975; Baader and Bauer, 1951; Kimmig and Schulz, 1957a, b; Bauer et al., 1961; Poland et al., 1971). Symptoms include headache (Ashe and Suskind, 1950; Bauer et al., 1961; Jirasek et al., 1974; Oliver, 1975; Kimmig and Schulz, 1957a, b; Poland et al., 1971), insomnia (Ashe and Suskind, 1950; Suskind et al., 1953; Oliver, 1975; Kimmig and Schulz, 1957a, b), nervousness or irritability (Ashe and Suskind, 1950; Suskind et al., 1953; Bauer et al., 1961; Oliver, 1975), depression and anxiety (Bauer et al., 1961; Jirasek et al., 1974), loss of libido, and encephalopathy (Jirasek et al., 1974; Kimmig and Schulz, 1957a) (Table 7-39).

Some reports indicate that symptoms referable to the CNS and PNS may persist in some exposed individuals for as long as 25 years (Suskind et al., 1953; Poland et al., 1971; Jirasek et al., 1973; Jirasek et al., 1974; Creso et al., 1978; Ashe and Suskind, 1950; Suskind et al., 1953). In 1953, Suskind et al. reported a variety of CNS-related symptoms in 36 workers from a plant in Nitro, West Virginia, who had developed chloracne and other symptoms after exposure to contaminants subsequent to a TCP reactor explosion in March 1949 (N=11) or during normal production process of TCP and 2,4,5-T (N=25) between 1948 and 1953 (Suskind et al., 1953). Such symptoms reported among this relatively young group (average age = 36 years, range = 22-63 years) included fatigue (N=21), nervousness and irritability (N=17), and decreased libido (N=13). No attempt was made to determine whether these symptoms also occurred among exposed individuals without chloracne or among the nonexposed plant population.

Between 1968 and 1969, Jirasek et al. (1974) "observed very closely" (by clinical evaluation) a group of 55 workers exposed to 2,3,7,8-TCDD in the production of 2,4,5-T, hexachlorobenzene, and pentachlorophenol. As described in the report, intoxication with

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2,3,7,8-TCDD "occurred gradually from 1965 to 1968" although further quantification of exposure is not described. Psychiatric examination revealed the following: severe neurotic symptoms (64%), neurasthenia syndrome with depressive component (11%), depressive syndromes (8%), pseudoneurasthenia syndromes in patients with arteriosclerosis of the CNS (14%), and normal psychiatric examination (3%). One patient died at age 57 years with rapidly progressive dementia secondary to an atypical arteriosclerosis involving brain and other organs.

Ten years after the initial examination, Pazderova-Vejlupkova et al. (1981) evaluated the health status of 44 of the original 55 workers. They found the following on psychiatric examination: 58% continued to have neurotic symptoms without depressive or anxiety components, 18% developed severe neurasthenia syndromes with signs of dementia, and 24% were normal.

Poland et al. (1971) administered the Minnesota Multiphasic Personality Inventory (MMPI) to 52 male production workers who were exposed at that time to TCP, 2,4,5-T, 2,4-D, and other chemicals. Severity of acne correlated significantly with a high score on the hypomania scale of the MMPI. When production workers were compared with 17 presumably unexposed administrative workers, the two groups differed on only one MMPI symptom scale; exposed production workers scored higher on the hypochondriasis scale.

Table 7-41 describes the results of neurologic and neurobehavioral assessments of TCP production workers, Ranch Hands, and U.S. Army Vietnam veterans.

Moses et al. (1984) found a significant excess among workers with chloracne compared to those without lesions for the following symptoms: insomnia, decreased libido, and difficulties with ejaculation or erection. There was no difference between the two groups for the symptoms of fatigue, irritability, nervousness, depression, or personality changes.

In the study by Suskind and Hertzberg (1984), psychological symptoms were evaluated by interview and peripheral nerve function by nerve conduction velocity of the peroneal motor and sural sensory nerve fibers. The complaint of loss of libido was more frequent among the exposed than unexposed even after stratification by age. The complaint of nervousness, depression, or anxiety was not significantly related to exposure (16.3% vs. 11.7%) in the crude analysis even though the sample size was sufficient to detect a twofold

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Table 7-41. Cross-Sectional Studies of Psychological and Neurologic Effects Among TCP Production Workers and Vietnam Veterans Exposed to 2,3,7,8-TCDD-Contaminated Materials

Author	Exposure	Study population	Comparison population	Evaluation	Findings
Moses et al., 1984 West Virginia	TCP ^a 2,4,5-T ^b 1949 TCP reactor explosion	226 workers invited based on union record of employment in production of TCP or 2,4,5-T Definition of exposure = "current or history of chloracne"	Workers without chloracne (N=109) Conduction velocities	Review of symptoms (ROS) obtained by examining physician	Significant diff. with and without chloracne for: insomnia; decreased libido, erection, or ejaculation; no change for fatigue, irritability, nervousness, depression, or personality changes. Decreased pin prick sensation in 18.3% with chloracne; no decreased pin prick sensation without chloracne
Suskind and Hertzberg, 1984 West Virginia	TCP 2,4,5-T	204 workers or maintenance workers in TCP or 2,4,5-T dept.	163 workers never in TCP or 2,4,5-T Mean age of exposed population was younger (56.7 vs. 46.2) (p<0.0001).	Interviews and clinical examination	Decreased libido was more frequent among exposed by Mantel-Haenszel Chi by age. Nervousness/depression/anxiety not increased in exposed (sample size sufficient to detect a 2-fold increase)

^aTCP = 2,4,5-trichlorophenol.

^b2,4,5-T = 2,4,5-trichlorophenoxyacetic acid.

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Table 7-41. (continued)

Author	Exposure	Study population	Comparison population	Evaluation	Findings
Sweeney et al., 1993 Alderfer et al., 1992 Missouri and New Jersey	TCP 2,4,5-T	280 production workers with daily exposure New Jersey 1951-1969 Missouri 1968-1972	261 unexposed community residents matched for age, race, gender	Symptom and medical history; neurologic examination; neurophysiologic tests of vibration and thermal sensitivity; Beck Depression Scale; SCL-90-R	No significant difference for symptoms or any examination variable No significant difference in mood disorders
Lathrop et al., 1984	Vietnam service 1962-1971	1,208 Ranch Hands assigned to aerial spraying herbicides and insecticides Republic of Vietnam	1,238 men who flew cargo missions in Southeast Asia Matched by month of birth, race, and occupational code	Self-report of psychological or emotional illness Diagnostic Interview Schedule (modified) Self-reported depression	No difference Significantly more fatigue, anger, erosion, anxiety, closest for high school-educated Ranch Hands; no difference for college-educated Greater for Ranch Hands

7-177

05/13/94

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Table 7-41. (continued)

Author	Exposure	Study population	Comparison population	Evaluation	Findings
Lathrop et al., 1984 (cont.)				Cornell Index (self-administered inventory of neuropsychiatric symptoms) (psychophysiologic)	After adjustment for education 4/10 parameters abnormal (nervousness, anxiety, startle, psychosomatic, gastrointestinal system); abnormal parameters inversely related to education level.
				MMPI	High school-educated Ranch Hands showed significant deficits on scales for hypochondria, masculinity/femininity; mania/hypomania but comparisons show more denial; MMPI scores influenced by education ($p < 0.01$)
				Halstead-Reitan Battery	No impairment in Ranch Hands
				Wechsler Adult Intelligence Scale (WAIS)	Scores related to educational level

7-178

05/13/94

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Table 7-41. (continued)

Author	Exposure	Study population	Comparison population	Evaluation	Findings
Roegner et al., 1991	Vietnam service	720 USAF Ranch Hands; serum 2,3,7,8-TCDD levels were measured (lipid adjusted)	779 who flew cargo missions in Southeast Asia; serum 2,3,7,8-TCDD levels were measured	Self-report SCL-90-R* Cornell Medical Index (CMI) Neurologic exam	No significant mental, emotional, or sleep disorders No significant differences with increasing serum levels Significantly higher mean schizoid and schizotypal scores in Ranch Hands over 33.3 pg/g 2,3,7,8-TCDD, but they did not relate to similar scales in the SCL-90-R Overall, no consistent relationship between neurologic abnormalities and TCDD level.

*SCL-90-R: Symptom Checklist Revised.

7-179

05/13/94

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increase. However, in the group over 50 years old there was a significant exposure effect (19% vs. 6.4%) for the complaint of nervousness, depression, or anxiety. The complaint of impotence was significantly related to exposure in the crude analysis, but after stratification by age this effect disappeared.

The effects of exposure to 2,3,7,8-TCDD on measures of current symptoms of depression were evaluated (Alderfer et al., 1992) as part of the NIOSH cross-sectional medical study (Sweeney et al., 1989). Symptoms of depressed mood were measured by the Beck Depression Inventory and the depression subscale of the Self-Report Symptom Checklist-90-Revised (SCL-90-R). Neither serum 2,3,7,8-TCDD levels nor status as a worker was associated with depressed mood as assessed by either the Beck Depression Inventory or the SCL-90-R depression subscale (Alderfer et al., 1992). This finding supports the conclusion that current serum levels of 2,3,7,8-TCDD are not associated with current depression among a population of workers that was highly exposed to TCDD. However, because this cross-sectional study was conducted many years after 2,3,7,8-TCDD exposure, this analysis could not address the question of whether 2,3,7,8-TCDD is associated with past depression that resolved before the study was performed. These findings are consistent with those of the U.S. Air Force study (Roegner et al., 1991) of personnel who applied Agent Orange during the Vietnam war: serum 2,3,7,8-TCDD was not associated with the depression subscale score of the SCL-90-R after controlling for covariates.

The Air Force study conducted neurologic and psychological assessments of the participating Ranch Hands and comparisons (Lathrop et al., 1984). In the first examination series published in 1984 (baseline), the psychological assessment included a self-report of psychological or emotional illness, the Diagnostic Interview Schedule (DIS), Cornell Medical Index (inventory of psychophysiologic symptoms), MMPI, Halstead-Reitan Battery (HRB), and Wechsler Adult Intelligence Scale (WAIS). In the 1987 follow-up examination, the psychological assessment included an interviewer-administered questionnaire in which each participant was asked about the occurrence of mental or emotional disorders and sleep disorders. The presence of posttraumatic stress disorder was based on a subset of questions from the MMPI; the WAIS IQ assessment was deleted and the Millon Clinical Multiaxial

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Inventory (MCMI) and the Symptom Checklist-90-Revised (SCL-90-R) were added (Lathrop et al., 1987).

Results of the 1984 baseline study revealed no difference between Ranch Hands and the comparison group for self-reported psychological or emotional illness. The DIS revealed significantly more fatigue, anger, erosion, and anxiety for high school-educated but not college-educated Ranch Hands. These outcomes were highly related to education. The Cornell Medical Index found 4 of 10 parameters abnormal for Ranch Hands--startle, psychosomatic, gastrointestinal nervousness, and anxiety. These parameters were inversely related to education level. On the MMPI, high school-educated Ranch Hands showed significant differences (more deficits) on subscales for hypochondria, masculinity/femininity, and mania/hypomania, but comparisons scored higher on the subscale for denial, a finding that might undermine the deficits noted for Ranch Hands. Again, MMPI scores were influenced by education level ($p < 0.01$) but not exposure level. For both the HRB and WAIS, there was no difference between Ranch Hands and comparisons, and the scores were related to education level.

In the 1987 reanalysis with serum 2,3,7,8-TCDD (Roegner et al., 1991), there was no significant difference between groups or relationship with serum 2,3,7,8-TCDD levels on reported (and verified) data on lifetime psychological illness or sleep disorders or any SCL-90-R. Some of the MCMI parameters appeared to be related to serum 2,3,7,8-TCDD levels (significantly higher mean schizoid and schizotypal scores and significantly lower mean histrionic score in the group above 33.3 pg/g than in comparisons). However, these findings were inconsistent with similar variables in the SCL-90-R and the self-reported histories.

Comprehensive neurologic and psychological assessments were conducted on participants of the Vietnam Experience Study (Centers for Disease Control Vietnam Experience Study, 1988a, b). The neurobehavioral tests evaluated aptitude, concept formation and problem-solving, memory, manual dexterity, verbal skills, visuo-motor skills, attention, and mental control. Among Vietnam veterans there was a significantly greater prevalence of alcohol abuse or dependence (Vietnam veterans, 13.7%; non-Vietnam veterans, 9.2%; OR=1.5, 95% CI=1.2-1.8), depression (Vietnam veterans, 4.5%; non-Vietnam veterans, 3.2%; OR=2.0, 95% CI=1.4-2.9), and a higher prevalence of poor psychological

status (Centers for Disease Control Vietnam Experience Study, 1988b). The poor psychological status tended to be most prevalent in Vietnam veterans who were not white, who enlisted before their 19th birthday, and whose enlistment test scores fell below the group median.

In the study of residents of the Quail Run Mobile Home Park, neurobehavioral tests evaluated reaction time, mood, memory, visuo-motor coordination, intelligence, and indicators of psychological stress (Hoffman et al., 1986). Differences between Quail Run residents and controls were observed in the vocabulary subtest of the WAIS (Quail Run = 34.7, controls = 41.1, raw scores; $p < 0.01$), tension/anxiety raw score (Quail Run = 13.7, controls = 11.1; $p < 0.01$), and anger/hostility scale (Quail Run = 11.6, controls = 8.9; $p < 0.05$) of the POMS inventory, and for the depression/dejection and fatigue/inertia scales (no data provided).

7.13.7.2. *Neurologic Status*

On neurologic examination, 11 of 60 West Virginia workers with chloracne exhibited decreased sensitivity to pin prick, whereas none of the 34 subjects without chloracne had decreased pin prick sensation ($p < 0.01$) (Moses et al., 1984). There were no other differences in performance of the neurologic examination noted in the text. When examined by Suskind and Hertzberg (1984), no significant differences were noted in the conduction velocities of either nerve fiber (sural sensory: exposed workers, mean = 42.06 ± 0.49 ; unexposed workers, mean 41.49 ± 0.54 ; peroneal motor: exposed workers, mean = 41.77 ± 0.47 ; unexposed workers, mean = 42.62 ± 0.52).

Among New Jersey and Missouri TCP workers, the overall neurologic status and peripheral nerve function were assessed for all 281 workers and 260 referents by self-reported medical history, neurologic examination, electrophysiologic tests of nerve conduction velocity, amplitude and latency, and vibratory and thermal threshold (Sweeney et al., 1993). No differences in neurologic status or nerve function between workers or referents were detected. Additionally, although the mean serum 2,3,7,8-TCDD level in the workers was 220 pg/g, there was no relationship between neurologic function and levels of serum 2,3,7,8-TCDD.

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The neurologic examination in the 1987 follow-up study evaluated cranial, CNS, and PNS function in participating Ranch Hands (Lathrop et al., 1987). In general, there was no difference in the prevalence of neurologic abnormalities in Ranch Hands and comparisons. However, Ranch Hands with serum 2,3,7,8-TCDD levels above 33.3 pg/g tended to have a higher proportion of individuals with abnormal coordination than the comparisons (Ranch Hands, 2.7%; comparisons, 0.4%; adjusted RR=18.3, $p < 0.001$) (Roegner et al., 1991).

Overall neurologic status of Army Vietnam veterans did not differ from that of non-Vietnam veterans (Vietnam veterans, 1.0%; non-Vietnam veterans, 0.8%; OR=1.2, 95% CI=0.6-2.3) (Centers for Disease Control Vietnam Experience Study, 1988b). Only self-reported symptoms related to nerve disorders were significantly more prevalent among Vietnam veterans than non-Vietnam veterans (Vietnam veterans, 8.2%; non-Vietnam veterans, 6.5%; OR=1.2, 95% CI=1.0-1.6).

Table 7-40 describes the results of neurologic and neurobehavioral studies of Seveso and Missouri residents. While three studies evaluated the neurologic status of residents of Seveso (Pocchiari et al., 1979; Filippini et al., 1981; Assennato et al., 1989), no studies evaluated neurobehavioral changes. In an effort to quantify exposure-related neurologic disorders among Seveso residents and among workers from the Icmesa plant, two government-sponsored screenings were conducted in 1977 and 1978 on 308 residents of Seveso and 200 workers. Among these workers, approximately 4% (N=8) were found to have damage to nerve fibers of multiple (unspecified) nerves, controlling for confounding factors such as alcohol abuse, diabetes, kidney disease, and neurotoxic medication use (Pocchiari et al., 1979). The report did not describe the extent of worker exposure to 2,3,7,8-TCDD. Other potential neurotoxic occupational exposures do not appear to have been considered. Three workers were described as having polyneuropathies of the lower limbs.

In 1981, prevalence risk ratios (PRR) for neuropathy were calculated separately for the 308 Seveso residents (Filippini et al., 1981). PRRs for neuropathy were determined for residents who exhibited clinical indication of 2,3,7,8-TCDD exposure, defined as the presence of elevated liver enzyme levels (GGT, ALT, AST) (which are also indicative of nonspecific insults to the liver) or chloracne, and for those who exhibited conditions that are

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risk factors for neuropathy, e.g., alcoholism, inflammatory disease, diabetes, or potential occupational exposure to neurotoxins. Seveso residents who had clinical indication of 2,3,7,8-TCDD exposure (chloracne, or elevated liver enzymes GGT, AST, ALT) or who had risk factors for neuropathy were found to have significantly greater prevalence of neuropathy than residents without either manifestation (PRR exposure = 2.8, 95% CI=1.2-6.5; PRR [possible 2,3,7,8-TCDD-predisposing factors] = 2.6, 95% CI=1.2-5.6) (Filippini et al., 1981). Additional analysis identified that individuals who met the definition for chloracne or abnormal levels of hepatic enzymes were significantly more at risk than residents without either condition.

Residents of Seveso who developed chloracne after the reactor release (N=193) were invited to a series of three follow-up screenings in 1982-1983, 1983-1984, and 1985 (Assennato et al., 1989). A control group from a nearby but uncontaminated area was also examined. Conduction velocities of the median motor, peroneal motor, and sural sensory fibers were conducted in 1982-1983 and 1985 (Assennato et al., 1989). No increases in the prevalence of abnormal electrophysiologic measures were observed in the chloracne group when compared with controls without chloracne. In addition, there was no change in the conduction velocities for each fiber from the 1982-1983 to 1985 studies.

Quail Run residents reported significantly more "numbness" or "pins and needles" in the hands or feet (28.6%) than the controls (18.1%) ($p < 0.05$), but there were no differences in mean threshold scores for the more objective neurosensory tests. Residents also reported more persistent severe headaches (Quail Run, 26.0%; control, 14.2%; $p < 0.05$).

Participants in the study by Webb et al. (1989) did not complete neurobehavioral tests, but they were examined by a neurologist. The results were unremarkable. Of the 38 participants, two with levels above background had abnormal pin prick sensitivity (≤ 20 pg/g, N=2; 20-60 pg/g, N=1; ≥ 60 pg/g, N=1), three had abnormal vibration thresholds (≤ 20 pg/g, N=2; 20-60 pg/g, N=1; ≥ 60 pg/g, N=2), and four had abnormal reflexes (≤ 20 pg/g, N=2; 20-60 pg/g, N=2; ≥ 60 pg/g, N=2). The results of other components of the neurologic examination were not reported and are assumed to be normal.

7.13.7.3. *Comment*

The overall results of these case reports and epidemiologic studies demonstrate that exposure to 2,3,7,8-TCDD-contaminated materials is associated with symptoms referable to the central and peripheral nervous systems shortly following exposure and, in some cases, lasting many years (Tables 7-40 and 7-41). Symptoms include fatigue, nervousness, anxiety, and decreased libido (Ashe and Suskind, 1950; Suskind et al., 1953; Oliver, 1975; Kimmig and Schulz, 1957a, b; Bauer et al., 1961; Jirasek et al., 1974). One case report found mania/hypomania on the MMPI (Poland et al., 1971). These symptoms are consistent with mood disorder. While one study reported neurasthenia with signs of dementia lasting 10 or more years following exposure (Jirasek et al., 1974), the U.S. Air Force study of Vietnam veterans used neurobehavioral testing but was unable to demonstrate cognitive or other functional CNS deficits. However, this negative study did not investigate the relationship between serum 2,3,7,8-TCDD levels and neurobehavioral deficits. NIOSH investigated measures of depressed mood many years after exposure to 2,3,7,8-TCDD among production workers and found no relationship between depressive symptoms and serum 2,3,7,8-TCDD levels (Alderfer et al., 1992).

Overall neurologic status of workers, community residents, and Vietnam veterans exposed to 2,3,7,8-TCDD and evaluated from 5 to 37 years after last exposure appears to be normal. These data suggest that, although exposure to 2,3,7,8-TCDD may have been extensive as in the case of the exposed workers, Ranch Hands, and Seveso residents and case reports describe many related symptoms, the effects may have been transient. If so, studies conducted years after the last exposure would not detect such changes. These results suggest that, in adults, no long-term neurologic effects were caused by even high exposure to 2,3,7,8-TCDD-contaminated materials. However, there is very little information with which to examine the effects of exposure on the developing human neurologic system.

7.13.8. *Circulatory System*

The relationship between human exposure to 2,3,7,8-TCDD-contaminated chemicals and disorders of the circulatory system has been explored in a variety of reports. A number of early case reports have described effects on the cardiovascular system among individuals

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reportedly exposed to chemicals contaminated with 2,3,7,8-TCDD. Myocarditis (Goldman, 1972), myocardial infarctions (Walker and Martin, 1979; Bauer et al., 1961), ectasia of the coronary arteries (England, 1981), and rapidly progressive atherosclerosis (Jirasek et al., 1974; Pazderova-Vejlukova et al., 1981) have been reported.

Studies have described mortality from diseases affecting the circulatory system among populations exposed to 2,3,7,8-TCDD (Bond et al., 1987; Coggon et al., 1991; Fingerhut et al., 1991b; Zober et al., 1990; Bueno de Mesquita et al., 1993; Bertazzi et al., 1989, 1992; Collins et al., 1993) (Table 7-42). The circulatory system includes ICD-9 codes 390-459 (International Classification of Diseases 9). The results are limited because the studies were primarily designed to test hypotheses relating to cancer and, secondarily, to characterize mortality from causes other than cancer, without detailed consideration of confounders or extent of exposure to 2,3,7,8-TCDD. In addition, because the study populations have differing age distributions, direct comparison of the SMRs is not valid. However, any trends suggested by the direction and magnitude of the SMRs are important.

Among TCP production workers, mortality from all diseases of the circulatory system was similar to mortality in the general population, as described by an SMR of 100, and in workers from The Netherlands (Plant A) (SMR=98, 95% CI=65-142) (Bueno de Mesquita et al., 1993), the United States (Nitro, West Virginia) (SMR=90, 95% CI=80-100) (Collins et al., 1993), and Great Britain (SMR=116, 95% CI=91-146) (Coggon et al., 1991) (Table 7-42). In two studies, mortality in workers with chloracne was not significantly different from that of the national comparison group (U.S. workers, SMR=95, 95% CI=79-113; German workers, SMR=121, 90% CI=83-170) (Bond et al., 1987; Zober et al., 1990).

Mortality from more specific end points, such as ischemic heart disease, all heart disease, and cerebrovascular disease, was noted in a few studies. The SMR from ischemic heart disease was 102 (95% CI=63-158) among Dutch workers (Bueno de Mesquita et al., 1993) and 96 (95% CI=51-164) in U.S. workers (Midland, MI) (Bond et al., 1987). And, in an analysis that included all diseases of the heart combined among 5,200 U.S. production workers, the SMR was 96 (95% CI=87-106) (Fingerhut et al., 1991b). Cerebrovascular disease mortality was slightly elevated among Dutch TCP production workers (SMR=117, 95% CI=38-274) (Bueno de Mesquita et al., 1993) and increased by more than twofold in

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Table 7-42. Mortality from Diseases of the Circulatory System in Populations Exposed to 2,3,7,8-TCDD

Author	Population	Outcome	No. of deaths	SMR ^a	95% CI	Cohort size	Years of follow-up
Fingerhut et al., 1991b	2,4,5-TCP and 2,4,5-T production workers, USA	Diseases of the heart (ICD 390-398, 402-404, 410-414, 420-429)	393	96	87-106	5,172	1942-1987
Fingerhut et al., 1991b	2,4,5-TCP and 2,4,5-T production workers, USA	Diseases of the circulatory system (ICD 401, 403, 405, 415-417, 430-438, 440-459)	67	77	60-98	5,172	1942-1987
Zober et al., 1990	2,4,5-T production workers with chloracne, Germany	Diseases of the circulatory system (ICD 390-458)		121	83-170 ^b	127	1953-1987
Coggon et al., 1991	2,4,5-T synthesis or formulation, Great Britain	Diseases of the circulatory system	74	116	91-146	2,239	1975-1987
Bond et al., 1987	U.S. chemical production workers: TCP + 2,4,5-T with chloracne (Michigan)	Diseases of the circulatory system	19	102	61-159	322	1940-1982
		ASHD ^c	13	96	51-146		
		Vascular lesions of CNS	4	208	57-539		
Bond et al., 1987	U.S. chemical production: workers without chloracne (TCP + 2,4,5-T) (Michigan)	Diseases of the circulatory system	130	95	79-113	2,026	1940-1982
		ASHD ^c	106	110	90-133		
		Vascular lesions of CNS	10	64	31-117		

7-187

05/13/94

DRAFT--DO NOT QUOTE OR CITE

Table 7-42. (continued)

Author	Population	Outcome	No. of deaths	SMR ^a	95% CI	Cohort size	Years of follow-up
Bueno de Mesquita et al., 1993	2,4,5-TCP and 2,4,5-T production workers, The Netherlands	Diseases of the circulatory system (ICD 390-458)	28	98	65-142	549	1955-1985
Bueno de Mesquita et al., 1993	2,4,5-TCP and 2,4,5-T production workers, The Netherlands	Ischemic heart disease (ICD 410-414)	20	102	63-158	549	1955-1985
Bueno de Mesquita et al., 1993	2,4,5-TCP and 2,4,5-T production workers, The Netherlands	Cerebrovascular disease (ICD 430-438)	5	117	38-274	549	1955-1985
Michalek et al., 1990	U.S. Air Force Ranch Hand personnel	Diseases of the circulatory system	25	110	60-150	1,261	1961-1987
Centers for Disease Control Vietnam Experience Study, 1988c	U.S. Army Vietnam veterans	Diseases of the circulatory system (ICD 390-459)	12	0.49 ^d	0.25-0.99	9,324	1965-1983
Fett et al., 1987b	Australian Vietnam veterans; served > 12 months	Diseases of the circulatory system	20 ^d	1.7	0.9-3.0	Vietnam veterans = 19,205 Non-Vietnam veterans = 25,677	1966-1985

7-188

05/13/94

DRAFT--DO NOT QUOTE OR CITE

Table 7-42. (continued)

Author	Population	Outcome	No. of deaths	SMR ^a	95% CI	Cohort size	Years of follow-up
Bertazzi et al., 1989	Residents of Seveso, Italy, ages 20-74, Zone A (high TCDD region)	Diseases of the circulatory system (ICD 390-459) ISHD ^f Cerebrovascular disease	11	Males: 1.75 ^d	1.0-3.2	556 ^e	1976-1986
			6	Females: 1.89 ^d	0.8-4.2		
			2	Males: 1.25 ^d	0.5-3.3		
			5	Males: 3.3 ^d	1.4-8.0		
Bertazzi et al., 1992	Residents of Seveso, Italy, ages 1-19 years	Diseases of the circulatory system (ICD 390-459)	0	Males: not reported	---	306 ^e	1976-1986
			2	Females: 1.63 ^d	0.3-8.1		

^aSMR = Standardized mortality ratio.

^b90% confidence interval.

^cAtherosclerotic heart disease.

^dRelative risk.

^eZone A males and females combined.

^fIschemic heart disease.

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Michigan TCP production workers with chloracne (SMR=208, 95% CI=57-539) (Bond et al., 1987).

The SMRs for circulatory system diseases reported in the various mortality studies are close to 100, suggesting that the "healthy worker effect" is not seen in these studies. Generally, because employed workers are healthier than the general population, the SMR for cardiovascular disease in employed populations tends to be lower than 100 (McMichael, 1976; Fox and Collier, 1976). The absence of a healthy worker effect, in light of the positive animal data, suggests that more detailed analyses should be conducted for cardiovascular outcomes in these populations.

Among 1,261 Ranch Hand personnel, mortality from circulatory disease was nonsignificantly elevated (SMR=110, 95% CI=60-150) compared with that of a comparison population of 19,101 other Air Force veterans who were not exposed to herbicides (Michalek et al., 1990). Similar nonsignificant increases in the relative mortality ratio (RMR) were observed for circulatory diseases (RMR=1.6, 95% CI=0.8-3.2) in Australian Vietnam veterans (N=19,205; 260 deaths) compared to 25,677 (263 deaths) non-Vietnam veterans who only served in Australia (Fett et al., 1987b). In contrast, the unadjusted relative risk of 0.49 (95% CI=0.25-0.99) for all circulatory diseases suggested a deficit of deaths from this cause among 9,325 Vietnam Army veterans (246 deaths) compared to 8,989 non-Vietnam veterans (200 deaths) (Centers for Disease Control Vietnam Experience Study, 1988c).

Bertazzi and colleagues examined the mortality experience of Seveso residents ages 1-19 years (Bertazzi et al., 1992) and ages 20-74 years (Bertazzi et al., 1989) 10 years after the contamination of the town by 2,3,7,8-TCDD-contaminated effluent. In the younger population, two deaths from circulatory diseases occurred only in female residents (RR=1.63, 95% CI=0.3-8.1). In the older population, circulatory disease mortality of residents from Zone A (the most highly contaminated region) was elevated in both males (RR=1.75, 95% CI=1.0-3.2) and females (RR=1.89, 95% CI=0.8-4.2). In males, the highest death rate occurred during the first quinquennium, 1976-1981 (RR=2.04, 95% CI=1.0-4.2), and, in females, the highest death rate occurred during the second quinquennium, 1982-1986. The authors suggest that the study was limited by the small number of subjects and the crude measure of 2,3,7,8-TCDD exposure. The authors could

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not attribute the increased mortality from circulatory disease to 2,3,7,8-TCDD exposure but suggested that the "high stress and pollution" imposed on the residents of Zone A may have been a contributing factor.

Several cross-sectional medical studies have also examined the association between 2,3,7,8-TCDD exposure and effects on the cardiovascular system (Suskind and Hertzberg, 1984; Moses et al., 1984; Bond et al., 1983; Centers for Disease Control Vietnam Experience Study, 1988a; Roegner et al., 1991). Statistically significant associations relative to 2,3,7,8-TCDD exposure were found only in the Ranch Hand study for diastolic blood pressure, arrhythmias detected on the electrocardiogram (ECG), and peripheral pulse abnormalities (Roegner et al., 1991). However, there is some doubt that the significant findings are dose related because significant increases in the mean diastolic blood pressure were found in Ranch Hands with serum 2,3,7,8-TCDD levels from 15 to 33.3 pg/g but not in the Ranch Hands with higher serum 2,3,7,8-TCDD levels. The adjusted odds ratios for ECG-diagnosed arrhythmias among Ranch Hands were not entirely consistent with a dose-response relationship. For each serum 2,3,7,8-TCDD category the odds ratios were: serum 2,3,7,8-TCDD levels ≤ 10 pg/g, OR=1.33 (95% CI=0.68-2.58, $p=0.40$); 15-33.3 pg/g, OR=0.83 (95% CI=0.31, 2.23, $p=0.71$); and for serum 2,3,7,8-TCDD levels above 33.3 pg/g the OR was 2.34 (95% CI=1.00-5.51, $p=0.051$). The proportion of individuals in this group with arrhythmias (5.2%) was not much higher than in Ranch Hands whose serum levels were ≤ 10 pg/g (4.7%). Finally, relative to the comparison group, the proportion of abnormal peripheral pulses in all Ranch Hands, regardless of serum level, was elevated.

No excess abnormalities or disorders of the circulatory system or heart were found in several groups of TCP production workers, although their potential for exposure to 2,3,7,8-TCDD-contaminated chemicals was high (Suskind and Hertzberg, 1984; Moses et al., 1984; Bond et al., 1983). The prevalence of hypertension or coronary artery disease (both self-reported), abnormal ECG findings, atherosclerotic changes (not specified) on chest X-ray, or blood pressure elevation was not elevated in West Virginia TCP production workers (Suskind and Hertzberg, 1984). Similarly, when Moses et al. (1984) examined TCP production workers with chloracne and compared them with workers not affected by chloracne, they found no increased risk for self-reported abnormal ECG, self-reported angina, or self-

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reported myocardial infarction and no difference in the physical examination of the cardiovascular system. Finally, Bond et al. (1983) found no increased risk for self-reported hypertension among workers involved in the production of trichlorophenol and 2,4,5-T.

7.13.8.1. *Comment*

The picture relating exposure to 2,3,7,8-TCDD and diseases of the circulatory system is mixed. Animal data indicate that high doses of 2,3,7,8-TCDD affect cardiac and vascular integrity (Allen and Carstens, 1967; Allen et al., 1977; Norback and Allen, 1973). Data from a few animal studies suggest that relatively high doses of 2,3,7,8-TCDD cause damage to the myocardium and heart valves in rats (Kociba et al., 1978; Buu-Hoi, 1972) and to the arterial walls in rabbits (Brewster et al., 1987). Other research found that 2,3,7,8-TCDD may alter cardiac function in rats and guinea pigs (Hermansky et al., 1988; Kelling et al., 1987; Canga et al., 1988), causing reduced spontaneous and isoproterenol-induced heart contractility; this suggests that 2,3,7,8-TCDD may increase the risk of arrhythmias among the dosed animals. The authors postulate that 2,3,7,8-TCDD alters cyclic-AMP concentrations, altering the responsiveness of cardiac cells to β -adrenergic stimuli (Brewster et al., 1987). In contrast, histopathologic changes were not observed in the cardiovascular system of hamsters, which appears to be resistant to the effects of 2,3,7,8-TCDD at levels of 3,000 $\mu\text{g}/\text{kg}$ of 2,3,7,8-TCDD (Olson et al., 1980). Other experimental studies suggest an association between 2,3,7,8-TCDD and alterations in lipid levels (Poli et al., 1980; Albro et al., 1978; Bombick et al., 1984; Swift et al., 1981).

Findings from mortality and morbidity studies of production workers are not definitive, and suggest the need for analyses more specifically examining possible effects on the circulatory system and heart. The outcomes examined in the animal and human studies are different. Animal studies describe morphologic and chemical changes in the vascular and cardiac cells caused by 2,3,7,8-TCDD. On the other hand, the diseases and causes of death in the human studies assessed the possible consequences of exposure on long-term pathologic changes to the tissues, which cause cell and, sometimes, organ and system failure. Using the animal data, it is possible to project the long-term consequences of exposure to the organ or system. However, the animal studies do not account for the possibility of intervening events,

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such as the repair of tissue after exposure ends, or other events that reduce the hypothesized end point to a much less drastic outcome.

Circulatory diseases and diseases of the heart have not been rigorously investigated as hypothesized health outcomes of exposure to 2,3,7,8-TCDD. Therefore, mortality studies have not considered the possible confounding effect of other variables, including smoking, lipid levels, and other conditions, that influence circulatory diseases and disorders of the heart. Furthermore, the mortality studies tended to examine the circulatory system as a whole, without consideration of the possible pathophysiology of 2,3,7,8-TCDD exposure on the various components of the system, e.g., vessels versus cardiac muscle damage. Finally, mortality studies count only those conditions that ultimately caused the death of the individual. Events such as myocardial infarctions, which may debilitate the individual but not cause death, may be missed if death is caused by another circumstance. Therefore, the effect of 2,3,7,8-TCDD on the coronary arteries might be missed because it was not coded as the underlying cause of death on the death certificate.

With the exception of the Ranch Hand study (Roegner et al., 1991), cross-sectional analyses of other more highly exposed groups were limited by their lack of good exposure data and their inability to examine the relationship between serum 2,3,7,8-TCDD levels and diseases of the circulatory system or heart. Such studies may also be limited by the fact that they include a survivor population, as described in the introduction.

Further research would be useful to define the relationship between the pathologic end points observed in animals after high, single doses of 2,3,7,8-TCDD and the disease outcomes observed in humans after high, long-term exposure. To identify whether 2,3,7,8-TCDD has an effect on the human vasculature, additional work is needed to determine whether certain doses of 2,3,7,8-TCDD cause changes in the human vascular system; to determine whether there are changes in the action of chemicals associated with human cardiac muscle contraction caused by 2,3,7,8-TCDD exposure; and to assess mortality and morbidity in individuals with potential for 2,3,7,8-TCDD exposure while carefully controlling for other risk factors and using more accurate measures of exposure.

7.13.9. Pulmonary Effects

Studies of long-term exposure to 2,3,7,8-TCDD in Sprague-Dawley rats (Kociba et al., 1979; Van Miller et al., 1977), B6C3F1 mice (NTP, 1982a), Swiss-Webster mice (NTP, 1982b), and rhesus monkeys (Allen et al., 1977) have reported changes in bronchiolar or alveolar tissue ranging from epithelial hyperplasia and metaplasia to squamous cell carcinomas. The hyperplastic and metaplastic changes observed in exposed animals are similar to the pathologic picture of chronic bronchitis in humans (American Thoracic Society, 1962).

Case reports have described temporary respiratory irritation (Zack and Suskind, 1980) and tracheobronchitis (Goldman, 1972) among chemical workers exposed to 2,3,7,8-TCDD-contaminated herbicides following industrial accidents. In addition, Baader and Bauer (1951) reported chronic bronchitis in seven workers involved in pentachlorophenol production, which resolved in all but two workers within 2 weeks after production was discontinued.

There is conflicting evidence from controlled epidemiologic studies regarding an association between chronic respiratory system effects and human exposure to substances contaminated with 2,3,7,8-TCDD. One study of workers involved in the production of TCP and 2,4,5-T suggested that 2,3,7,8-TCDD exposure increases the risk for abnormal ventilatory function (Suskind and Hertzberg, 1984). This study found a statistically significantly increased risk for an abnormal forced expiratory volume at 1 second (FEV_1) ($p < 0.01$), an abnormal forced vital capacity (FVC) ($p < 0.001$), and an abnormal FEV_1/FVC ratio ($p < 0.05$) among workers who were smoking at the time of the study. For workers, the percent predicted spirometric parameters for FEV_1 , FVC, and FEV_1/FVC were 99.4%, 92.7%, and 76.5% and for referents, 104.4%, 97.6%, and 79.9%, respectively. The only other study of TCP and 2,4,5-T production workers that reported ventilatory function findings found no association between serum 2,3,7,8-TCDD levels and declines in ventilatory function (Calvert et al., 1991). The disparity in results between the two studies may be related to the age of the unexposed populations and potential exposures of the exposed. In the Suskind and Hertzberg study, the exposed workers were, on average, 10 years older than the unexposed workers. Although the authors indirectly adjusted for age by analyzing age-adjusted ventilatory measures, it is not clear if these adjustments can completely control

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for a 10-year difference in age. In the study by Calvert et al. (1991), the difference in mean age between the exposed and unexposed groups was 0.6 years. The second difference involves the potential for exposure to 2,4,5-T acid dust at the plants studied. The 2,4,5-T acid that was produced at the plant studied by Suskind and Hertzberg was finished as a powder. At the plants studied by Calvert et al. (1991), the 2,4,5-T acid was finished as a liquid. Therefore, the potential for exposure to 2,4,5-T acid dust was greater at the plant studied by Suskind and Hertzberg (1984). Although we are not aware of any published reports supporting an association between ventilatory function and 2,4,5-T acid exposure, a respiratory burden of particles, in the absence of a specific toxic agent, can be a probable cause of ventilatory function declines (Becklake, 1985).

The Ranch Hand study also examined the association between serum 2,3,7,8-TCDD level and respiratory system effects (Roegner et al., 1991). This study found significant declines in the mean FEV_1 and the mean forced expiratory volume (FVC) for Ranch Hands with serum 2,3,7,8-TCDD levels above 33.3 pg/g (adjusted mean FEV_1 = 91.3%; mean FVC = 87.4) compared to a nonexposed comparison group (adjusted mean FEV_1 = 93.5%; mean FVC = 91.7) (Roegner et al., 1991). The 2,3,7,8-TCDD-related declines were small and were interpreted by the authors to be "subtle" and "not clinically significant." As expected, smoking appeared to have the greater influence on lung function.

7.13.9.1. *Comment*

In conclusion, case reports indicate that intense acute exposure to 2,3,7,8-TCDD can produce respiratory irritation. However, the findings from controlled epidemiologic studies do not support an association between 2,3,7,8-TCDD exposure and chronic effects on the respiratory system.

7.13.10. Renal Effects

There is little evidence in the animal or human data to suggest that exposure to 2,3,7,8-TCDD is related to renal or bladder dysfunction. In a single case report, a child exposed to 2,3,7,8-TCDD after contact with soil sprayed with contaminated waste oil was diagnosed with focal pyelonephritis (Kimbrough et al., 1977). After diagnosis and treatment,

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the condition resolved with no reported recurrence. No major renal or bladder dysfunctions were noted among Air Force Ranch Hands (Lathrop et al., 1984, 1987; Roegner et al., 1991) or among TCP production workers from West Virginia (Suskind and Hertzberg, 1984) or New Jersey (Poland et al., 1971).

7.13.11. Reproductive Effects

Animal studies of reproductive effects have focused primarily on maternal 2,3,7,8-TCDD exposure and teratogenesis (Courtney and Moore, 1971; Courtney, 1976; Giavinni et al., 1983; Khera and Ruddick, 1973; Neubert and Dillman, 1972). Some ambiguity is introduced into the interpretation of results because of differences in study design, dosages, and species sensitivity. However, prenatal exposure to 2,3,7,8-TCDD has been associated with cleft palate and kidney abnormalities in rats (Khera and Ruddick, 1973) and mice (Courtney and Moore, 1971; Neubert and Dillman, 1972). Fetal resorption (Courtney, 1976) and reduced fertility (Giavinni et al., 1983) have also been observed in studies of maternal exposure.

Fewer studies have focused on the effects of dioxin on the male reproductive system or on the results of matings in which only the males were exposed to dioxin. Studies of male exposures have not provided evidence of changes in sperm characteristics or effects on the offspring (Lamb et al., 1980; Murray et al., 1979). Chapter 5 contains a more detailed account of the animal literature.

Thus, experimental research has emphasized maternally mediated reproductive effects of 2,3,7,8-TCDD while in humans, studies of paternal exposures have predominated. Moreover, assessment of exposed male animals has most commonly examined the effects of 2,3,7,8-TCDD on spermatogenesis, fertility, and sex organ development (Theobald and Peterson, in press), whereas studies of human males have mainly targeted effects on congenital malformations and recognized spontaneous abortions. Experimental research designed to corroborate human investigations may provide critically needed data to plug the gaps in our understanding of the mechanisms through which 2,3,7,8-TCDD exposure may operate to produce adverse reproductive events in humans.

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The origin of concerns regarding a potential link between exposure to chlorinated dioxins and adverse reproductive events can be traced to early animal studies reporting increased incidence of developmental abnormalities in rats and mice exposed early in gestation to 2,4,5-T (Courtney and Moore, 1971). This was of grave concern, as the U.S. military's most widely used herbicide during the Vietnam conflict at that time, Agent Orange, was composed of approximately equal proportions by weight of the n-butyl esters of 2,4-D and 2,4,5-T. The latter is contaminated by 2,3,7,8-TCDD during manufacture.

One dilemma encountered when attempting to review the epidemiologic literature dealing with dioxins and reproductive effects is the categorization of studies of sufficient similarity to allow for comparative analysis. These studies vary greatly in the nature (occupational, environmental) and route (inhalation, digestion, absorption) of exposure; in the reproductive outcomes examined (often multiple end points were considered, and case definition differed across studies); in the assessment of parental exposure (maternal, paternal, or both); and in the timing of exposure relative to the pregnancy.

The examination of reproductive and developmental disorders poses several challenges to the researcher as compared with other health outcomes. First, to understand both normal and pathologic reproduction, evaluation should include paternal and maternal, and sometimes fetal, contributions. The recent increased interest in male-mediated reproductive toxicity emphasizes the need to consider the couple as the unit of analysis in many reproductive study settings.

The second challenge to researchers is the interrelatedness of the spectrum of reproductive end points available for study. Fecundity (the capability to conceive), fertility (the capability to produce live children), and early pregnancy loss (those conceptions that do not survive to be recognized by usual diagnostic methods) as related to 2,3,7,8-TCDD exposure have not been evaluated. Studies investigating early fetal loss and reduced fertility among couples where exposure to 2,3,7,8-TCDD is an issue are not available. Clearly, these end points affect the rates of reproductive outcomes occurring later in the reproductive spectrum.

Another feature of reproductive effects is the changing vulnerability of the developing organism throughout gestation. Exposure to a single teratogen throughout pregnancy may

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result in different effects at various stages of gestation. The window of susceptibility varies among different teratogens; therefore, knowledge of the timing of exposure is critical in these studies.

Finally, although not restricted to studies of reproductive events, care must be given to the collection and analysis of confounding variables. These factors may need to be obtained for both mother and father, with attention to the timing of specific characteristics, such as changes in smoking or occupation during pregnancy. These are some points to keep in mind as the reader reviews the studies of 2,3,7,8-TCDD and reproductive effects presented here.

The reproductive effects of dioxins in humans were succinctly and elegantly reviewed by Hatch in 1984 (Hatch, 1984b). In her review, Hatch employed type of exposure, i.e., populations that were exposed occupationally, environmentally, or through industrial accident or military service, as the classification scheme for the research presented. The same approach has been followed in this review. The earlier investigations of 2,3,7,8-TCDD and reproductive effects, i.e., those conducted prior to 1984, are presented separately from the more recent studies. The development of assays in the mid-1980s to quantitate 2,3,7,8-TCDD in serum and adipose tissue, allowing individual measurements of exposure, warrants this dichotomy of the research.

7.13.12. Review of the Literature Prior to 1984

7.13.12.1. *Occupational Studies*

Epidemiologic studies of occupational dioxin exposure and reproductive effects have focused on potential paternally mediated effects, with exposures occurring at varying intervals relative to conception. Townsend et al. (1982) interviewed 370 wives of employees exposed to dioxins at the Dow Michigan Division in Midland, Michigan (63% of those eligible for the study), and 345 control wives of Dow employees who were not exposed to dioxin (62% of the eligible control pool). Exposure classification was determined by an industrial hygienist familiar with the processes performed at the plant. Employees were considered exposed to dioxin if they had been assigned for at least 1 month to specific jobs

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associated with chlorophenol processes between 1939 and 1975. All outcomes were reported by the employee's spouse.

There was no systematic attempt to ascertain the reason(s) for the high refusal rate in both cohorts. "Unsolicited reasons" for refusal included divorce, death of spouse, or no pregnancies; no breakdown by cohort was provided. The possibility of differential rates of infertility or early pregnancy loss, as reflected by the reported absence of pregnancy, was not addressed in this study.

For the multiple end points of spontaneous abortion, stillbirth, birth defects, infant mortality, and childhood morbidity, no significant association between dioxin exposure and any adverse event was identified (Table 7-43). Given the very long interval when both exposure and event could have occurred and the minimum requirement for paternal exposure being 1 month at any time during the interval, an effect of exposure, if it existed, would have been diluted by this approach unless the damage was "irreparable," as it was defined in this report. The authors do not discuss whether time since last exposure was considered in the analysis.

In another study of occupational exposure to 2,4,5-T, Smith et al. (1982) evaluated 548 professional chemical sprayers and their spouses in New Zealand, ascertaining spraying activities from the males and reproductive histories from their spouses through a mailed questionnaire. A group of 441 agricultural contractors and their spouses served as controls. This study had impressive response rates of 89% for the exposed and 83% for the nonexposed groups. The investigators noted that wives of the chemical sprayers anecdotally reported assisting their husbands in spraying activities, some performing this task during their pregnancy. No associations between herbicide exposure and the outcomes of spontaneous abortion (OR=0.89, 95% CI=0.6-1.3) or congenital malformations (OR=1.2, 95% CI=0.6-2.4) were identified.

The questionnaires were completed in 1980 and elicited information on spraying activities and reproductive events that occurred between 1969 and 1980, a rather long period of time for recall of these events. Reported pregnancy outcomes were categorized into three groups based on whether the fathers had sprayed any chemicals at any time during, or prior to, the calendar year in which the pregnancy occurred and whether 2,4,5-T had been used.

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Table 7-43. Results of Studies Examining the Effect of Dioxin on Reproductive Outcomes in Humans, 1984-1992

Author	Exposed group	Control group	Type of exposure	Data source: exposure/outcome	Outcome	Outcome in exposed (N)	Outcome in unexposed (N)	OR	95% CI
Smith et al., 1982	548 male pesticide applicators who sprayed 2,4,5-T and other pesticides	441 agricultural contractors	Spraying of 2,4,5-T	Mailed survey/mailed survey	Total births	1,172	1,122		
					Congenital defect	13	9	1.19 ^a	0.58-2.45
					Miscarriage ^b	43	40	0.89	0.61-1.30
					Stillbirth	3	0	—	—
Townsend et al., 1982	370 male chemical workers exposed to 2,4,5-T only and their spouses	345 employees not exposed to 2,4,5-T and their spouses	Working with chlorophenol processes	Interviewer-administered questionnaire/ interviewer-administered questionnaire	# Conceptions	418	2,031	— ^c	
					All fetal deaths	50	246	1.02	0.71-1.47
					Stillbirth	7	33	0.97	0.38-2.36
					Spontaneous abortions	43	213	0.96	0.65-1.42
					Infant deaths	6	39	0.82	0.30-2.09
					Health defects	32	155	0.93	0.60-1.43
					Congenital malformation	21	87	1.08	0.63-1.83

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Table 7-43. (continued)

Author	Exposed group	Control group	Type of exposure	Data source: exposure/outcome	Outcome	Outcome in exposed (N)	Outcome in unexposed (N)	OR	95% CI
Townsend et al., 1982 (cont.)	Male chemical workers exposed to any dioxins				Total conceptuses	737	2,031		
					All fetal deaths	100	246	1.03*	0.78-1.37
					Stillbirth	15	33	1.06	0.54-2.09
					Spontaneous abortions	85	213	1.03	0.77-1.39
					Infant deaths	9	39	0.63	0.27-1.39
					Health defects	52	155	0.85	0.60-1.21
					Congenital malformation	30	87	0.85	0.53-1.35
Mastroiacovo et al., 1988	2,900 births in Zones A, B, and R, Seveso, Italy, 1977-1981	12,391 births in study area outside Zones A, B, and R	TCDD cloud released from chemical plant accident	TCDD soil analysis/ Seveso Congenital Malformations Registry	Total birth defects	137	605	0.97	0.83-1.13
					Multiple birth defects	10	38	1.12	0.63-2.02
					Syndromes	5	29	0.74	0.33-1.63
					Major birth defects	67	343	0.83	0.67-1.04
					Minor birth defects	70	262	1.14	0.92-1.42
Stehr et al., 1986	68 persons residing in areas of high TCDD concentration	36 persons with no known contact with contaminated soil	Contact with TCDD-contaminated soil	EPA soil analyses for TCDD/interview	Infertility (males)	-	-	-	NS
					Impotence	-	-	-	NS
					Infertility (females)	-	-	-	NS
Hoffman and Stehr-Green, 1989	154 residents of Quail Run Mobile Home Park	155 residents nonexposed mobile park home	Contact with soil sprayed with TCDD for dust control	EPA soil analyses for TCDD/interview	Fetal deaths	-	-	-	NS
					Spontaneous abortions	-	-	-	NS
					Congenital malformations	-	-	-	NS

7-201

05/13/94

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Table 7-43. (continued)

Author	Exposed group	Control group	Type of exposure	Data source: exposure/ outcome	Outcome	Outcome in exposed (N)	Outcome in unexposed (N)	OR	95% CI
Stockbauer et al., 1988	402 pregnancies to exposed mothers	804 pregnancies to unexposed mothers (matched on maternal age and race, hospital and year of birth, plurality)	Contact with soil sprayed with TCDD for dust control	EPA soil analyses for TCDD/vital statistics and hospital records	Birth defects (all)	17	42	0.78	0.40-1.47
					Major birth defects	15	35	0.84	0.40-1.66
					Multiple birth defects	2	11	0.34	0.03-1.65
					Fetal deaths	4	5	1.60	0.32-7.43
					Infant deaths	5	5	2.00	0.46-8.69
					Perinatal deaths	6	9	1.33	0.39-4.20
					Low birth weight	27	36	1.59	0.89-2.81
					Very low birth weight	1	4	0.50	0.01-5.05
					IUGR	14	26	1.09	0.50-2.28
Erickson et al., 1984	4,929 infants from the Metropolitan Atlanta Congenital Defects Program	3,029 infants from Georgia Vital Statistics Records	Vietnam military service	Self-reported, and Exposure Opportunity Index/Birth defects registry and vital statistics	Total birth defects (96 subcategories also examined)	428	4,387	0.97	0.83-1.14
CDC, 1988d, 1989	7,924 Vietnam veterans	7,364 non-Vietnam veterans	Vietnam military service	Military records/self-reports	Total birth defects	826	590	1.3	1.2-1.4
					Spontaneous abortion	1,566	1,190	1.3	1.2-1.4
					Stillbirth	126	131	0.9	0.7-1.1
					Low birth weight	100	87	1.1	0.8-1.4
					Childhood cancer	25	17	1.5	0.8-2.8
					Sperm abnormalities:				
					concentration	51	20	2.3	1.2-4.3
Substudy #1, CDC 1988d, 1989	1,791 offspring of Vietnam veterans	1,575 offspring of non-Vietnam veterans	Vietnam military service	Military records/self-report and hospital records verification	motility	91	58	1.2	0.8-1.8
					morphology	51	29	1.6	0.9-2.8
					Total birth defects	130	112	1.0	0.8-1.4
					Low birth weight	51	37	1.1	0.7-1.8
					Perinatal mortality	58	54	1.0	0.7-1.5
					Suspected birth defects	21	21	0.9	0.5-1.7

7-202

05/13/94

DRAFT--DO NOT QUOTE OR CITE

Table 7-43. (continued)

Author	Exposed group	Control group	Type of exposure	Data source: exposure/outcome	Outcome	Outcome in exposed (N)	Outcome in unexposed (N)	OR	95% CI
Substudy #2, CDC 1988d, 1989	127 offspring of Vietnam veterans	94 offspring of non-Vietnam veterans	Vietnam military service	Military records/self-report and hospital record verification	Cerebrospinal malformations	26	12	1.8	0.8-4.0
Stellman et al., 1988	2,858 Vietnam veterans	3,933 non-Vietnam veterans	Vietnam military service	Survey/survey	Difficulty conceiving	349	363	1.2	NS
					Time to conception	4.4 years	4.4 years	—	NS
					Birth weight	—	—	—	NS
					Spontaneous abortion	231	195	1.3	1.4-2.0
Aschengrau and Monson, 1989	201 spontaneous abortion cases at Boston Hospital for Women	1,119 full-term births at Boston Hospital for Women	Vietnam military service	Military records/hospital records	Spontaneous abortion	8	44	0.9	0.42-1.9
Aschengrau and Monson, 1990	966 infants with late adverse pregnancy outcomes at Boston Hospital for Women	998 normal term infants at Boston Hospital for Women	Vietnam military service	Military records/Hospital records	Total birth defects	55	656	1.3	0.9-1.9
					≥ 1 Major malformation	18	151	1.8	1.0-3.1
					Minor malformation	11	189	0.9	0.5-1.7
					Stillbirths	5	51	1.5	0.4-3.9
					Neonatal deaths	3	36	1.2	0.2-4.2
Wolfe et al., 1992b	2,533 conceptions among 791 Ranch Hand personnel	2,074 conceptions among 768 non-Ranch Hand personnel	Spraying/handling of Agent Orange	Serum TCDD levels/hospital and medical records	Total birth defects				
					≤ 10 ⁻⁶	202.1 ¹	208.0	0.96 ^{m-a}	0.69-1.34
					15-≤ 33.3	293.1		1.58	1.10-2.27
					> 33.3	193.8		0.92	0.64-1.32

7-203

05/13/94

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Table 7-43. (continued)

Author	Exposed group	Control group	Type of exposure	Data source: exposure/ outcome	Outcome	Outcome in exposed (N)	Outcome in unexposed (N)	OR	95% CI
Wolfe et al., 1992b (cont.)					Nervous system anomalies				
					≤ 10 ^a	0.0 ^d	3.1	—	
					15- ≤ 33.3	5.7		1.88 ^{a,e}	0.20-18.3
					> 33.3	13.2		4.37 ^{a,e}	0.87, 21.8
					Respiratory system anomalies				
					≤ 10 ^a	7.1 ^l	2.0	3.5 ^{a,e}	0.49-25.0
					15- ≤ 33.3	5.7		2.83	0.26-31.4
					> 33.3	4.4		2.17	0.20-24.0
					Digestive system anomalies				
					≤ 10 ^a	21.3 ^l	24.5 ^d	0.83	0.31-2.23
					15- ≤ 33.3	34.5		1.30	0.48-3.51
					> 33.3	17.6		0.64	0.21-1.91
					Genital anomalies				
					≤ 10 ^a	3.5 ^d	18.3 ^l	0.19	0.03-1.43
					15- ≤ 33.3	51.7		2.92	1.29-6.61
					> 33.3	13.2		0.72	0.21-2.46
					Urinary system anomalies				
					≤ 10 ^a	14.2 ^l	12.2 ^l	1.16	0.37-3.63
					15- ≤ 33.3	34.5		2.88	1.07-7.79
					> 33.3	22.0		1.82	0.63-5.22
					Musculoskeletal deformities				
					≤ 10 ^a	120.6 ^d	134.6 ^d	0.88 ^{a,e}	0.59, 132
					15- ≤ 33.3	143.7		1.08	0.68-1.71
					> 33.3	105.7		0.76	0.48-1.21

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Table 7-43. (continued)

Author	Exposed group	Control group	Type of exposure	Data source: exposure/outcome	Outcome	Outcome in exposed (N)	Outcome in unexposed (N)	OR	95% CI
Wolfe et al., 1992b (cont.)					Anomalies of the skin				
					≤ 10 ^b	17.7 ^d	21.4 ^d	0.76	0.25-2.26
					15-≤33.3	34.5		1.83	0.72-4.70
					> 33.3	8.8		0.46	0.11-1.95
					Circulatory system and heart anomalies				
					≤ 10 ^b	14.2 ^d	16.3	0.85	0.27-2.69
					15-≤33.3	46.0		2.16	0.81-5.74
					> 33.3	8.8		0.32	0.04-2.52

^aRelative risk.

^bRate: 86/1,000 births in applicators; 93/1,000 births in agricultural contractors (controls).

^cAdjusted for mother's age at time of birth, birth control methods, labor and delivery complications, medical conditions and medications during pregnancy, smoking and alcohol use during pregnancy, high job risk, and gravidity.

^dRate: Zone A: 0; Zone B: 57.5/1,000 births; Zone R: 45.1/1,000 births; zone non-ABR: 48.8/1,000 births.

^e90% CI.

^fZones A and B.

^gZones A and B vs. non-ABR.

^hControls matched on maternal age, race, hospital of birth, plurality, and year of birth.

ⁱOdds ratio adjusted for veteran's age at birth, year of entry in army, enlistment status, general technical test score, military occupational specialty, years between entry and birth, maternal age, and gravidity.

^jChildren born after the father was stationed in Southeast Asia (SEA).

^kLogit (p) = $\beta_0 + \beta_1 d_1 + \beta_2 d_2 + \beta_3 d_3$, where p = probability of an adverse reproductive outcome; d₁, d₂, d₃ are indicators for the dioxin categories: Unknown (Ranch Hands with up to 10 pg/g current dioxin), Low (Ranch Hands with more than 15 pg/g of lipid and up to 33.3 pg/g of lipid current dioxin), and High (Ranch Hands with more than 33.3 pg/g of lipid current dioxin).

^lRate of abnormalities.

^mUnadjusted.

ⁿAdjusted analysis not statistically significant.

^oNo adjusted analysis: total defects < 10.

7-205

05/13/94

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There were 1,122 births reported among the 441 control spouses and 1,172 among the 548 spouses of the exposed sprayers. If all other factors were considered equivalent in both cohorts (maternal age, socioeconomic status, and maternal smoking histories were shown to be similar), then there appear to be 220 fewer births observed in the exposed group than might be expected.

This study is limited by the lack of information on the total number of conceptuses and the high probability of exposure to chemicals other than 2,4,5-T. While exposure levels were not quantitated in this study, a later study of a subset from this same population was conducted to estimate 2,3,7,8-TCDD exposure in this group.

In 1988, nine pesticide applicators with the greatest number of years and months per year of pesticide application were selected for serum 2,3,7,8-TCDD analysis (Table 7-44). The mean serum 2,3,7,8-TCDD level, adjusted for total lipids, among the cases (53.3 pg/g) was 10 times that of age-matched controls (5.6 pg/g). However, exposure in the reproductive study was based on self-reports of pesticide application, and research has demonstrated repeatedly that self-reports do not correlate with documented serum 2,3,7,8-TCDD levels (Needham et al., 1991). Therefore, it would be helpful if serum levels could be obtained from a subset of those applicators with lower self-reported exposures.

A clinical epidemiology study conducted in 1979 examined workers involved with the manufacture of 2,4,5-T between 1948 and 1969 in Nitro, West Virginia (Suskind and Hertzberg, 1984). All active and retired plant employees exposed to the 2,4,5-T process during that 22-year interval comprised the eligible pool of "exposed" subjects. The control group consisted of current and former plant employees who were never associated with the 2,4,5-T process, according to company records. The response rates for these cohorts were 61% (N=204) and 46% (N=163), respectively.

A reproductive history was obtained from these male employees during an interview and clinical examination. No attempt was made to verify reports of live births, infant deaths, miscarriages, birth defects, and stillbirths with the spouses or through medical records. There were no significant differences in rates of any adverse outcome by exposure status. Given the poor response rates, crude measure of exposure, and lack of verification of

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Table 7-44. 2,3,7,8-TCDD Levels (pg/g of Lipid) for Selected Populations

Author	Study population	Specimen	Range	Mean	Median
Mocarelli et al., 1991	Seveso, Italy residents: 10 Zone A 10 former Zone A 10 non-ABR Zone	Serum	828 - 56,000 1770 - 10,400 nd-137	19,144 5,240 -	14,000 4,540 -
Patterson et al., 1986b	39 Missouri residents with history of TCDD exposure	Adipose tissue	2.8 - 750	79.7	17.0
	57 Missouri residents with no known TCDD exposure		1.4 - 20.2	7.4	6.4
Smith et al., 1992	9 New Zealand pesticide applicators	Serum	3.0 - 131.0	53.3	37.6
	9 controls		2.4 - 11.3	5.6	9.3
CDC, 1988	646 Vietnam ground combat troops with service in heavily sprayed areas	Serum	nd - 45	4.2	3.8
	97 non-Vietnam veterans		nd - 15	4.1	3.8
Kahn, 1988	10 "heavily exposed" Vietnam veterans	blood (per lipids)	-	46.3	25.1
		adipose tissue	-	41.7	15.4
	10 Vietnam veterans with "little or no" exposure	blood (per lipids)	-	6.6	5.3
		adipose tissue	-	5.1	5.4
	7 non-Vietnam veterans	blood (per lipids)	-	4.3	3.9
		adipose tissue	-	3.2	3.5

7-207

05/13/94

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Table 7-44. (continued)

Author	Study population	Specimen	Range	Mean	Median
Schechter et al., 1989	26 Vietnam veterans	adipose tissue	nd - 11	5.8	-
Kang, 1991	36 Vietnam veterans	adipose tissue	-	13.4	10.0
	79 Non-Vietnam veterans		-	12.5	11.4
	80 Civilians		-	15.8	11.8
Roegner et al., 1991	872 Ranch Hands	serum	0-617.8	-	12.8
	1060 Controls		0-54.8	-	4.2
Phuong, 1989b	Vietnamese Populations: 9 OB/GYN patients from a South Vietnam hospital	adipose tissue	nd - 103	23	11.3

paternally reported reproductive histories, this study was not likely to detect an association between 2,3,7,8-TCDD and reproductive events if one existed.

7.13.12.2. *Environmental Studies*

The problem of documentation of exposure is perhaps of even greater concern in studies of subjects environmentally exposed to dioxins that are lacking individual 2,3,7,8-TCDD measures. The route of exposure (inhalation, ingestion, absorption), length and intensity of exposure, and the timing of the exposure are more difficult to estimate in free-living populations compared with workers in an occupational setting.

Selection bias is also a critical concern in environmental studies because there is no equivalent to company records in the occupational setting that defines the population at risk. Proximity of residence to a contaminated site was generally the best option available for identifying the population at risk of exposure. Issues such as length of time at that residence, the amount of time the subject spent in the home, and the occurrence of the contamination episode relative to the time spent at home may have greatly affected the degree of exposure.

Volunteer bias, which is inherent in studies that rely on subjects responding to publicized requests for participation, is an additional concern in studies of this nature. Moreover, it is extremely difficult to conduct epidemiologic investigations under crisis situations such as the industrial accident that occurred in Seveso, Italy. Given these limitations, the efforts of these investigators have provided valuable impetus to the refinement of study designs and the development of more sophisticated techniques to explore this issue.

7.13.12.3. *The Seveso, Italy, Dioxin Accident of 1976*

The 1976 accident at a chemical plant near Meda, Italy, in which effluent containing 2,3,7,8-TCDD among other contaminants was released, has been well described (Pocchiari, 1979; Reggiani, 1978). Following the reactor release, an extensive surveillance system was initiated to monitor the health of the exposed population. Rates of reproductive events were examined by "zones" of exposure, as described above.

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The Seveso Congenital Malformations Registry enrolled all live births and stillbirths occurring January 1, 1977, through December 31, 1982, to women residents of Zones A, B, R, and non-ABR (Mastroiacovo et al., 1988). A total of 15,291 births (live and still) and 742 birth defects were recorded: Zone A, 26 births and no birth defects; Zone B, 435 births and 25 birth defects (57.5/1,000 births); Zone R, 2,439 births and 110 birth defects (45.1/1,000 births); Zone non-ABR, 12,391 births and 605 birth defects (48.8/1,000 births). Birth defects were confirmed by medical records. Relative risk estimate for total defects comparing Zones A+B with Zone non-ABR is 1.2 (90% CI=0.88-1.64) (Table 7-43). The rate of birth defects occurring early in the observation period (first quarter of 1977) did not differ from the rate of birth defects occurring later in the observation period (data not shown), and there were no discernible patterns of defects within or between the exposure groups.

To document an increase in the rate of an event, sound baseline information is required. No reliable information regarding rates of birth defects in this area was available until the establishment of the Seveso Congenital Malformations Registry 6 months after the exposure incident. Thus the potential contribution of 2,3,7,8-TCDD to the congenital malformation rate cannot be separated from improved case ascertainment.

The influence of both spontaneous and induced abortions on the birth defect rate is likewise unknown. As with congenital malformations, reliable background data for spontaneous abortion in the study area were not available. The impact of induced abortions sought after the accident is difficult to estimate, particularly since abortion was illegal in Italy in 1976. The birth rate for the entire study area declined between 1976 and 1980, and the small number of conceptions available for study limits the power of these studies to detect an association between 2,3,7,8-TCDD exposure and spontaneous abortion and birth defects.

Another adverse outcome that has not been described in most epidemiologic studies is the effect of 2,3,7,8-TCDD on the chromosome. Pocchiari et al. described one study in which 30 therapeutic and four spontaneous abortions from the Seveso area were examined (Pocchiari, 1979). There were no indications of mutagenic, teratogenic, or embryotoxic effects that could be attributed to 2,3,7,8-TCDD exposure. However, it was difficult to determine maternal exposure status for any of the cases. A more recent study examined the

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association between 2,3,7,8-TCDD and cytogenetic abnormalities in fetuses from abortions induced shortly after the Seveso accident (Tenchini et al., 1983). The frequency of aberrant cells and the mean number of aberrations per damaged cell were significantly higher in exposed versus unexposed fetuses. However, the paper omits important data, including whether the fetus was conceived before or after 2,3,7,8-TCDD exposure, how long after exposure the abortion occurred, the zone in which the mother and father resided at the time of conception and pregnancy, and tissue 2,3,7,8-TCDD concentration in the fetuses. No other studies have examined fetal tissue in this manner to corroborate these data. This approach offers exciting possibilities for future research, however, as genetic techniques in the area of DNA-adduct analyses become more widely available.

7.13.12.4. *Studies of Exposure to Agent Orange by Military Veterans and Vietnamese Civilians*

The problem of exposure documentation has also been a highly controversial issue in studies of potential exposure to Agent Orange in Vietnam and adverse health effects among Vietnam veterans and residents of Vietnam and their offspring. An early study among the Vietnamese population encompassed a 10-year period in Vietnam from 1960 to 1969 (Cutting et al., 1970). Exposure was dichotomized into pre- or light-spraying years from 1960 to 1965 and heavy-spraying years from 1966 to 1969. A total of 480,087 births, 16,166 stillbirths, 2,866 hydatidiform moles, and 2,355 congenital malformations of all types were examined in this study. Pregnancy outcome data were collected from 22 hospitals.

Increases in the rates of stillbirths, molar pregnancies, and congenital malformations were noted in the coastal plain and delta areas following heavy spraying, although the authors emphasized the slight downward trend observed for all outcomes in the countrywide rates. Several biases in this sampling approach severely limit the interpretation of the study's findings. The births examined were not representative of the births in the country during that period. In addition, the hospital records were incomplete, and transport of the mothers to the selected hospitals resulted in uncertainty regarding maternal residence during the pregnancy.

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A second investigation conducted in Vietnam used the HERBS tape (military records detailing Agent Orange spraying missions) covering the period from 1965 to 1971 to determine maternal exposure status according to area of residence (Kunstadter, 1982). HERBS data were matched to hospital records indicating the date of birth to imply "estimated date of conception" and to maternal residence at birth to imply "potential for maternal exposure." Birth outcome data were collected from hospital records, which as in the above study were subject to inaccuracies and incompleteness.

No association between spraying of Agent Orange and any type of birth defect or perinatal mortality was noted. Although cleft lip defects increased in proportion to other malformations during the heavy spraying period, the total number of birth defects declined and continued to decrease after spraying activities ceased.

Finally, Australian investigators examined the relationship between service in Vietnam during 1962-1972 and birth defects (Report to the Minister for Veterans' Affairs, 1983). Cases were infants born with any of a defined set of congenital malformations in any of 34 hospitals in New South Wales, Victoria, and the Australian Capital Territory from 1966 to 1979. Control infants were matched to cases on maternal age, hospital, and time of birth, yielding 8,500 matched pairs for analysis.

Fathers of case and control infants were matched against a list of members who had served in the Australian Army during the specified time period. No associations were detected for Vietnam service and total birth defects ($OR=1.02$, 95% $CI=0.8-1.3$) or for any of the approximately 100 birth defects examined. Length of service in Vietnam, time between deplanement and conception, and Vietnam service prior to and following conception were considered in the analysis.

A critical point that should be emphasized regarding the Australian study is the assessment of service in Vietnam as the exposure of interest. The author clearly stated that investigations had indicated that exposure to herbicides was "infrequent and probably very low in Australian troops in Vietnam; the study does not exclude possible effects of herbicides in situations of substantial exposure" (Report to the Ministry for Veterans' Affairs, 1983).

A series of unpublished studies conducted by Vietnamese investigators was reviewed by Hatch (1984a) and should be mentioned in this review. While these reports also have

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limitations, including incomplete background data for rates of reproductive events and sparsity of epidemiologic details provided in the studies, the assessment of Vietnamese populations offered the opportunity to evaluate pregnancies with various patterns of parental exposure.

Investigations conducted in northern Vietnam assessed pregnancies with no maternal exposure to spraying activities; paternal exposure was presumed to have occurred only when the father had performed military service in the south.

Studies of couples in southern Vietnam compared reproductive outcomes observed in sprayed versus nonsprayed areas and represent potential associations between either maternal and/or paternal herbicide exposure and risk of adverse pregnancy outcome.

Three studies examined presumed paternal herbicide exposure and birth defects among pregnancies in northern Vietnam. Lang and Van compared the frequency of birth defects during the period 1975-1978 as a function of the father having served in south Vietnam (Hatch, 1984a). Among 2,547 offspring whose fathers had never served in the south, the birth defect rate was 6 per 1,000 (N=15). Among 511 offspring whose fathers had served in the south, the rate was 29 per 1,000 (N=15). Similar results were obtained in a study by Lang et al. at unspecified agricultural and handicraft cooperatives in northern Vietnam (Hatch, 1984a). The congenital malformation rate among "exposed" pregnancies (paternal service in southern Vietnam) was 23-26 per 1,000 (71 or 82 of 3,147). In comparison, the rate among the unexposed pregnancies was 5 per 1,000 (10 of 2,172).

In what is perhaps the most stringent of the Vietnamese studies by Can et al., 40,064 women from three rice-growing districts in northern Vietnam were assessed (although few details are provided on the method of selection) (Hatch, 1984a). All of the women were married and pregnant at least once during the war and had no history of tuberculosis, syphilis, or malaria or of using antibiotics or hormones during pregnancy. "Detailed" interviews were conducted by physicians and midwives, and district health records were consulted in an attempt to validate reported pregnancies and outcomes. Only pregnancies conceived during the conflict were considered in this study.

There were a total of 121,993 pregnancies among the 29,041 women whose spouses were "nonexposed" and 32,069 pregnancies among the 11,023 women whose spouses had

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served in the south. Service in south Vietnam was associated with an increased rate of spontaneous abortion ($p=0.05$). The increased rate of congenital malformations among the exposed pregnancies (0.6%) compared with the unexposed (0.4%) was marginally significant ($p=0.10$).

In attempt to confirm this finding, these investigators conducted a case/control study in which they examined a random sample of 61 families of children who had survived with a birth defect. A control group of 183 families of normal children matched on maternal age, number of deliveries, living environment, and age was selected. Forty-nine percent of children with a birth defect had a father who had served in south Vietnam compared with 21% of the children without malformations, yielding an odds ratio of 3.6.

Additional unpublished studies conducted in Vietnam by Vietnamese investigators were reviewed by Constable and Hatch (1985). The reader is referred to this source for details of the studies. It was concluded that studies of presumptive paternal "herbicide" exposure prior to or at conception were suggestive of a relationship with congenital malformations. The evidence for an association with spontaneous abortion was "less convincing," and for molar pregnancies no relationship appeared to exist. Those studies that examined both maternal and paternal "herbicide" exposure were also suggestive of a relation to birth defects as well as spontaneous abortion, stillbirths, and molar pregnancies. Follow-up studies by these Vietnamese investigators supported these earlier findings (Huong et al., 1989; Phuong et al., 1989b).

In the next section, the impact of assays to measure individual levels of 2,3,7,8-TCDD is discussed, and studies of 2,3,7,8-TCDD exposure and reproductive effects that have been published since 1984 are presented.

7.13.13. Review of the Literature From 1984 to 1992

During the interval since 1984, assays that had been developed to measure TCDD in serum and adipose tissue were being tested and refined. Several investigators have since used these assays in (usually small) subsets of their study populations to describe exposure to 2,3,7,8-TCDD in their total sample and also in attempts to validate their assumptions regarding magnitude of exposure for their study subjects. Subsets were generally selected to

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represent subjects designated as "high" versus "low" exposure by the study investigators, utilizing various information sources including self-reports, company or military records, etc. Table 7-44 presents the results of the exposure analyses (Mocarelli et al., 1991; Patterson et al., 1986b; Smith et al., 1992; Centers for Disease Control Veterans Health Studies, 1988; Fingerhut et al., 1991a; Schecter et al., 1989; Kahn et al., 1988; Kang et al., 1991; Roegner et al., 1991; Phuong et al., 1989b).

These data illustrate wide variability in groups presumed to have been exposed to 2,3,7,8-TCDD at levels above background (≤ 20 pg/g). For example, the mean and median serum levels of Vietnam ground combat troops with service in areas heavily sprayed with Agent Orange did not exceed the levels found in the U.S. general population. There is evidence for significantly higher exposure to 2,3,7,8-TCDD among certain subgroups of Vietnam veterans (Kahn et al., 1988; Roegner et al., 1991) as well as residents of Vietnam (Phuong et al., 1989b), Seveso (Mocarelli et al., 1991), and Missouri (Patterson et al., 1986b), and occupational groups (Fingerhut et al., 1991a; Beck et al., 1989).

It is also important to note that with the exception of the Ranch Hand study, these subsets were selected to describe 2,3,7,8-TCDD exposure in the total study sample and not for an examination of the relationship between 2,3,7,8-TCDD and reproductive events. In addition, the data from the Ranch Hand population indicated a serum 2,3,7,8-TCDD half-life of 7.1 years (Pirkle et al., 1989), but serum samples in this group were collected and analyzed at 11- and 15-year intervals following exposure. Questions regarding the impact of initial dose, age, gender, and pregnancy itself on half-life in humans remain unanswered at this time.

7.13.14. Environmental Studies

7.13.14.1. *The Times Beach, Missouri, 2,3,7,8-TCDD Episode*

In 1971, a waste oil dealer in Missouri disposed of waste sludge containing approximately 29 kg 2,3,7,8-TCDD by mixing it with waste oils as a dust control spray, which was subsequently distributed throughout the state. General media announcements from health officials were made, urging persons potentially exposed to 2,3,7,8-TCDD to participate in a survey and health screening process. People were warned of their potential

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exposure by virtue of their residence, employment, or engagement in recreational activities in the contaminated sites. A pilot study was initiated in 1983, in which a subset of those persons who had responded to the media announcements was assessed (Stehr et al., 1986). Approximately 800 completed questionnaires were screened to select participants; it was not clear if this number represents all of the questionnaires that had been returned up to that time.

This phase of the study was intended to identify potential problems for future investigations. Persons determined to be at "high" versus "low" risk for dioxin exposure, based on their completed surveys, were selected. "High risk" was defined as either (1) reported residence or occupation in areas with TCDD levels between 20 and 100 ppb for at least 2 years or in areas with TCDD levels > 100 ppb for at least 6 months, or (2) participation in activities requiring close contact with soil in areas with TCDD concentrations as described for similar periods of time. "Low-risk" persons were determined to have had no access to or "regular high-soil-contact activities" in any known contaminated area. Controls were frequency matched with the high exposed group on type of exposure site, age, sex, race, and socioeconomic status.

Sixty-eight "high-risk" persons (83% of those eligible) and 36 "low-risk" persons (90% response) were evaluated through physical, neurological, and dermatological examinations, laboratory tests, and interviews.

Information on reproductive outcomes was obtained during the interview administered to the subjects "or their nearest relative." None of the outcomes observed differed significantly by exposure status (Table 7-43), although high-risk women had a later mean age at menarche ($p=0.06$). A total of 30 births were available for assessment in this sample.

The following year, a more intensive study was undertaken to test the results found in the pilot study (Hoffman et al., 1986). The exposed group consisted of residents of the Quail Run Mobile Home Park in Gray Summit, Missouri, where TCDD levels in the soil were measured at up to 2,200 ppb. Data on 95 of the approximately 207 households in the park were available; 154 persons (74%) who were both "eligible and interested" agreed to participate. The unexposed group consisted of 155 residents of a nonexposed trailer park, representing 77% of those "both eligible and interested" in participation.

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The protocol was similar to that employed in the pilot study described above. The authors reported that "no differences were found between the exposed and unexposed groups in the frequency of reproductive disorders or adverse pregnancy outcomes, such as fetal deaths, spontaneous abortions, and children with congenital malformations." No sample sizes or statistical test results for any of the outcomes were reported. Clearly, these studies of Missouri residents were not designed to investigate the relationship between dioxin exposure and reproductive outcomes, and not very much can be learned about this association from these results.

In a retrospective cohort study by Stockbauer et al. (1988), the association between 2,3,7,8-TCDD and reproductive outcomes was examined among residents of contaminated areas in Missouri. All live births and stillbirths that occurred in the nine residential areas identified as contaminated with TCDD during the period 1972-1982 were identified through Missouri vital statistics records. A total of 402 births were examined from six residential areas. TCDD levels in the soil from these six areas ranged from 241 to 2,200 ppb. 2,3,7,8-TCDD levels were not reported for the three areas where no births occurred during this interval, which would have been of interest to note.

A reference group of 804 unexposed births, matched for maternal age and race, hospital and year of birth, and plurality, was selected from the vital statistics records. Medical records were abstracted to ascertain birth defects in the matched sets. In addition, the births were linked to a statewide birth defect register that had been recently initiated. Data on several potentially confounding variables were obtained from birth certificates.

The exposed mothers tended to be less educated, had more children, were more likely to be in the extremes of the prepregnant weight distribution, and were more likely to smoke cigarettes. Statistical testing for these differences was not reported. Moreover, statistical adjustment for these potential confounders was performed only in the birth weight analyses, although there was little change in the birth weight risk ratios after adjustment.

Increased risk ratios were reported in the exposed group for infant death (OR=2.0), fetal death (OR=1.6), and perinatal death (OR=1.3), low birth weight (AOR=1.5), and several subcategories of malformations, although none of these findings achieved statistical significance (Table 7-43).

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Two approaches were used to determine a dose-response relationship. In the first, the study data set was matched against the Missouri central listing of dioxin-exposed persons, which yielded 98 of the exposed mothers and none of the control mothers. These 98 women were then dichotomized into "high" (N=20) or "low" (N=78) exposure groups. High exposure was defined as residence for at least 6 months in areas with ≥ 100 ppb TCDD in the soil or ≥ 2 years in areas with TCDD levels from 20 to 100 ppb. Low exposure was defined as residence in areas with similar TCDD levels as described above but for less than the required time period or residence at sites with 1-19 ppb TCDD. No evidence for a dose-response relationship was observed with this analysis.

The second approach categorized the births into two different intervals relative to the spraying of the soil with the TCDD-contaminated sludge in 1971 to 1973. When births in the 1972-1974 period were compared with births from 1975 to 1982, the authors reported that "the only birth outcomes with higher risk ratios in the earlier time period were very low birth weight, birth defects, and major birth defects." None of these observations were statistically significant, but sample sizes and testing results were not provided in the paper.

The authors acknowledged the possibility of exposure misclassification and also the "modest" power of the study to detect associations due to the small sample size.

7.13.14.2. *Studies of Vietnam Experience in Ground Troops and Ranch Hands Published 1984-1992*

7.13.14.2.1. *Evaluation of exposure*

Evidence from earlier studies to determine if Agent Orange exposure increased the risk of adverse pregnancy outcomes among Vietnam veterans has been described as "sparse, sometimes off the point, sometimes conflicting..." (Hatch and Stein, 1986). The general dissatisfaction with these studies had a common factor: the lack of a valid measure of dioxin exposure. Once the assays to document individual 2,3,7,8-TCDD exposure became available and served as the gold standard for assessing the exposure assumptions made by study investigators, this concern regarding exposure misclassification was shown to be justified. Until 1992, when the first study to examine reproductive outcomes among Vietnam veterans based on individual exposure measurements of 2,3,7,8-TCDD was published, this remained

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the major criticism of the research. However, even though 2,3,7,8-TCDD serum levels were available for reanalysis of the 1984 data of the Ranch Hand study, the lack of a good estimate of 2,3,7,8-TCDD level at the time of conception continues to instill uncertainty about the findings.

7.13.14.2.2. *Exposure indices*

7.13.14.2.2.1. The CDC case-control study's exposure opportunity index. In the early 1980s, the CDC conducted a large case-control study that examined the relationship between service in Vietnam and risk of congenital malformations (Erickson et al., 1984). Although service in Vietnam was the major exposure variable, two additional measures of exposure were assessed. Vietnam veterans were asked if they believed they had been exposed to Agent Orange. In addition, an exposure opportunity index (EOI), developed by the Army Agent Orange Task Force, assigned a score estimating the likelihood of exposure based on places and times of Vietnam service. These scores ranged from minimal (1) to high (5) opportunity for Agent Orange exposure.

The CDC then reported on a study, using the serum assay as the standard, to evaluate the validity of both self-reported exposure to Agent Orange and use of military records to formulate the EOI used in the case-control study of military service in Vietnam and birth defects. The results revealed a poor correlation (no correlation coefficient was provided) between both of these exposure estimates and serum TCDD levels (Centers for Disease Control Veterans Health Study, 1988). In addition, the distributions of serum 2,3,7,8-TCDD levels were nearly identical (median = 3.8 ppt) among 646 ground combat troops who had served in heavily sprayed areas compared with 97 veterans who had never served in Vietnam.

In a separate study, Kahn et al. (1988) measured serum 2,3,7,8-TCDD levels in 10 Vietnam veterans who reported that they had handled Agent Orange "regularly" while in Vietnam, 10 Vietnam veterans with little or no exposure to Agent Orange, and 27 Vietnam-era veterans. The levels of serum 2,3,7,8-TCDD among those men who had handled Agent Orange were significantly elevated (median = 25.1 pg/g blood fat) compared with the

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Vietnam control veterans (median = 5.3 pg/g) and the Vietnam-era veterans (median = 3.9 pg/g) ($p < 0.01$).

7.13.14.2.2.2. *The Baseline Ranch Hand study's exposure index.* In 1984, the U.S. Air Force released preliminary results of a 20-year study designed to examine the personnel responsible for conducting the aerial spraying of herbicides in Vietnam (Lathrop et al., 1984). The analyses in this baseline study were based on cohort status, i.e., Ranch Hands ("exposed") versus controls ("nonexposed"). In an attempt to determine a link between exposure and clinical end points, an exposure index was developed to estimate individual 2,3,7,8-TCDD exposure. The exposure index developed for this study was defined as the product of a TCDD weighting factor and the gallons of TCDD-contaminated herbicides sprayed during the veteran's tour divided by the number of Ranch Hands sharing his duties during his tour.

In 1988, a report describing a U.S. Air Force and CDC collaborative pilot study utilizing the serum 2,3,7,8-TCDD assay among 200 Air Force Ranch Hand personnel was published. It was noted that the Ranch Hand personnel had significantly higher serum 2,3,7,8-TCDD levels than controls (Wolfe et al., 1988). These data also indicated that the Ranch Hands as a whole were not as highly exposed to 2,3,7,8-TCDD as compared with the NIOSH cohort (Piacitelli et al., 1992) and the Seveso population (Mocarelli et al., 1991).

In 1992, the report describing reproductive outcomes among Ranch Hand personnel became available (Wolfe et al., 1992b). In addition to outcome verification, serum 2,3,7,8-TCDD levels were measured in a sample of Ranch Hands ($N=791$) and the comparison population ($N=942$). A comparison of the exposure index used in the baseline Ranch Hand study with individual 2,3,7,8-TCDD levels revealed "considerable misclassification" among the study subjects.

As a result of these investigations, it became clear that the likelihood of exposure misclassification in studies of the relationship between 2,3,7,8-TCDD and reproductive events, without direct measures of individual exposure, casts considerable doubt as to the validity of the findings.

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An equally important finding resulting from these investigations was the inability of the exposure indices to classify exposure status in individuals (Needham et al., 1991). 2,3,7,8-TCDD analyses in small subsets of the total study sample, selected on the basis of presumed exposure as defined by an "exposure index" (either military or government records, self-reports, or 2,3,7,8-TCDD levels in soil samples), have demonstrated that the exposure classification schemes employed in these studies were not valid. Therefore, studies that defined exposure as paternal military service in Vietnam should be evaluated without inference to 2,3,7,8-TCDD exposure.

7.13.15. Study Results

7.13.15.1. *Atlanta Congenital Defects Program Study*

In 1984, the CDC released the findings from the large case-control study that examined the relationship between service in Vietnam and risk of congenital malformations (Erickson et al., 1984). Case-group babies were infants with serious structural malformations born between 1968 and 1980 and registered with the Metropolitan Atlanta Congenital Defects Program (MACDP). Of 7,133 eligible cases, maternal interviews were obtained for 4,929 (69%). Control babies were selected from Georgia vital statistics records and were frequency matched to cases on race and year and hospital of birth. Of 4,246 eligible controls, maternal interviews were obtained for 3,029 (71%). Paternal interview rates were 56% and 57%, respectively.

While response rates were similar by case status overall, among nonwhites significantly more cases were not interviewed. The major reason for nonparticipation was the inability to locate subjects rather than subjects refusing to enroll.

Among the children with congenital malformations, 428 (9%) were fathered by Vietnam veterans and 4,387 (91%) were fathered by non-Vietnam veterans; identical percentages were noted among the control infants. The odds ratio for service in Vietnam and birth defects of any type was 0.97 (95% CI=0.83-1.14). Odds ratios were also calculated for 96 separate categories of birth defects, with no significant associations observed.

The EOI developed for this study (as described above) also was not associated with total birth defects. However, significant associations were observed for spina bifida, cleft lip

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with or without cleft palate, neoplasms, and coloboma. With the exception of the last defect, all three also showed evidence of a dose-response relationship.

For the analysis that examined potential associations between self-reported Agent Orange exposure and birth defects, the frequency of exposure among fathers and each type of malformation was compared with the frequency among fathers and all other defects in an attempt to reduce recall bias. Twenty-five percent (N=74) of the Vietnam veterans reported that they believed they were exposed to Agent Orange during their service in Vietnam. The analysis of self-reported exposure and birth defects was negative on all counts, in contrast to the EOI analysis, which found significant associations as described above. However, the small numbers of cases of many individual defects resulted in a virtual lack of power to detect any associations for these specific defects.

7.13.15.2. CDC Vietnam Experience Study

As part of a separate, large, multifaceted study mandated by Congress, the CDC evaluated the risk of service in Vietnam and adverse reproductive outcomes (Centers for Disease Control Vietnam Experience Study, 1988d, 1989). The Vietnam Experience Study protocol involved two phases. In the first phase, a random sample of male veterans who met eligibility criteria related to military service was selected for a telephone interview.

Of the eligible "exposed" group, i.e., those veterans who had served in Vietnam, 84% (N=7,924) agreed to participate, and 84% (N=7,364) of the nonexposed (those who had not served in Vietnam) were enrolled. Of these 15,288 veterans who completed the telephone interview, a random sample was selected for the second phase, which consisted of a comprehensive medical examination. For this phase, the response rates were 75% (N=2,490) for the Vietnam veterans and 63% (N=1,972) for the non-Vietnam veterans group.

During the telephone interview, veterans were questioned about miscarriage, induced abortion, ectopic pregnancy, live births, stillbirths, birth defects, as well as leukemia and other childhood cancers, and major health problems or impairments during the first 5 years of life.

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A preliminary analysis of the interview data revealed that the Vietnam veterans had reported 40-50% more birth defects than the non-Vietnam veterans. In addition, a difference between the cohorts for certain subcategories of malformations, including spina bifida, cleft lip with or without cleft palate, and hydrocephalus, was noted. Therefore, a substudy was conducted to compare the rates of total birth defects in Vietnam and non-Vietnam veterans as these events were recorded on hospital records.

The eligible sample for this substudy consisted of 2,282 veterans who had not yet received their medical examinations, as it would be easier to collect the additional information required and permission to obtain hospital records for their offspring from this group. Hospital records were obtained for 92% (N=1,791) of the offspring of Vietnam veterans and 91% (N=1,575) of the offspring of non-Vietnam veterans.

When birth defects were identified from hospital records, there was no association of Vietnam service with total, major, minor, or suspected birth defects. From the telephone interview data, the odds ratio for Vietnam service and total birth defects was 1.3 (95% CI=1.2-1.4); in the hospital records substudy, the odds ratio was 1.1 (95% CI=0.7-1.8). It was concluded that this finding supported the explanation of differential reporting in the telephone interview.

The odds ratios for selected categories of birth defects calculated from both the telephone interview and hospital records study are presented in Table 7-45. The rate of birth defects increased for both cohorts when malformations were identified in the medical records.

The extent of differential reporting between the two cohorts has also been described (Centers for Disease Control Vietnam Experience Study, 1989). Overall, the authors concluded that agreement between veterans' reports and hospital records for the presence of a birth defect was "relatively poor" for both cohorts. Positive predictive value, sensitivity, and the kappa statistic were slightly lower among Vietnam veterans (Table 7-46). It was further stated that there was no evidence of selection bias or participation bias in this substudy because no differences were noted between cohorts in health histories and demographic or military covariates, and the participation of both groups of veterans was high. However, the subjects in this substudy were selected from the group of veterans who completed the

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Table 7-45. Odds Ratios for Selected Categories of Birth Defects for the Telephone Interview and Hospital Records Study in the Vietnam Experience Study, 1989

Telephone Interview				
Defect Category	Rate (per 1,000) Vietnam veterans	Rate (per 1,000) Controls	OR	95% CI
Total	64.6	49.5	1.3	1.2-1.4
Hospital Records Study				
Total	72.6	71.1	1.0	0.8-1.4
Major	28.5	23.5	1.1	0.7-1.8
Minor	32.4	34.3	1.00	0.7-1.5

Adapted from the Centers for Disease Control Vietnam Experience Study (1988d).

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Table 7-46. Results of the Misclassification Analyses for Birth Defects in the Hospital Records Substudy, Vietnam Experience Study, 1989

Vietnam veterans		Non-Vietnam veterans	
PPV:	24.8%	PPV:	32.9%
NPV:	95.2%	NPV:	95.8%
Sensitivity:	27.1%	Sensitivity:	30.3%
Specificity:	94.7%	Specificity:	96.2%
% Agreement:	90.6%	% Agreement:	92.4%
Kappa index:	20.9%	Kappa index:	27.6%

PPV = Positive predictive value.

NPV = Negative predictive value.

Adapted from the Centers for Disease Control Vietnam Experience Study (1989).

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examination. The response rate for this phase of the study was not high, as noted above, with 75% of Vietnam veterans and only 63% of the non-Vietnam veterans participating in the medical examination. While characteristics of the subset who agreed to undergo physical examination did not differ from the telephone interview sample, no data on those who refused the exam and no reasons for refusing to participate were provided in this paper.

Adjusted odds ratios for two additional outcomes verified in the hospital records substudy, low birth weight (AOR=1.1, 95% CI=0.8-1.4) and perinatal mortality (AOR=1.6, 95% CI=0.8-3.1), did not differ by Vietnam service status. A race-specific analysis of total, major, and minor defects showed an increased risk for total and minor birth defects among black veterans. The adjusted odds ratio was 3.3 (95% CI=1.5-7.5) for total defects and 2.9 (95% CI=1.1-8.0) for minor malformations. This finding is based on very small numbers, however, and on multiple occurrences of two minor defects in two families.

From data obtained during the telephone interview, the adjusted odds ratio for Vietnam service and spontaneous abortion was 1.3 (95% CI=1.2-1.4). Although an excess among Vietnam veterans was noted across all three trimesters, only the association in the first trimester was significant. There was no attempt to confirm this end point by using hospital records. No significant differences were observed for the reproductive outcomes of induced abortion (AOR=1.0, 95% CI=0.9-1.2), stillbirths (AOR=0.9, 95% CI=0.7-1.1), ectopic pregnancies (AOR=1.0, 95% CI=0.7-1.2), or childhood cancers (OR=1.5, 95% CI=0.8-2.8).

In addition to the reported excess of total birth defects in the telephone interview, Vietnam veterans also reported more neural tube defects and hydrocephalus than the non-Vietnam veterans. A second substudy was undertaken to examine the increase in cerebrospinal malformations (CSMs). In this substudy, an attempt was made to obtain hospital records for all of the offspring identified in the telephone interview as meeting one of the following criteria: (1) offspring with a CSM as reported by a veteran, (2) those with a reported condition that suggested a CSM, or (3) all reported stillbirths.

Of the 403 offspring reported to have a CSM, 109 were ineligible, 14% (N=58) because of conception prior to father's military service, and 12.6% (N=51) were classified

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as miscarriages. Again, the issue of the impact of spontaneous abortion on rates of birth defects is introduced.

The CSM substudy was limited by a poor response rate among the non-Vietnam veterans. The sample thus consisted of 127 offspring of Vietnam veterans (82.5%) and 94 children of the non-Vietnam veterans (67%). Compared with fathers who participated in the CSM study, nonparticipants were more likely to be nonwhite, less educated, unmarried, younger at the time of their child's birth, and have lower general technical test scores. There were also differences in paternal covariates between the participating cohorts that could confound the findings. Compared with Vietnam veterans, non-Vietnam veterans were better educated, had higher general technical test scores, were more likely to be married, were older when their child was born, were less likely to consume alcohol, and were more likely to have had a nontactical primary military occupational specialty (MOS) in the Army, and to have served in both the later and earlier time periods.

The number of CSMs reported by the veterans that were verified by hospital records was examined separately by stillbirth and live birth status. Among the reported stillbirths, five CSMs were documented among children of Vietnam veterans and six among the non-Vietnam veterans. Ten of these 11 CSM had not been reported by the veterans. Positive predictive values derived from this analysis were 6.5% for Vietnam veterans and 8.1% for non-Vietnam veterans.

Among the live births, 21 of 49 reported CSM cases were noted on hospital records for the Vietnam veterans; 6 of the reported 20 cases were observed among the non-Vietnam veterans group, yielding positive predictive values of 42.9% and 30%, respectively.

Tables 35 and 36 in the unpublished technical report of the study list the fathers' descriptions of the birth defects in their offspring obtained through the telephone interview by cohort (Centers for Disease Control Vietnam Experience Study, 1989). It was intriguing to note that among Vietnam veterans, for 22 of the 55 reported cases of birth defects, the hospital record finding was "none." In five additional cases, the hospital record finding was "none of the nervous system." Of these 27 nondocumented reports of birth defects, 3 cases were reported as having died within the first year of life. Death during the first year of life was also reported for one of the eight unverified CSMs in the non-Vietnam veterans cohort.

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In summary, the CDC investigators concluded that for "most reproductive and child health outcomes studied, Vietnam veterans were more likely to report an adverse event than were non-Vietnam veterans." In the two substudies conducted to compare rates that were identified through hospital records, no significant differences in adverse outcomes between the two cohorts were determined. However, the ability of this study to address the issue of 2,3,7,8-TCDD exposure and reproductive outcome is severely limited. The question of bias still remains in the two substudies. In addition, exposure was defined as service in Vietnam, which does not provide much insight into the question of the reproductive toxicity of 2,3,7,8-TCDD.

7.13.15.3. *American Legion Study*

The relation of self-reported exposure to Agent Orange and reproductive outcomes was part of a study conducted among 6,810 American Legionnaires who had served during the Vietnam war (Stellman et al., 1988). Information was obtained through a questionnaire mailed to 2,858 veterans (42%) who had served in Southeast Asia and 3,933 veterans (58%) who had served elsewhere. No association between Agent Orange exposure and difficulty with conception, time to conception of the first child, or infant birth weight was observed. However, the proportion of spontaneous abortion was significantly higher among the spouses of veterans who served in Vietnam (7.6%) compared with controls (5.5%) ($p < 0.001$). These figures were well below the background rate for recognized spontaneous abortion (15-20%) in the general population.

The significance of these findings is limited by the lack of verification of self-reported exposure, the low response rate, the lack of outcome verification through medical records, and the selection of veterans from the American Legion organization, as they may not be representative of all veterans who served during the Vietnam conflict.

7.13.15.4. *Boston Hospital Study*

A case-control study to investigate the relationship between paternal military service in Vietnam and risk of spontaneous abortion was conducted at Boston Hospital for Women (Aschengrau and Monson, 1989). Cases identified through hospital records were

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spontaneous abortions ≤ 27 weeks gestation that occurred between July 1976 and February 1978 (N=201). Paternal identifying information from the hospital birth records was linked with national and state military records to identify those fathers who had served in Vietnam. Frequency of service in Vietnam was compared for cases and all full-term live birth controls (N=1,119) born at the hospital during this time period. No association was detected (OR=0.88, 95% CI=0.42-1.86).

These same investigators conducted a subsequent study that expanded the outcomes examined to include late adverse pregnancy outcomes (Aschengrau and Monson, 1990). Case infants identified through hospital records included 857 congenital malformations, 61 stillbirths, and 48 neonatal deaths that occurred at the hospital during August 1977 and March 1980. "Exposure" was defined by using the same method as in the previous study. Frequency of paternal service in Vietnam was compared for cases and 998 normal term infant controls. Again, no associations with any of these later adverse outcomes were detected (Table 7-43).

7.13.15.5. *The Ranch Hand Study*

7.13.15.5.1. *Baseline study - 1984.* This initial report of the health of Ranch Hand personnel used cohort status (Ranch Hand vs. comparisons) as the basis for evaluating effects and exposure. This group of exposed veterans included those who served in Vietnam during 1962-1965, when Herbicides Purple, Pink, and Green were sprayed. These herbicides had higher TCDD concentrations (33 ppm, 66 ppm, and 66 ppm, respectively) than Herbicide Orange with 2 ppm TCDD (Lathrop et al., 1984).

The protocol consisted of a comprehensive personal and family health questionnaire and a physical examination, including an in-depth laboratory analysis. The response rates for each phase of the protocol were quite different both within and between cohorts. Participation in the questionnaire phase was 97% (N=1,174) for the Ranch Hands and 93% (N=956) for controls. In the physical examination phase, participation dropped to 87% (N=1,045) for the Ranch Hands and 76% (N=773) for controls.

Nonresponders were "on the average" younger than participants. Ranch Hand enlisted personnel had higher participation rates than officers, and black Ranch Hand officers

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had lower participation rates than nonblack officers. The difference in the response rates for the physical examination phase of the protocol was ascribed partially to the active encouragement of the Ranch Hand Association for participation and the intense media coverage that the study received. The authors stated that the "majority" of reasons given for nonparticipation were "no time-no interest" and passive refusal.

The reproductive outcomes evaluated in this phase of the study were ascertained through questionnaires obtained from both the veterans and their spouses or partners. A total of 7,399 conceptions were analyzed in this report. There were 3,293 conceptions among 1,174 Ranch Hands and 4,106 among the 1,531 controls.

No significant differences were reported for four "measures of fertility": (1) the number of childless marriages, (2) the number of couples having achieved their desired family size, (3) the number of childless marriages per total number of marriages, and (4) the number of conceptions per years spent together, which included nonmarital relationships. The fertility analysis was performed on the total number of conceptions reported and was not adjusted for any confounding variables. Moreover, exposure in this analysis was defined by a simple dichotomy of Ranch Hands versus controls.

To examine the relationship between Ranch Hand status and spontaneous and induced abortion, stillbirths, and live births, exposure was stratified by pre- and post-Southeast Asia (SEA) service. The unadjusted analysis indicated that Ranch Hands had increased spontaneous abortion rates in both pre-SEA duty ($p=0.06$) and post-SEA duty ($p=0.13$). The report qualified this by stating that these inferences based on analyses that were not adjusted for "key factors affecting pregnancy outcome are of questionable value," although no similar qualification was given for the fertility analysis. After adjustment for maternal age, smoking, and alcohol use and paternal age, no significant difference was observed for spontaneous abortion.

Among the live births with complete data obtained to allow for adjustment of cofactors, no difference in risk of prematurity was noted. However, the estimate of gestational age was based on parental report, which is not a sensitive measure of gestational length, and it was not clear whether prematurity was analyzed as only a dichotomous variable (<37 weeks, ≥ 37 weeks) or as a continuous variable. No analyses for birth weight

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differences by exposure status were performed, which was unfortunate given the controversy regarding the finding of lower birth weight among infants exposed to PCDDs and PCDFs (Rogan, 1979; Yen et al., 1989; Rogan et al., 1988).

Unadjusted analyses were conducted to examine the relationship between exposure and neonatal death, infant death, physical handicaps, birth defects, and learning disabilities. These analyses were stratified by pre- and post-SEA service periods. The results indicated that Ranch Hands were more likely to report physical handicaps ($p=0.07$), birth defects ($p=0.08$), and neonatal deaths ($p=0.02$) in the post-SEA analysis. After adjustment for the maternal and paternal covariates described above, the relationship with birth defects achieved statistical significance ($p=0.04$); the other relationships were not statistically significant.

Twelve of the 76 birth defects reported to have occurred among the Ranch Hands after post-SEA service were skin anomalies (ICD Code 757). When these anomalies are excluded, this relationship is no longer statistically significant ($p=0.14$) although "still of interest."

Finally, semen samples from Ranch Hands ($N=560$) and controls ($N=409$) were analyzed for sperm count and morphology. The response rates for this parameter were 72.5% and 76.5%, respectively, although some of the samples submitted were ineligible for analysis because of prior vasectomies and orchiectomies. Linear regression techniques examined sperm count as a continuous variable and percentages of sperm with abnormal morphology as dependent variables. Independent variables were age and exposure to industrial chemicals. No differences in either parameter were identified.

This finding contrasts with the semen analysis results obtained among 324 Vietnam veterans and 247 non-Vietnam veterans in the Vietnam Experience Study (Centers for Disease Control Vietnam Experience Study, 1988a). That analysis indicated that Vietnam veterans had significantly lower sperm concentrations ($OR=2.3$, 95% $CI=1.2-4.3$), below the clinical reference value (20 million cells/mL), than the non-Vietnam veterans. In addition, Vietnam veterans had a significantly lower average proportion of "normal" sperm heads. These analyses were adjusted for six covariates, although industrial chemicals were not among them.

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7.13.15.5.2. *The Ranch Hand study - reproductive outcomes 1992.* The significant association between Ranch Hand status and birth defects found in the previous study was sufficiently troubling to launch a massive project to verify all reported conceptions and pregnancy outcomes through medical record abstraction. In addition, in 1987 serum 2,3,7,8-TCDD levels were obtained from a subset of Ranch Hands and controls. In 1992, the Air Force released the results of the first study that examined the relationship between direct measure of individual serum 2,3,7,8-TCDD levels and verified reproductive outcomes (Wolfe et al., 1992b). A total of 4,607 conceptions were examined in this study; 2,533 were contributed by 791 Ranch Hands, and 2,074 were contributed by 768 controls.

Ranch Hand personnel were shown to have significantly higher 2,3,7,8-TCDD levels compared with the controls. The median values were 12.8 pg/g and 4.2 pg/g, respectively. The 98th percentile for Ranch Hands was 166.4 pg/g; for controls, 10.4 pg/g. 2,3,7,8-TCDD levels were determined in 1987. These results were used to estimate initial doses received during the veterans' tour in Southeast Asia but not the 2,3,7,8-TCDD level at the time of conception.

The fertility analysis performed in the earlier study was not repeated according to level of serum 2,3,7,8-TCDD, which was a disappointing omission. There was a significant variation in the association between 2,3,7,8-TCDD and miscarriage with time since SEA tour (≤ 18.6 years or > 18.6 years) and time of conception (pre- or post-SEA tour) among Ranch Hands with current 2,3,7,8-TCDD levels > 10 ppt ($p=0.01$) (Table 7-47). This was attributed to the low miscarriage rate among the pre-SEA Ranch Hands with current 2,3,7,8-TCDD levels > 33.3 pg/g lipids. In examining post-SEA conceptions only, a linear trend can be seen for spontaneous abortions and increasing 2,3,7,8-TCDD levels among Ranch Hands who had "late tours" in SEA, i.e., less than or equal to 18.6 years had elapsed between their tour of duty and current 2,3,7,8-TCDD levels. The opposite trend is noted in Ranch Hands with "early tours," i.e., more than 18.6 years had elapsed between the end of duty and the 1987 blood draw. It was concluded that 2,3,7,8-TCDD did not affect the rates of miscarriage because it seemed "implausible that dioxin would act differently in the two groups."

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Table 7-47. Rates of Miscarriage (per 1,000) by Pre- and Post-Vietnam Tour Status and Time Since Tour of Duty, Among 1,475 Ranch Hands with > 10 pg/g Serum Dioxin, Ranch Hand Study, 1992^a

Time of conception	Time since tour (years)	Miscarriage rate (no./n) current dioxin			p-Value
		10-14.9 pg/g	15-33.3 pg/g	> 33.3 pg/g	
Pre-tour	≤ 18.6	142.0 (23/162)	146.8 (32/218)	48.8 (2/41)	0.014 ^b
	> 18.6	123.9 (14/113)	159.4 (33/207)	166.7 (16/96)	
Post-tour	≤ 18.6	92.1 (7/76)	136.6 (22/161)	168.5 (15/89)	
	> 18.6	237.3 (14/59)	198.6 (29/146)	121.5 (13/107)	

^aAdapted from Wolfe et al., 1992b.

^bComparison of pre- and posttour data.

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An alternative explanation might be that there is a relationship, but it cannot be detected by this type of analysis. To evaluate the relationship between 2,3,7,8-TCDD level and spontaneous abortion, 2,3,7,8-TCDD level at the time of conception must be considered. Assuming a half-life of 7 years in humans (Pirkle et al., 1989), it would seem reasonable, for example, to assume that the two groups of Ranch Hands with 2,3,7,8-TCDD levels of 10-14.9 pg/g of lipids with post-SEA conceptions may have had very different 2,3,7,8-TCDD levels at the time their children were conceived. This is possible because the early tour veterans had more time to decrease their body burden of 2,3,7,8-TCDD before their bloods were drawn in 1987 than did their late tour counterparts.

Table 7-43 illustrates the risk estimates for reproductive outcomes in the Ranch Hand study. Interestingly, the only statistically significant associations between 2,3,7,8-TCDD and adverse events (total birth defects, genital anomalies, and urinary system anomalies) occurred among Ranch Hands with 2,3,7,8-TCDD levels of 15-33.3 pg/g of lipids and not among those in the >33.3 pg/g group.

The report stated that the "expected dose-pattern" for 2,3,7,8-TCDD and total adverse reproductive outcomes (miscarriage, tubal pregnancy, other noninduced abortive pregnancy, or stillbirth) is the "linear one in which the highest anomaly rate occurs at the highest levels of dioxin." This statement raises at least two questions. If a linear response is assumed, might this imply that very early pregnancy losses occur at the highest 2,3,7,8-TCDD levels, so that the conceptus would not survive long enough to be clinically recognized? Or, are very early pregnancy losses and clinically recognized spontaneous abortions two separate entities with different thresholds? Such a scenario has been suggested to explain changes in spontaneous abortions observed after exposure to radiation in Hiroshima (Miller and Blot, 1972).

These questions are of interest because the rate of each of these end points may directly affect the rates of all subsequent reproductive outcomes that are available for examination. The miscarriages assessed in this study are most likely late spontaneous abortions, as 99.6% of reported miscarriages were verified through medical records.

An analysis in which the 1987 dioxin levels are used to estimate dioxin level at time of conception would be a worthwhile effort. If a relationship between paternal 2,3,7,8-

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TCDD level and adverse reproductive outcome does exist, this may help determine the dose-response pattern of the relationship.

No evidence was found to support an association between 2,3,7,8-TCDD and total adverse outcomes. These findings should be viewed with caution in view of the above comments concerning the unexplored area of events early in gestation.

Overall, there was little convincing evidence to support an association between birth weight, examined as both a continuous variable and dichotomized ($<2,500$ g or $\geq 2,500$ g), and paternal 2,3,7,8-TCDD level. Analyses that adjusted for covariates included maternal and paternal age, maternal alcohol use and smoking, and race of the father. No assessment of 2,3,7,8-TCDD and prematurity was reported.

The potential association between cohort status and birth defects was examined for all defects combined and 12 additional categories of malformations. The only categories with sufficient numbers of verified post-SEA cases to detect a relative risk of 2 were total birth defects (229 cases among 1,045 Ranch Hands and 289 cases among 1,602 controls) and musculoskeletal deformities (132 cases among Ranch Hands and 180 among controls).

A significant variation was observed in the association between total birth defects ($p=0.03$), defects of the respiratory system ($p=0.03$), and urinary system abnormalities ($p=0.04$), by Ranch Hand versus control status with time of conception (pre- or post-SEA). All of these findings were due to a lower rate among Ranch Hands in the pre-SEA conceptions and a higher rate among the post-SEA conceptions for the Ranch Hands.

Analyses of birth defects by 2,3,7,8-TCDD level did not find any "consistent patterns" to support an association. For example, among children of enlisted flying and enlisted ground personnel, children of Ranch Hands with 2,3,7,8-TCDD levels ≤ 10 pg/g lipids had higher rates (433 per 1,000 and 317 per 1,000) than children of controls with background 2,3,7,8-TCDD levels < 10 pg/g lipids (229 per 1,000). However, rates of children of enlisted ground personnel with 2,3,7,8-TCDD levels ≥ 33.3 pg/g lipids were not significantly elevated. Again, these analyses were not based on 2,3,7,8-TCDD level at time of conception. Moreover, if the higher 2,3,7,8-TCDD levels were related to early pregnancy loss, these results would make more biological sense, as the abnormal conceptuses due to 2,3,7,8-TCDD exposure would have been lost before the pregnancy was recognized.

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There was also a significant association between 2,3,7,8-TCDD levels and neonatal death (OR=5.5, 95% CI=1.5-20.7). Insufficient numbers (N=13) precluded the calculation of an adjusted odds ratio for this finding.

Finally, no association was detected between 2,3,7,8-TCDD level and either sperm count or percentage of abnormal sperm. These analyses were based on semen samples that had been collected in 1982.

7.13.15.6. *Reproductive Hormones*

In laboratory rats, 2,3,7,8-TCDD has been related to decreased testosterone levels with evidence that dioxin decreases testosterone synthesis (Kleeman et al., 1990; Mebus et al., 1987; Moore and Peterson, 1988; Moore et al., 1985).

A reported symptom of men who were exposed to 2,3,7,8-TCDD-contaminated materials as a result of daily exposure and industrial accidents is reduced libido (Baader and Bauer, 1951; Bauer et al., 1961; Suskind et al., 1953). Two independently conducted studies of West Virginia TCP workers noted that exposed study subjects also reported this condition approximately 50% more often than either the unexposed controls or individuals without chloracne (Moses et al., 1984; Suskind and Hertzberg, 1984). Endocrine studies or evaluations of conditions or situations that may lead to a reduction in libido were not conducted.

In the NIOSH study of TCP production workers, questions regarding libido were not asked, however, reproductive hormone levels were measured and related to serum 2,3,7,8-TCDD levels. In linear regression analyses, serum 2,3,7,8-TCDD was positively and significantly related to serum levels of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) and inversely related to total testosterone after adjustment for potential confounders ($p < 0.05$) (Egeland et al., 1994). The prevalence of abnormally low testosterone was two to four times higher among workers with serum 2,3,7,8-TCDD levels of 20-75 pg/g (OR=3.9, 95% CI=1.3, 11.3), 76-243 pg/g (OR=2.7, 95% CI=0.9, 8.2), or ≥ 244 pg/g (OR=2.1, 95% CI=0.8, 5.8) than among unexposed referents (4.8%) (mean serum 2,3,7,8-TCDD = 7 pg/g). Workers in these same serum 2,3,7,8-TCDD quartiles had a higher prevalence of abnormally high LH than workers with serum 2,3,7,8-TCDD levels of

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244 pg/g to 3,400 pg/g, but the differences between each serum 2,3,7,8-TCDD category and referents were not significant.

The Ranch Hand veterans study provides the only other human data available that evaluated the relationship between serum 2,3,7,8-TCDD and testosterone (Roegner et al., 1991). Ranch Hand veterans with current serum dioxin levels exceeding 33.3 pg/g were reported to have a lower mean total serum testosterone level (515.0 ng/dL) than the nonexposed comparison group (525.2 ng/dL), but the difference was statistically nonsignificant. No association was observed with FSH and LH. This same exposure group had a nearly fourfold excess of unspecified testicular abnormalities.

Testosterone, FSH, and LH were also measured in U.S. Army veterans and non-Vietnam veterans (Centers for Disease Control Vietnam Experience Study, 1988a). No significant differences in hormone means were noted between the two groups. Additionally, the proportions of values outside the reference range were also similar.

7.13.15.7. *Comment*

The human data offer some evidence of alterations in male reproductive hormone levels associated with substantial occupational exposure to 2,3,7,8-TCDD. The results support the animal literature in which dioxin-related effects have been observed on the hypothalamic-pituitary-Leydig-cell axis and on testosterone synthesis.

7.14. NONCANCER EFFECTS OF INGESTION OF RICE OIL CONTAMINATED WITH POLYCHLORINATED DIBENZOFURANS, QUATERPHENYLS, AND BIPHENYLS IN JAPAN (YUSHO) AND TAIWAN (YU-CHENG)

This section briefly reviews the noncancer effects observed in Yusho (Japan) and Yu-Cheng (Taiwan) victims, individuals exposed, by ingestion, to large concentrations of compounds structurally related to dioxins, namely polychlorinated dibenzofurans, quaterphenyls, and biphenyls. The history of each incident, the chemicals in question, and levels of exposure are described in this chapter. In addition, other reviews have summarized the numerous papers dedicated to Yusho and Yu-Cheng (Lü and Wong, 1984; Kuratsune, 1989; Rogan, 1989).

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Reports describing effects among individuals who ingested the contaminated rice oil both in Taiwan and Japan are limited to acute rather than chronic effects. Studies have not comprehensively evaluated long-term effects despite the facts that almost 25 years have passed since the Yusho incident and 15 years since the Yu-Cheng incident and that serum levels of some contaminants are available for both populations. Recent epidemiologic studies have concentrated on the development of offspring of Yu-Cheng mothers. These children were exposed in utero at the time the contaminants were ingested or were conceived after the poisoning and were exposed to residual contaminants transplacentally or through breast milk (Chen et al., 1992; Guo et al., 1992; Lai et al., 1993; Hsu et al., 1993; Guo et al., 1993).

7.14.1. Acute Effects in Adults and Children Directly Exposed to Contaminated Rice Oil

In both groups, the most notable acute effects are dermatologic and neurologic signs and symptoms of fatigue, headaches, and gastrointestinal distress (nausea, vomiting, abdominal pain) (Kuratsune, 1989; Rogan, 1989).

7.14.1.1. *Yusho*

The initial recognition of Yusho occurred in 1968. As of 1983, a total of 2,060 individuals were identified as part of the Yusho population (Masuda et al., 1985). Five years after exposure ended, the mean concentrations of PCBs in the adipose tissue, liver, and blood of Yusho cases were 1.9 ppm, 0.08 ppm, and 6.7 ppb (Masuda et al., 1985), respectively, which were about twice the levels in the control group. Adipose tissue levels of PCDFs ranged from 6 to 13 ppb (Masuda et al., 1985). Sixteen years after exposure, mean PCQ level in adipose tissue of Yusho cases was 207 ppb, approximately 100 times the level in Japanese controls (Kashimoto et al., 1985).

In addition to the major health effects, other possible outcomes were examined. Effects observed shortly after exposure included elevated triglyceride levels and effects on female reproductive hormones noted by changes in menstrual and basal body temperature patterns and lowered excretion of estrogens and pregnanediol in exposed women (Kuratsune, 1989). However, fertility and other measures of reproductive function were not evaluated.

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Evidence of chronic bronchitis and respiratory infections still remained 14 years after exposure ended (Nakanishi et al., 1985). However, more than 10 years postexposure, PCB levels were not related to levels of serum triiodothyronine (T_3), thyroxine (T_4), and thyroxine-binding globulin (TBG) (Murai et al., 1987). Although the liver is the suspected target organ for halogenated hydrocarbons and marked proliferation in the endoplasmic reticulum was observed, clinical evidence of liver damage, such as alterations in liver enzymes or liver disease, was not observed (Kuratsune, 1989).

Dermatologic effects were the most evident signs, characterized by hyperpigmentation of the nails, gingivae, and face and by nail deformities, horny plugs, comedones, acneform eruptions, cysts, and other abnormal keratotic changes (Urabe and Asahi, 1985). Acneform eruptions were observed on the face, cheeks, auricles, retroauricular areas, inguinal regions, and external genitalia (Urabe and Asahi, 1985). More than 80% of Yusho cases experienced one or more dermatologic effects (Kuratsune, 1989), which diminished in severity over time (Urabe and Asahi, 1985).

Ophthalmologic effects were characterized by swelling and hypersecretion of the meibomian glands and pigmentary changes of the conjunctiva (Kuratsune et al., 1972). More than 80% of Yusho cases exhibited ocular changes, which, in some cases, appeared to persist 15 years after exposure ended (Kuratsune, 1989).

Thirty percent of the cases reported having at least one symptom consistent with neurologic involvement, such as limb paresthesia and spasms, weakness, headaches, and fatigue (Kuratsune, 1972). As summarized by Kuratsune (1989), Kuriowa et al. (1969) found mostly sensory deficits, identified through slowed nerve conduction velocities in 23 cases. Follow-up of these cases indicated that the neurologic symptoms disappeared over time; however, conduction velocities were not repeated.

A number of studies examined the immune status of Yusho cases (Kuratsune, 1989). Significant decreases in mean IgA and IgM and increases in IgG were noted in 28 cases tested in 1970 ($p < 0.05$) (Nakanishi et al., 1985). Within 2 years, means levels of all three immunoglobulins returned to normal. Small increases in the percentage of CD4 cells, small decreases in the percentage of CD8 cells, and enhanced lymphocyte stimulation were also noted in Yusho cases (Nakanishi et al., 1985).

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Studies of offspring of Yusho cases have been limited to descriptions of effects on newborns exposed in utero. An early description of 13 children born to exposed mothers noted two stillborn infants, one of whom was diffusely and deeply hyperpigmented (Rogan, 1982). Neonates described in other reports were darkly pigmented and had marked secretions of the conjunctival palpebra, gingival hyperplasia, hyperkeratosis, calcification of the skull, low birth weight, and natal teeth (Yamashita and Hayashi, 1985). The abnormal pigmentation disappeared after 2 to 5 months. No other physical abnormalities (neurologic, cardiovascular, or malformations) were identified.

7.14.1.2. *Yu-Cheng*

The initial recognition of Yu-Cheng occurred in 1979. As of 1983, approximately 2,000 individuals were found to have been exposed to the contaminated rice oil. Within the first year of exposure, mean serum PCB, PCDF, and PCQ levels for 15 cases were 60 ppm (range 4-188 ppm), 0.14 ppb (range <0.005-0.27 ppb), and 19.3 ppb (range 0.9-63.8), respectively (Kashimoto et al., 1985). Analysis of PCB levels in 1980-1981 in 165 cases (mean 38 ppb, range 10-720) (Rogan, 1989) and in 1985 in 32 cases (mean 15.4 ppb, range 0.6-86.8) (Lundgren et al., 1988) suggested that some PCBs were being eliminated. It is not clear from the reports if the samples were drawn from distinctly different individuals or included some of the same individuals.

The ophthalmologic and dermatologic changes observed in Yu-Cheng cases were very similar in character and anatomical distribution to those noted in Yusho cases (Lü and Wu, 1985). In 89 cases followed for up to 17 months, dermatologic conditions of 38% of the cases improved, 54% remained the same, and 7% showed deterioration of their conditions (Lü and Wong, 1984).

Like Yusho cases, Yu-Cheng cases examined within 2 years of exposure for nerve function exhibited slowing of sensory nerve conduction. They also exhibited motor nerve slowing and mixed deficits (Chen et al., 1981, 1983, 1985; Chia and Chu, 1984). Twenty percent of a population of 27 individuals also had abnormal EEGs (Chia and Chu, 1984). However, the authors suggest that any correlation between PCB exposure and the abnormal EEGs may be spurious due to low PCB levels in the cerebrospinal fluid (0.5-2.3 ppb)

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(measured in four subjects), despite much higher blood PCB levels of 48-64 ppb. A sample of 28 individuals with peripheral neuropathy in 1980 was reexamined in 1982 and was found to have normal EEGs and some recovery of sensory and motor nerve conduction velocity (Chia and Chu, 1985).

In 1981, immunologic function was assessed on different subsets of Yu-Cheng cases and summarized by Lü and Wong (1984). In 30 cases compared with unexposed controls, both IgA and IgM were significantly decreased, while IgG did not differ from controls. In this same group, percentages of active T cells and T cells were significantly increased ($p < 0.05$), while total lymphocyte count and percentage of B cells were unchanged. Significant increases in helper T cells (T4) but not suppressor T cells (T8) were also observed. In another group of cases, response to lymphocyte-stimulating mitogens was mixed and the findings unclear. In 143 cases, reaction to streptococci antigen appeared to be significantly ($p < 0.05$) depressed relative to controls.

Altérations in porphyrin levels and liver enzymes have been identified as acute reactions to exposure to halogenated polycyclic hydrocarbons, including PCBs. Porphyrin levels were measured in two exposed groups (Chang et al., 1980; Gladen et al., 1988). In 1980, statistically significant elevations in 24-hour urinary excretion of uroporphyrin (exposed = $41.23 \mu\text{g} \pm 24.56$; unexposed = $13.57 \mu\text{g} \pm 11.76$, $p < 0.01$) and δ -aminolevulinic acid (exposed = $1.002 \text{ mg} \pm 0.600$; unexposed = 0.715 ± 0.337 , $p < 0.05$) were noted among 69 subjects (Chang et al., 1980). Coproporphyrin and porphobilinogen levels were increased in the exposed group but were not significantly elevated. The second study group was composed of 75 children born between June 1978 and March 1985 to mothers who ingested contaminated rice oil (Gladen et al., 1988). Spot urines were collected in 1985. Mean total porphyrin (exposed = $95.2 \mu\text{g/L}$; unexposed = $80.7 \mu\text{g/L}$) and coproporphyrin (exposed = $72.4 \mu\text{g/L}$; unexposed = $59.8 \mu\text{g/L}$) excretion was elevated in the exposed, possibly due to extremely high levels ($> 200 \mu\text{g/L}$) in eight exposed children and two controls (Rogan et al., 1988). However, no porphyria cutanea tarda, a severe form of porphyria, was observed in either group of children. Moderate, but statistically significant, increases were observed in AST and ALT levels in 23 cases tested 1 year after exposure (Lü and Wong, 1984). LDH and bilirubin levels were not significantly elevated. As in Yusho

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cases, triglyceride levels were significantly increased by approximately twice the level in unexposed controls.

7.14.1.3. *Effects Observed in Offspring of Yu-Cheng Cases*

A concerted effort has been made to evaluate the overall development of children exposed prenatally to contaminated rice oil ingested by their mothers. Two studies are currently being conducted: one to evaluate children and controls born between 1978 and 1985, and the second, to evaluate children born in 1985 and later. The purpose of the two studies is to determine if there are differences among children exposed in utero by the mothers' ingestion of the contaminated rice oil or shortly afterward and children conceived 6 or more years after direct exposure ended (Chen et al., 1992, 1993).

In terms of musculoskeletal development, several studies have documented delays and abnormalities (Yu et al., 1991; Rogan, 1989; Guo et al., 1993; Chen et al., 1993). In one of the first studies conducted in 1985, Rogan and colleagues (1988) examined 117 children born since the mothers' exposure in 1979 and 107 unexposed controls. In this study, babies of exposed mothers were consistently smaller and shorter at birth than controls and had similar characteristics: natal teeth, neonatal conjunctivitis, and pigmentation. Exposed mothers reported a mean birth weight 479 g lower than that reported by control mothers; no validation of these reports using medical records was undertaken. As older children, they exhibited a variety of signs and symptoms: fragile chipped teeth and gum hypertrophy, pigmented and deformed fingernails and toenails, and abnormal lung auscultation. In this same study, neurologic developmental assessments were also conducted to evaluate development (Yu et al., 1991). Forty-nine percent of Yu-Cheng children compared with 22% of controls were developmentally delayed in 32 of 33 developmental milestones, 12% had clinical evidence of developmental or psychomotor delays compared with 2% of controls, and 7% of Yu-Cheng children versus 3% of controls had speech problems. These delays were noted at all ages and persisted over 2 years of testing. Delay was greater in children of smaller size and in children who had exhibited neonatal symptoms of intoxication.

In 1991, the musculoskeletal development of 56 Yu-Cheng children (age range 6-10 years) and their matched controls was again assessed. Only children born first after the

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mother's exposure were shorter in stature and had decreased lean muscle mass and soft tissue content (Guo et al., 1992). A subsequent examination of 110 Yu-Cheng children and 108 controls, ages 8 through 14 years, found Yu-Cheng girls to be significantly shorter than controls, matched to the exposed children by age, sex, maternal age, parents' combined educational level, occupation, and neighborhood (Guo et al., 1993). As measured by the Tanner scale, sexual maturation was not slower in Yu-Cheng boys or girls. However, Yu-Cheng boys aged 11-14 had significantly shorter penis length, but testicular and scrotal development did not differ from the controls. Penile length was not related to sexual development as measured by the Tanner scale.

With a validated and standard battery of tests, cognitive and behavioral development of Yu-Cheng offspring was studied yearly from 1985 through 1991. Throughout the testing period, Yu-Cheng children scored consistently lower in the Stanford Binet IQ (SB-IQ) and 4-5 points lower than controls (with the same matching criteria as the above study) in three subscales of the Wechsler Intelligence Scale for Children, Revised (WISC-R): verbal IQ (VIQ), performance IQ (PIQ), and full-scale IQ (FIQ) (Chen et al., 1992; Lai et al., 1993). Yu-Cheng children are also reported to exhibit more health, habit, and behavior problems as reported by parents responding to the Rutter's scale and to manifest higher activity based on teachers' responses to the Teacher's Activity Check List (Hsu et al., 1993).

Analysis of physical and cognitive development began in October 1991 of 104 children whose mothers were exposed and 109 children whose fathers but not mothers were exposed and of three matched controls born after 1985 (Guo et al., 1993). Like children born before 1985, the later-born children were shorter in stature and lower in weight than controls, although the authors indicate that the differences have disappeared. Yu-Cheng children are reported to have higher activity levels but do not have temperament, physical, habit, or behavioral problems. Overall, scores on all tests in paternally exposed children were similar to those of the controls. However, maternally exposed children scored lower on the SB-IQ and on all subscales of the WISC-R.

7.14.1.4. *Comment*

Data from Yusho and Yu-Cheng strongly implicate direct ingestion of contaminated rice oil with numerous acute effects of the skin and peripheral and central nervous systems. The data on immunologic function suggest possible effects, but the numbers of subjects in the various studies were too small to determine an exposure-response relationship. Similarly, data on elevated triglyceride and liver enzyme levels are from a small number of cases and, therefore, the relationship between exposure and the effect is unclear. Furthermore, there are few data to evaluate the long-term effects of these very high exposures. Because many of the 4,000 individuals who were exposed in these two episodes were children, longitudinal studies would be invaluable in assessing the long-term health effects of these exposures.

One difficulty in evaluating the various reports relating to Yusho and Yu-Cheng is the inability to determine if the effects are generalizable to the entire exposed population. It is the impression of this reviewer that many of the cases reported in the literature were those that exhibited the severest signs and symptoms and probably had the highest body burden of contaminants. Some reports included small numbers of cases and controls, relative to the size of the exposed population, and others had no controls. Finally, while much work has gone into determining the severe acute effect, it would be interesting to know what chronic, age, and gender-specific effects are now being exhibited in the approximately 4,000 individuals directly exposed to the contaminated rice oil.

Another difficulty is the presence of several chlorinated hydrocarbons in the contaminated oil, which results in uncertainty as to which contaminant or combination of contaminants is responsible for the noted effects. The data on the offspring of exposed Yu-Cheng mothers and fathers are fascinating and disturbing. It is apparent that parental exposure--more specifically, maternal exposure--to PCBs, PCDFs, and PCQs is directly linked to in utero exposure of the fetus, affecting cognitive, selective musculoskeletal, and possibly, sexual development of the offspring. The ongoing assessment of development in the Yu-Cheng children is imperative to determine the long-term consequences of these exposures on the children's future quality of life and to emphasize the lesson that parental exposures greatly affect the fetus. Prospective studies of fertility and reproductive

experience in these offspring would provide insight into possible intergenerational effects of exposure to these compounds.

7.15. SUMMARY

The data presented in this chapter describe nonmalignant effects in epidemiologic studies of populations with the potential for exposure to chemicals contaminated with 2,3,7,8-TCDD. The purpose of this review is to highlight the salient results of the studies and to assess whether the observed effect was related to exposure to 2,3,7,8-TCDD.

In summary, based on the results of two or more studies, recent evidence suggests that chloracne, elevated GGT levels, an increased risk of diabetes, and altered reproductive hormone levels (luteinizing hormone, follicle-stimulating hormone, and testosterone) appear to be long-term consequences of exposure to 2,3,7,8-TCDD (Table 7-48). In contrast, multiple studies show possible acute effects but few chronic exposure-related effects for dermatologic end points other than chloracne, such as eye lid cyst, hypertrichosis, hyperpigmentation, actinic keratosis, and Peyronie's disease; for liver diseases such as cirrhosis, liver enlargement, and hepatic enzyme levels (LDH, AST, ALT, and D-glucuric acid) other than GGT; and for porphyrias and renal, neurologic, and pulmonary disorders. Circulatory and heart diseases, reproductive outcomes, immunologic disorders, lipid levels, and thyroid function require further study before their respective relationships to 2,3,7,8-TCDD can be more definitively assessed. Studies are under way in several locations that may yield useful information regarding these outcomes.

In the best of circumstances when reviewing studies, it would be ideal if all studies examined the same end points in the same manner, had sufficient statistical power to detect truly positive findings, had good estimates of extent of exposure, and had consistent exposure-response relationships. In the absence of ideal situations, epidemiologists examine the evidence of studies using "six tenets of judgment" (Hatch and Stein, 1986; Hill, 1965) to assess the collective wisdom of the study results. These tenets are: temporality (sequence of events), degree of exposure, strength, consistency and specificity of association, and biologic plausibility.

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Table 7-48. Summary of Effects Observed in Humans

System	Acute	Chronic
Dermatologic	conjunctivitis red and irritated eyes blepharitis	chloracne
Liver	temporary enlargement	— ^a
Liver enzymes	↑ GGT ↑ AST ↑ ALT ↑ D-glucaric acid excretion	↑ GGT
Urinary porphyrins	+/- ^b PCT uroporphyrin urobilinogen coproporphyrin	--
Lipids	↑	--
Cholesterol	↑	--
Triglycerides	↑	--
Other GI	+/- RUQ ^c pain loss of appetite nausea	--
Thyroid	↑ T ₄ ↑ T ₄ /TBG in some studies	--
Diabetes	no data	+/-
Immune	no data	+/- ↑ NK cells +/- ↑ IgA
Neuro	+/- ↓ libido ↑ irritability ↑ nervousness ↓ pin prick sensation	--
Circulatory	--	--
Pulmonary	irritation	--
Renal	--	--
Reproductive	--	+/-
Reproductive hormones	no data	↑ LH in ♂ ↑ FSH in ♂ ↓ testosterone in ♂
Chromosome	+/-	no data

^aNo effect noted.

^bSome positive and some negative studies.

^cRight upper quadrant.

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In evaluating many of the studies that examined the relationship between serum 2,3,7,8-TCDD, there are several common threads that bear noting. They will be discussed first to avoid repetition throughout the summary.

In terms of temporality, all studies reviewed in this chapter were conducted after the presumed exposure occurred. Some of the studies were completed shortly after the exposure, as in Seveso (Mocarelli et al., 1986; Filippini et al., 1981); others were conducted many years after the groups' last exposure to evaluate the more chronic health outcome (Suskind and Hertzberg, 1984; Moses et al., 1984; Bond et al., 1987; Roegner et al., 1991; Assennato et al., 1989; Calvert et al., 1991, 1992, 1993; Alderfer et al., 1992; Sweeney et al., 1990; Egeland et al., 1994; Sweeney et al., 1992; Webb et al., 1989; Ott et al., 1993b). One dilemma in assessing the effect of past exposures is ascertaining whether an effect observed many years postexposure is due to the exposure itself or to an exposure or event that occurred during the intervening period. Another problem is determining what, in the analysis, the investigator considered the most important of the possible confounding exposures. Finally, restricting examination of events to those that occurred after the exposure does not in and of itself satisfy this time order criterion. Several factors must be considered, such as the half-life of the contaminant in the body and the concentration at the time of the event. Consistency in the results of similarly designed studies of 2,3,7,8-TCDD-exposed populations should help strengthen the conclusion of an effect or no effect.

Determination of the extent of exposure throughout the studies was varied. When the risk of disease increases with the dose or gradient of exposure, the evidence for causation is strengthened. It should be emphasized that there are many possible dose-response patterns, which may result in different threshold levels for different end points. Due to the exposure misclassification bias present in most of the dioxin research, with the exception of a few studies, it is not valid to attempt to determine dose-response relationships. To summarize, four studies evaluated the relationship between nonmalignant effects and body levels of 2,3,7,8-TCDD: the Ranch Hand study of U.S. Air Force personnel (Roegner et al., 1991); the study of 50 Missouri residents (Webb et al., 1989); the evaluation of the BASF accident cohort (Ott et al., 1993b); and the NIOSH study of 281 TCP production workers (Sweeney et al., 1989, 1990, 1993; Calvert et al., 1991, 1992, 1993; Alderfer et al., 1992; Egeland et

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al., 1994). In 1988, the workers in the NIOSH study had the highest serum levels (mean = 220 pg/g) in these four studies.

In two populations, Seveso and U.S. Army veterans (Mocarelli et al., 1991; Centers for Disease Control Veterans Health Studies, 1988), serum 2,3,7,8-TCDD levels were measured, but the levels were not used to examine dose-response relationships. In the Veterans Health Studies, more than 99% of the serum 2,3,7,8-TCDD levels of the sample of both Vietnam and non-Vietnam veterans were at the background level (4 pg/g). Therefore, comparisons were made between the two groups as a whole.

Serum levels of Seveso residents were obtained for a small proportion (N=20) of the total number of residents of Zone A within 1 year of the reactor release (Mocarelli et al., 1991). The data suggest that the levels may be related to a number of factors, including age (younger children were outside at the time of the release), whether the resident was inside or outside, ingestion of local produce, number of days of residence in the area after the release, to name a few. The data suggest that the potential for substantial exposure was high for individuals residing in the area. The range of levels in the 20 Zone A residents was 820 pg/g to 56,000 pg/g (median = 7,400 pg/g).

The majority of the remaining studies examined the differences between individuals identified as exposed or unexposed or with or without chloracne. Most of these studies did not evaluate other parameters that might explain differences in effects between exposed and unexposed, for example, the length of exposure. However, one study assessed dose-response relationships based on a statistical algorithm of intensity and highest dose of TCDD exposure (Bond et al., 1989).

In terms of the magnitude (or strength) of the association, this criterion refers to the degree to which the measure of association (e.g., odds ratio or relative risk) exceeds the null value of 1. The stronger the association between exposure and effect, the more convincing is the argument for causation. There is no definitive cutpoint to numerically define a meaningful measure of association. Other factors, such as the prevalence of the exposure in the population, affect the significance of the measure. Because so many adverse health conditions are multifactorial in etiology, a general rule of thumb is that a relative risk less than 2 renders a cause-effect relationship less likely.

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A critical element that should always accompany the effect measure is a confidence interval. Placement of an interval around the measure enables quantitation of the result for a more meaningful interpretation. An odds ratio of 30 is quite impressive, but if the 95% confidence interval is 0.9-200, the magnitude of the association is less impressive!

If an association between a factor and a disease is demonstrated across a variety of studies employing different designs and different populations (consistency), then the argument for causation is strengthened. Replication of an association under different conditions decreases the likelihood that confounding is responsible for the observed association. Consistency is a powerful criterion for causation but only when "the variables under test (exposure, outcomes) are similar enough" to justify the comparison of the various studies' findings (Hoffman et al., 1986).

It should also be determined a priori that each study included in the critical evaluation process is in adherence to basic epidemiologic principles governing study design and analysis. Deficient studies with suspect results should be excluded. While this is not to imply that such studies have no worth, as invaluable information has often been derived from these studies that improve on subsequent examinations of the issue, they have no place in the evaluation process. Unfortunately, in studies of 2,3,7,8-TCDD and effects in humans, the probability of exposure misclassification forces exclusion of much of the research to date.

Specificity refers to the uniqueness of the association between a factor and an outcome. If the relationship were absolute, then only factor X would be related to only effect Y. It is indeed rare to encounter this type of association, which renders this criterion generally less useful in the evaluation process.

Finally, according to the criterion of biological plausibility, the observed association between exposure and effect should be consistent with existing theory and information from other scientific disciplines. Certainly one would feel more secure in the causation debate if the biological basis for an observed association can be explained. However, biological implausibility may simply reflect gaps in existing scientific knowledge that could explain the relationship.

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7.15.1. Effects Having a Positive Relationship With Exposure to 2,3,7,8-TCDD

The following section describes those end points for which there is good evidence from two or more studies suggesting an effect of exposure to 2,3,7,8-TCDD.

7.15.1.1. *Chloracne*

7.15.1.1.1. *Temporality.* Chloracne is one of the best known of the medical consequences of exposure to 2,3,7,8-TCDD-contaminated substances. In general, it has been observed in most incidents where substantial exposure has occurred, particularly among TCP production workers (Goldman, 1972; May, 1973; Bleiberg et al., 1964; Bond et al., 1987; Suskind and Hertzberg, 1984; Moses et al., 1984; Zober et al., 1990) and Seveso residents (Reggiani, 1978; Caramaschi et al., 1981; Ideo et al., 1985; Mocarelli et al., 1986; Assennato et al., 1989). As previously stated, chloracne appears within several weeks to months from the time of exposure, often resolving after discontinuation of exposure (Moses et al., 1984; Suskind and Hertzberg, 1984), although for some it may remain for extended periods after exposure ended (Moses et al., 1984).

7.15.1.1.2. *Degree of exposure, consistency of the association.* The amount of exposure necessary for development of chloracne has not been resolved, but studies suggest that high exposure (both high acute and long-term exposure) to 2,3,7,8-TCDD increases the likelihood of chloracne, as evidenced by chloracne in TCP production workers and Seveso residents who have documented high serum 2,3,7,8-TCDD levels (Beck et al., 1989; Fingerhut et al., 1991; Mocarelli et al., 1991; Neuberger et al., 1991) or in individuals who have a work history with long duration of exposure to 2,3,7,8-TCDD-contaminated chemicals (Bond et al., 1989). The absence of substantial chloracne in U.S. Army Vietnam veterans whose mean serum 2,3,7,8-TCDD levels were at background (4 pg/g) (Centers for Disease Control Vietnam Experience Study, 1988d) and U.S. Air Force Ranch Hands whose serum 2,3,7,8-TCDD levels fell intermediate to those of workers and Army Vietnam veterans (Roegner et al., 1991) suggests that there is a higher incidence of the disorder among those with higher serum 2,3,7,8-TCDD levels.

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

7.15.1.1.4. *Specificity of the association.* Chloracne is associated with exposure to other polyhalogenated chemicals, including dibenzofurans, PCBs, naphthalenes, and others (Taylor, 1979). The likelihood of exposure to other polyhalogenated chemicals in the populations studied is probably low, particularly among the Seveso children, whose exposure was to TCP reactant effluents that were primarily contaminated with 2,3,7,8-TCDD. The issue is more relevant in chemical workers, who by virtue of their occupation, have the potential for exposure to other chemicals. Yet, much of the documented chloracne appeared shortly after TCP reactor releases (Ashe and Suskind, 1950; Goldman, 1972; May, 1973) or during TCP or 2,4,5-T production (Bond et al., 1989), suggesting that 2,3,7,8-TCDD was the chloranegenic agent.

7.15.1.1.5. *Biologic plausibility.* Animal studies have been effective in describing the relationship between 2,3,7,8-TCDD and chloracne, particularly in rhesus monkeys (McNulty, 1977; Allen et al., 1977; McConnell et al., 1978). Subsequent to exposure to 2,3,7,8-TCDD, monkeys developed chloracne and swelling of the meibomian gland, a modified

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sebaceous gland. The histologic changes in the meibomian gland are physiologically similar to those observed in human chloracne (Dunagin, 1984).

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

7.15.1.2. *Gamma Glutamyl Transferase (GGT) Levels*

7.15.1.2.1. *Temporality, degree of exposure, and strength and consistency of association.*

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

In contrast, although background levels of serum 2,3,7,8-TCDD suggested minimal exposure to Army Vietnam veterans, GGT was increased, at borderline significance, among Vietnam veterans compared to non-Vietnam veterans (Centers for Disease Control Vietnam Experience Study, 1988a). In addition, despite the increases observed in some occupational cohorts, other studies of TCP production workers from West Virginia or Missouri residents measured but did not report elevations in GGT levels (Suskind and Hertzberg, 1984; Webb et al., 1989).

7.15.1.2.2. *Specificity.* In clinical practice, GGT is often measured because it is elevated in almost all hepatobiliary diseases and is used as a marker for alcoholic intake (Guzelian,

1985). In individuals with hepatobiliary disease, elevations in GGT are usually accompanied by increases in other hepatic enzymes, e.g., AST and ALT, and metabolites, e.g., uro- and coproporphyrins. Significant increases in hepatic enzymes other than GGT and metabolic products were not observed in individuals whose GGT levels were elevated 10 or more years after exposure ended, suggesting that the effect may be GGT specific. However, in the Seveso male children and those with chloracne (both sexes), ALT was significantly increased concomitantly with GGT within 1 year of the reactor release (Mocarelli et al., 1986; Caramaschi et al., 1981). In a longitudinal analysis, both enzymes returned to normal levels within 5 years after exposure (Mocarelli et al., 1986; Assennato et al., 1989).

These data suggest that in the absence of increases in other hepatic enzymes, elevations in GGT are associated with exposure to 2,3,7,8-TCDD, particularly among individuals who were exposed to high 2,3,7,8-TCDD levels. The fact that investigators observed a decline in enzyme levels in Seveso children but a continued elevation in TCP workers may reflect differences in how exposure occurred (i.e., acute but high doses in Seveso versus continuous or frequent long-term, medium to high doses in TCP workers) or differences in the metabolism of the maturing versus mature system, or a bit of both.

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

Among humans, increased levels of GGT may suggest activity such as cholestases, liver regeneration, or drug or xenobiotic metabolism. In human adults, 2,3,7,8-TCDD is stored in the adipose tissue and has a half-life of approximately 7 years (Pirkle et al., 1989). Continued GGT activity in adults with serum 2,3,7,8-TCDD levels many times over background levels may reflect continuous, low-level metabolism of 2,3,7,8-TCDD.

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

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

7.15.1.3. *Diabetes and Fasting Serum Glucose Levels*

7.15.1.3.1. *Strength and consistency of association.* The epidemiologic evidence for an increased risk of diabetes and for an elevated prevalence of abnormal fasting serum glucose levels is based on two cross-sectional medical studies conducted at least 15 years and up to 37 years after last exposure to 2,3,7,8-TCDD-contaminated chemicals (Sweeney et al., 1992; Wolfe et al., 1992) and supporting evidence from a third study of the BASF accident cohort (Ott et al., 1993). All three studies found that individuals at the highest serum 2,3,7,8-TCDD levels had a slight but statistically significant or borderline significant increased risk for developing diabetes or having an elevated fasting serum glucose. Further study is needed to corroborate the findings of these three cross-sectional studies.

7.15.1.3.2. *Specificity.* Diabetes mellitus is a heterogeneous disorder that is a consequence of alterations in the number or function of pancreatic beta cells responsible for insulin secretion and carbohydrate metabolism. Depending on its type, diabetes has been attributed to endogenous factors such as genetic predisposition, to autoimmune processes, and to exogenous factors such as viral infections (Yoon et al., 1987) and chemical exposures, notably a rat poison (Miller et al., 1978) and some medications (Wilson and LeDoux, 1989), environmental toxins (Diabetes Epidemiology Research International, 1987), age, obesity,

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reduced physical exercise, diet, socioeconomic status, increased insulin resistance by the beta cells, and possibly parity (Pareschi and Tomasi, 1987).

Both the NIOSH and Ranch Hand studies used strict criteria to identify cases. In the NIOSH study, the relationship between exposure to 2,3,7,8-TCDD and possible alterations in glucose metabolism was assessed using two outcome measures: case definition of diabetes and fasting serum glucose levels. Participants met the case definition of diabetes if their fasting serum glucose level was 7.8 mmol/L or greater on two consecutive occasions (National Diabetes Data Group, 1979) or if they reported a positive history of physician-diagnosed diabetes after the date of first employment at the plant. In the Ranch Hand study, diabetic status was assessed by measuring fasting serum glucose and 2-hour postprandial glucose and using a case definition of diabetes. Diabetes was defined as having a verified history of diabetes or an oral glucose tolerance test of ≥ 11.1 mmol/L (200 mg/dL) (Roegner et al., 1991). Both studies used acceptable definitions for epidemiologic studies of diabetes. In the NIOSH study, the definition may exclude some individuals who would have otherwise been classified as diabetic with the glucose tolerance test. However, there should be no differential bias in identifying cases in workers or the unexposed referents.

7.15.1.3.3. *Biologic plausibility.* The effects of 2,3,7,8-TCDD on glucose metabolism have been evaluated only in a few laboratory studies (Zinkl et al., 1973; McConnell et al., 1978a; Gasiewicz et al., 1980; Schiller et al., 1986; Ebner et al., 1988; Gorski et al., 1990). Although these studies suggest that 2,3,7,8-TCDD may alter glucose metabolism, for the most part the animal studies do not corroborate the direction of the findings of two cross-sectional medical studies (Wolfe et al., 1992; Sweeney et al., 1993). Long-term feeding studies to evaluate the relationship between glucose levels, the development of diabetes, and 2,3,7,8-TCDD dose would be helpful in assessing the effect of exposure on the physiologic integrity of the islet cells. In addition, such studies may identify other factors that may affect either directly or indirectly the function of the islet cells.

In summary, both the NIOSH and Ranch Hand studies show that highly 2,3,7,8-TCDD-exposed individuals may be at a slightly greater risk for developing diabetes and

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experience a greater prevalence of elevated fasting glucose levels, which may be a precursor of conversion to a diabetic state. However, in the NIOSH study, the traditional risk factors for diabetes--age, body mass index or weight, and family history of diabetes--appear substantially more influential than 2,3,7,8-TCDD in the development of diabetes. These factors could not be evaluated in the data presented in the publicly available material.

7.15.1.4. Reproductive Hormones

7.15.1.4.1. Strength and consistency of association. Levels of reproductive hormones have been measured with respect to exposure to 2,3,7,8-TCDD in three cross-sectional medical studies. Testosterone, LH, and FSH were measured in TCP and 2,4,5-T production workers (Egeland et al., 1994) and in Army Vietnam veterans (Centers for Disease Control Vietnam Experience Study, 1988d), and testosterone and FSH in Ranch Hands (Roegner et al., 1991). The risk of abnormally low testosterone was two to four times higher in exposed workers with serum 2,3,7,8-TCDD levels above 20 pg/g than in unexposed referents (Egeland et al., 1994). The risk in the Ranch Hands, who had lower exposures than the production workers, was 1.3 times (OR=1.3) that of the comparison group (Roegner et al., 1991). No significant associations were found between Vietnam experience and altered reproductive hormone levels (Centers for Disease Control Vietnam Experience Study, 1988d). Only the NIOSH study found an association between serum 2,3,7,8-TCDD level and increases in serum LH.

7.15.1.4.2. Specificity. The NIOSH study excluded from analysis participants who had conditions that might have influenced gonadotropin and/or testosterone levels: history of prostate cancer, thyroid or other hormone usage, or liver cirrhosis. Similarly, in the Ranch Hand study, individuals with orchiectomies or who were taking testosterone medication were excluded from the analysis of testosterone; no participants were excluded from the analyses of FSH. The CDC study of Vietnam veterans did not describe the exclusions.

7.15.1.4.3. Biologic plausibility. In rats, 2,3,7,8-TCDD has been shown to decrease testosterone levels (Moore et al., 1985; Moore and Peterson, 1988; Mebus et al., 1987)

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through a decrease in testosterone synthesis (Kleeman et al., 1990) or by decreasing the production of pregnenolone from cholesterol (Ruangwies et al., 1991). In addition, 2,3,7,8-TCDD has been shown in rats to reduce the responsiveness of the pituitary to testosterone (Bookstaff et al., 1990a) and of the Leydig cells to LH stimulation (Moore et al., 1991).

The findings of the NIOSH and Ranch Hand studies are plausible given the pharmacological and toxicological properties of 2,3,7,8-TCDD. A mechanism responsible for the effects may involve the ability of 2,3,7,8-TCDD to influence hormone receptors. The aromatic hydrocarbon (Ah) receptor to which 2,3,7,8-TCDD binds resembles steroid hormone receptors in both structure and mode of action. Studies suggest that 2,3,7,8-TCDD modulates hormone receptors, including estrogens (Romkes and Safe, 1988; Romkes et al., 1987), prolactin, and its own Ah receptor (Poland and Glover, 1980; Morrow et al., 1986). However, the effect of 2,3,7,8-TCDD on testosterone receptors has not been evaluated.

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

7.15.2. Possible Acute Effects of Exposure to 2,3,7,8-TCDD

The following section reviews end points that were described in groups shortly after exposure to 2,3,7,8-TCDD but were not observed as chronic effects in studies conducted many years after exposure ceased. Also reviewed are end points observed as long-term effects in single studies.

7.15.2.1. *Dermatologic Conditions Other Than Chloracne*

Dermatologic conditions other than chloracne, such as hyperpigmentation, hypertrichosis, and eyelid cysts, have been related to exposure to 2,3,7,8-TCDD in early case reports (Ashe and Suskind, 1950; Suskind et al., 1953; Bleiberg et al., 1964; Poland et al., 1971; Bauer et al., 1961; Goldman, 1972; Jirasek et al., 1974; Oliver, 1975). However, these conditions may have been acute effects of 2,3,7,8-TCDD exposure that resolved over time or may be residual effects of chloracne because they appear to occur more frequently in

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individuals with persistent chloracne (Suskind and Hertzberg, 1984). These conditions were not observed in studies in which the cohorts were examined years after cessation of exposure, in individuals with the potential for high exposure, or in those with high adipose or serum 2,3,7,8-TCDD levels (Moses et al., 1984; Webb, 1989; Roegner et al., 1991).

Actinic keratosis, Peyronie's disease, and basal cell carcinoma may not be associated with 2,3,7,8-TCDD. All three conditions were observed in only one study (Suskind and Hertzberg, 1984; Lathrop et al., 1984) and were not observed in studies of individuals with similar potential for exposure (Ott et al., 1993b).

7.15.2.2. *Liver Enzymes Other Than GGT and Hepatomegaly*

A number of studies reported elevated liver enzymes, particularly AST and ALT, among individuals who were being exposed at the time of the measurement (May, 1973) or whose exposure was within a few years of the measurement (Jirasek et al., 1974; Mocarelli et al., 1986; Caramaschi et al., 1981). Follow-up studies or longitudinal analyses of exposed cohorts suggest that the increase in enzyme level resolves over time (Mocarelli et al., 1986; Assennato et al., 1989; Pazderova-Vejlupkova et al., 1981; May, 1982). In studies of exposed populations tested many years after exposure ceased, levels of AST and ALT were within normal range (Calvert et al., 1992; Webb et al., 1989; Roegner et al., 1991; Suskind and Hertzberg, 1984; Moses et al., 1984).

D-glucaric acid was tested in a number of 2,3,7,8-TCDD-exposed populations as an indicator of enzyme induction (Ideo et al., 1985; Martin, 1984; Roegner et al., 1991; Calvert et al., 1992). Shortly after the TCP reactor release, D-glucaric acid levels in Seveso children were elevated (Ideo et al., 1985). No other studies of exposed groups tested 5 to 37 years after exposure ceased found elevations of this enzyme (Martin, 1984; Roegner et al., 1991; Calvert et al., 1992).

These data suggest that certain hepatic enzymes are increased as a response to high, exogenous exposure to 2,3,7,8-TCDD. Once the exposure ends, the enzyme levels seem to decrease over time, as observed in the Seveso populations (Mocarelli et al., 1986; Ideo et al., 1985). Additional evidence of the acute nature of AST, ALT, and D-glucaric acid elevations is demonstrated by the lack of such increases in studies of highly exposed groups

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conducted long after exposure ceased (Calvert et al., 1992; Roegner et al., 1991; Martin, 1984).

As in the case of ALT, AST, and D-glucaric acid, hepatomegaly appears to be a condition reported in case reports after high exposure to 2,3,7,8-TCDD-contaminated chemicals, particularly among TCP production workers examined after a TCP reactor explosion and among Seveso residents (Ashe and Suskind, 1950; Suskind et al., 1953; Jirasek et al., 1974; Reggiani, 1980). Later studies conducted after exposure ceased failed to find excess dose-related hepatomegaly in the exposed populations (Bond et al., 1983; Suskind and Hertzberg, 1984; Moses et al., 1984; Calvert et al., 1992; Centers for Disease Control Vietnam Experience Study, 1988a; Roegner et al., 1991; Webb et al., 1989; Hoffman et al., 1986). However, the absence of an effect in cross-sectional studies does not confirm the lack of an effect in the past.

7.15.2.3. *Pulmonary Disorders*

Early case reports suggest that exposure to 2,3,7,8-TCDD chemicals may cause temporary respiratory irritation (Zack and Suskind, 1980) and tracheobronchitis (Goldman, 1972). The data from two cross-sectional medical studies provide weak evidence of slightly decreased lung function among exposed individuals (Suskind and Hertzberg, 1984; Roegner et al., 1991). In these studies, the effects may be due more to smoking (Roegner et al., 1991) or to a substantial age difference between the exposed and unexposed groups (Suskind and Hertzberg, 1984). One study of highly exposed TCP production workers found no relationship between serum 2,3,7,8-TCDD levels and chronic obstructive pulmonary disease, bronchitis, or decreased pulmonary function (Calvert et al., 1992).

In conclusion, case reports indicate that intense acute exposure to 2,3,7,8-TCDD can produce respiratory irritation. However, the findings from controlled epidemiologic studies conducted many years after exposure do not convincingly support an association between 2,3,7,8-TCDD exposure and chronic effects on the respiratory system.

7.15.2.4. *Neurologic Disorders*

The results of case reports and epidemiologic studies demonstrate that exposure to 2,3,7,8-TCDD-contaminated materials is associated with symptoms referable to the central and peripheral nervous systems shortly following exposure and, in some cases, lasting many years (Filippini et al., 1981; Ashe and Suskind, 1950; Moses et al., 1984). Overall, however, the neurologic status of workers, community residents, and Vietnam veterans exposed to 2,3,7,8-TCDD and evaluated from 5 to 37 years after last exposure appears to be normal (Centers for Disease Control of Vietnam Experience Study, 1988a; Lathrop et al., 1984; Sweeney et al., 1993). The data suggest that, although exposure to 2,3,7,8-TCDD may have been extensive as in exposed workers, Ranch Hands, and Seveso residents, the effects described in case reports may have been transient (Filippini et al., 1981; Lathrop et al., 1984; Centers for Disease Control Vietnam Experience Study, 1988a, b; Assennato et al., 1989; Alderfer et al., 1992; Sweeney et al., 1993). The findings of recent studies suggest that in adults there are no long-term neurologic effects caused by even high exposure to 2,3,7,8-TCDD-contaminated materials, but there is very little information with which to examine the effects of exposure on the developing human neurologic system.

7.15.2.5. *Porphyrias*

In rats and mice, exposure to 2,3,7,8-TCDD has been clearly shown to alter porphyrin metabolism (Goldstein et al., 1973; Smith et al., 1981; Jones and Chelsky, 1986; DeVerneuil et al., 1983; Cantoni et al., 1981; Goldstein et al., 1982). Whether 2,3,7,8-TCDD is associated with porphyrin changes in humans, particularly porphyria cutanea tarda (PCT), is a subject of unresolved debate. It has been suggested that the PCT and elevated urinary porphyrins observed in the New Jersey and Czechoslovakian workers during the years of operation of the plants were the result of exposure to hexachlorobenzene, which was produced at the same time as TCP (Pazderova-Vejlupkova et al., 1981; Jones and Chelsky, 1986). These statements have not been corroborated with strong studies. In the follow-up studies, urinary porphyrin levels of these TCP production workers were not elevated (Pazderova-Vejlupkova et al., 1981; Poland et al., 1971) or did not differ from levels in the

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control group (Calvert et al., 1993). Doss et al. (1984) also described transient elevations in coproporphyrins among 22 Seveso residents exposed to 2,3,7,8-TCDD.

Because 2,3,7,8-TCDD is a potent porphyrigen in rats and mice, it has been of interest to determine whether exposures may have contributed to the observed changes in porphyrin levels in human populations. The NIOSH study could not address the question of etiology or transient porphyria, but it did not find porphyria in highly exposed workers many years after their occupational exposure.

7.15.3. Effects For Which Further Research Is Needed

The following section describes end points for which the animal data have demonstrated exposure-related effects, but the human data are inconclusive and require further study.

7.15.3.1. *Diseases of the Circulatory System*

In general, the results of the cohort mortality studies of TCP production workers were remarkably similar. For all of the studies, the standardized mortality ratios for diseases of the circulatory system (ICD-9: ICD 390-459) were approximately 100, meaning that the death rate in the exposed population was nearly the same as that in the general population, controlling for age, race, gender, and calendar year (Fingerhut et al., 1991; Zober et al., 1990; Bueno de Mesquita et al., 1993; Bertazzi et al., 1989, 1992; Collins et al., 1993; Bond et al., 1989; Coggon et al., 1991). None of the SMRs above 100 were statistically significantly elevated.

Mortality from circulatory system diseases among Ranch Hands (SMR=110, 95% CI=60-150) (Michalek et al., 1990) and Australian Vietnam veterans (RR=1.7, 95% CI=0.9-3.0) (Fett, 1987b) was nonsignificantly elevated. There was a deficit of deaths from this cause among U.S. Army Vietnam veterans compared to non-Vietnam veterans (Centers for Disease Control Vietnam Experience Study, 1988c). Elevated mortality from circulatory diseases among Seveso residents is considered by the authors to be the result of environmental stresses and possibly other risk factors rather than exposure to 2,3,7,8-TCDD (Bertazzi et al., 1989). In addition, a comparison of Zone A residents (2,3,7,8-TCDD-

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contaminated region) with Zone B residents (less contaminated region) found the circulatory disease mortality rate to be higher in the residents of the less contaminated area.

Diseases of the circulatory system, particularly heart disease, are the leading causes of death among populations of most developed nations. Leading risk factors include age, cigarette smoking, elevated lipid levels, obesity, hypertension, diabetes, and physical inactivity (Smith et al., 1992). Among the studies that examined mortality from circulatory system diseases, none directly adjusted SMRs for known risk factors or attempted to evaluate jointly the contribution of known risk factors and 2,3,7,8-TCDD to the mortality rates (Fingerhut et al., 1991; Zober et al., 1990; Bueno de Mesquita et al., 1993; Bertazzi et al., 1989, 1992; Collins et al., 1993; Bond et al., 1989; Coggon et al., 1991). Therefore, given the strong contribution of these risk factors, it is not possible to rule out physical and personal risk factors in the etiology of diseases of the circulatory system and heart in these populations. However, the absence of a "health worker effect" for these causes of death suggests that future research be directed specifically at the relationship between circulatory and heart disease and exposure to 2,3,7,8-TCDD.

Cross-sectional morbidity studies have not found increases in the prevalence of circulatory or heart disease among TCP workers, Ranch Hands, or U.S. Army Vietnam veterans (Suskind and Hertzberg, 1984; Bond et al., 1987; Moses et al., 1984; Centers for Disease Control Vietnam Experience Study, 1988a). In some cross-sectional studies, risk estimates were adjusted for some risk factors depending on the study (Suskind and Hertzberg, 1984; Poland et al., 1971; Moses et al., 1984; Bond et al., 1983; Centers for Disease Control Vietnam Experience Study 1988a; Roegner et al., 1991). Ranch Hands were the only group to experience marginal differences in diastolic blood pressure, arrhythmias, and peripheral pulse abnormalities after adjusting for selected risk factors (Roegner et al., 1991).

The animal data suggest that at high levels of 2,3,7,8-TCDD, the vascular system, cardiac muscle, and valves and function may be affected by exposure (Kociba et al., 1978; Buu-Hoi et al., 1972; Brewster et al., 1988; Hermansky et al., 1988; Kelling et al., 1987; Canga et al., 1988). However, with the exception of the long-term feeding study (Kociba et

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al., 1978), the exposures in animals were single, high doses and the human exposures (except Seveso) were chronic, medium to high doses.

In summary, the animal studies suggest that 2,3,7,8-TCDD causes pathologic changes that may lead to later circulatory system disease. However, long-term studies of mature and aged animals have not been carried out to evaluate these hypotheses and to correlate the results of the animal with the human studies. Few epidemiologic studies were designed to control for many of the risk factors known to cause circulatory system and heart disease, but a consistent absence of the health worker effect for circulatory disorders and heart disease in numerous mortality studies suggests the need for additional research in this area. These studies should also include methods to quantify subject exposure to 2,3,7,8-TCDD.

7.15.3.2. Reproductive Effects

7.15.3.2.1. Consistency. A variety of study designs, including case-control, ecologic, cross-sectional, and historical cohort designs, have addressed the issue of 2,3,7,8-TCDD and reproductive effects in humans. Unfortunately, the different criteria for case definitions across studies make it difficult to compare the results. In addition, the method of case ascertainment for certain end points influences the rate of events observed. The Vietnam Experience Study substudies of veteran-reported birth defects compared with those identified through hospital records demonstrated that rates of self-reported outcomes differed by exposure status. Moreover, predictive value of self-reported events was poor in both cohorts. In contrast, rates of birth defects in the Ranch Hand study were similarly reported by the Ranch Hands and controls. Both groups underreported 7% of birth defects in children conceived prior to their SEA tour and 14% after their tour of duty.

7.15.3.2.2. Strength. With the exception of the finding that Vietnam veterans were more than twice as likely to have low sperm concentrations ($OR=2.3$, 95% $CI=1.2-4.3$), no effect measure greater than 2 was noted in any of these investigations. This is not surprising, given the limitations of the studies, particularly with regard to exposure misclassification. Therefore, the trends across these studies carry more import than "statistically significant" results.

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7.15.3.2.3. *Temporality, dose-response.* While these studies have restricted inclusion of reproductive events to those that occurred after exposure to 2,3,7,8-TCDD was suspected, no study has evaluated 2,3,7,8-TCDD levels at the time of the outcome. Determination of a 2,3,7,8-TCDD dose-response relationship with adverse reproductive outcomes would not be valid unless individual 2,3,7,8-TCDD levels were available. Elucidation of a dose-response pattern may be derived using data from the Ranch Hand study to calculate 2,3,7,8-TCDD levels at the time of the reproductive event, which would be an important contribution toward understanding this phenomenon. However, with regard to spontaneous abortion, this analysis would not be able to address those losses occurring early in gestation.

7.15.3.2.4. *Biological plausibility.* A growing body of animal research described in Chapter 5 lends biological plausibility to the association between dioxin and most of the reproductive end points evaluated in these studies, with the notable exception of molar pregnancies. There is growing evidence that dioxin affects testis and accessory gland weight, testicular morphology, spermatogenesis, and fertility in males. A model for a paternally mediated dioxin effect on congenital malformations has not been reported. Among female animals, the primary reproductive end points that have been examined include decreased fertility and pregnancy loss.

The mechanism by which 2,3,7,8-TCDD causes adverse reproductive and developmental effects has not been well described, although considerable insight has been gained from research focusing on the Ah receptor. While the Ah receptor has been linked with birth defects in several mouse strains, it appears that the mechanism of effect may be dependent on the outcome evaluated, as well as other dioxin congeners to which the population is exposed. Clearly, these relationships in humans have not been adequately investigated.

The discovery in the Times Beach, Missouri; CDC; and Ranch Hand studies that self-reported dioxin exposure and exposure indices developed from the analyses of 2,3,7,8-TCDD in soil and military records to estimate individual exposure are poorly correlated with serum 2,3,7,8-TCDD levels was a critical turning point in understanding the inconsistencies of the research to date regarding 2,3,7,8-TCDD and effects. Thus, because of the likelihood of

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exposure misclassification in those studies lacking direct measures of exposure, the findings have been severely limited.

7.15.3.3. *Specific Reproductive End Points*

1. Spontaneous abortions. Miscarriages were investigated in several studies with different designs and varied patterns of parental exposure. Events were generally ascertained by self- or spousal-report. When case ascertainment was through medical records, such as in the Ranch Hand study or the Vietnamese investigations, the events are by definition restricted to those miscarriages that were clinically recognized.

Research in the area of early pregnancy loss indicates that 30-50% of all conceptions are lost prior to or during implantation (Hertig et al., 1959). The rate of loss between implantation and expected first menstrual period ranges from 22% to 30% (Wilcox et al., 1988; Sweeney et al., 1988). Thus it is clear that restriction of the examination of pregnancy loss to those events that are ascertained through self-reports or medical records results in a large proportion of the outcome of interest being missed. In studies of environmental factors and spontaneous abortion in which information is lacking concerning the conditions surrounding conception, "the conflation of different doses with different effects can mislead" (Kline et al., 1989). Because of these discrepancies, it would not be meaningful to pool the results of the research on the association between dioxin exposure and miscarriage to judge the "consistency" of the association.

Overall, it must be acknowledged that the data compiled to date are inadequate to address this issue. To simply enumerate and compare the number of "positive" versus "negative" studies to ascertain consistency in the research would be inappropriate. The reasons for this have been described above in detail, with emphasis on the high (40-50%) exposure misclassification that has been documented in the majority of these investigations, the small sample sizes evaluated, lack of data on dioxin levels at the time of conception, and the unknown impact of early pregnancy loss on identification of birth defects. The animal and human evidence for a 2,3,7,8-TCDD-pregnancy loss relationship is sufficiently suggestive to warrant further investigation. Several studies of various designs and populations have demonstrated weak but consistent associations (Reggiani, 1978; Hatch,

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1984b; Constable and Hatch, 1985; Huong et al., 1989; Phuong et al., 1989; Stellman et al., 1988), whereas others have not (Townsend et al., 1982; Smith et al., 1982; Cutting et al., 1970; Kunstadter, 1982; Report to the Minister for Veterans' Affairs, 1983; Stockbauer et al., 1988; Erickson et al., 1984; Centers for Disease Control Vietnam Experience Study, 1989). (These studies include those that should be restricted to the assessment of military service in Vietnam and reproductive events.) The Ranch Hand study leaves several questions unanswered, including the determination of a dose-response level at the time of conception and adverse reproductive outcomes, as well as the impact of early pregnancy losses on rates of spontaneous abortion and birth defects that survive long enough to be "counted."

2. Congenital malformations. The confusing evidence regarding the relationship between dioxin exposure and birth defects results not only from the same limitations described above for the studies of miscarriage but also from the lack of power to evaluate specific types of malformations. To increase the power to detect a potential relationship, the studies have combined all birth defects together and calculated an odds ratio for total birth defects. Given emerging evidence for etiologic heterogeneity among subgroups of birth defects (Khoury, 1989), it is possible that this approach might dilute the effect measure.

These studies also should be stratified by type of parental exposure, i.e., paternal, maternal, or both. Biologically plausible mechanisms for birth defects resulting from paternal exposure to toxic substances have not been well researched. It has been known for many years that temporary infertility may occur after exposure of human males to certain toxic substances. However, animal research suggests that spermatogenesis is "particularly resilient" after exposure to these chemicals (Pearn, 1983). If dioxin were related to malformations among the offspring conceived after paternal service in Vietnam, it is implied that the effect must occur premeiotically. Some animal studies have found that spermatogonia and spermatocytes (premeiotic spermatogenic cells) were able to repair DNA after exposure to toxic agents, whereas spermatids and spermatozoa did not have this capability (Lee and Dixon, 1978).

A few studies (Hatch, 1984a; Constable and Hatch, 1985; Huong et al., 1989; Phuong et al., 1989a) have suggested an association, including those investigations of the Yusho and Yu Cheng incidents (Rogan, 1982; Yen et al., 1989; Rogan et al., 1988). Many

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studies have failed to find a relationship between dioxin and birth defects (Townsend et al., 1982; Smith et al., 1982; Cutting et al., 1970; Kunstadter, 1982; Stockbauer et al., 1988; Erickson et al., 1984; Centers for Disease Control Vietnam Experience Study, 1989; Mastroiacovo et al., 1988). Again, however, in view of the serious limitations of these studies of 2,3,7,8-TCDD and reproductive events, it must be concluded that the relationship between paternal dioxin exposure and congenital malformations remains unknown. However, if military service in Vietnam is the exposure of interest, there is little evidence to support an association with birth defects.

3. "Miscellaneous" end points. Additional reproductive outcomes that were evaluated in a subset of the studies include molar pregnancies (in the Vietnamese studies), infant birth weight, neonatal and infant death, and child cancer and mortality. Mainly because of small sample sizes, it is difficult to reach conclusions regarding neonatal, infant, and child mortality and childhood cancers. However, the increased risk for neonatal death observed in the Ranch Hand study, the only study with individual TCDD levels, should be further investigated. Available evidence does not support an association between paternal dioxin level and low birth weight (Wolfe et al., 1992b; Centers for Disease Control Vietnam Experience Study, 1989); a maternally mediated effect with documentation of TCDD exposure has not been examined.

There was evidence for a maternally mediated effect of dioxin exposure and birth defects in the Vietnamese studies. An attempt to confirm these findings by using recent advances in exposure measurement would be a worthwhile effort.

The Vietnam Experience Study found a significant relationship between service in Vietnam and sperm abnormalities, while the Ranch Hand study did not confirm these results when exposure was defined by both cohort status and 2,3,7,8-TCDD levels. However, the data on alterations in male reproductive hormone levels associated with occupational exposure to 2,3,7,8-TCDD emphasize that further research in these areas is required.

In conclusion, the research to date has been successful in resolving some confusion surrounding the conflicting evidence for an association of dioxin exposure and various reproductive end points in humans. High occurrence of exposure misclassification, differences in case definitions across studies, and small sample sizes have severely limited

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the power of these studies to address these questions. Additional research that includes a measure of dioxin level at the time of conception for both the father and mother is necessary if the effect of dioxins on the spectrum of reproductive outcomes is to be understood.

7.15.3.4. *Immunologic Effects*

Information on immunologic function in humans relative to exposure to 2,3,7,8-TCDD is scarce. The available epidemiologic studies do not describe a consistent pattern of effects among the studies. Two studies of German workers, one exposed to 2,3,7,8-TCDD and the other to 2,3,7,8-tetrabrominated dioxin and furan, observed dose-related increases of complements C3 or C4 (Zober et al., 1992; Ott et al., 1993b). Other studies of exposed groups have not examined complement components to any great extent.

More comprehensive evaluations of immunologic function with respect to 2,3,7,8-TCDD exposure are necessary to assess more definitively the relationships observed in nonhuman species. Longitudinal studies of the maturing human immunologic system may provide the greatest insight, particularly since animal studies have found significant results in immature animals and human breast milk is a source of 2,3,7,8-TCDD and other related compounds. Additional studies of highly exposed adults may also shed light on the effects of long-term chronic exposures. Therefore, there appears to be too little information to suggest definitively that 2,3,7,8-TCDD, at the levels observed, is an immunotoxin in humans.

7.15.3.5. *Lipids*

Animal studies indicate that 2,3,7,8-TCDD is associated with generally increased serum cholesterol and serum triglyceride levels. The effect of exposure to 2,3,7,8-TCDD-contaminated chemicals on lipids is not consistent in the available epidemiology studies. Elevations in total cholesterol and triglyceride levels were reported after high 2,3,7,8-TCDD exposure in TCP workers (Pazderova-Vejlupkova et al., 1981; Martin, 1984) and laboratory workers (Oliver, 1975). Despite their very high exposure to 2,3,7,8-TCDD-contaminated chemicals, neither adults nor children from Seveso had lipid levels above the referent level. Risk factors such as dietary fat intake, familial hypercholesterolemia, alcohol consumption,

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and exercise, which also affect cholesterol and other lipid levels, may be factors that were not considered in these studies.

Ranch Hands continue to have elevated lipid levels despite the extended length of time between exposure and testing. In the 1992 phase of the Ranch Hand study, additional parameters that may affect lipid levels were examined. Analysis of the lipid data collected during the NIOSH study also will contribute to the information on lipid levels in relation to serum 2,3,7,8-TCDD levels.

7.15.3.6. *Thyroid Function*

Many effects of 2,3,7,8-TCDD exposure in animals resemble signs of thyroid dysfunction. In the few human studies that examined the relationship between 2,3,7,8-TCDD exposure and thyroid function, the results are mostly equivocal (Centers for Disease Control Vietnam Experience Study, 1988a; Roegner et al., 1991; Suskind and Hertzberg, 1984). However, TBG appears to be positively correlated with current levels of 2,3,7,8-TCDD in the BASF accident cohort (Ott et al., 1993b). Little information on thyroid function has been reported for production workers and none for Seveso residents, two groups with documented high serum 2,3,7,8-TCDD levels. A study of nursing infants suggests that ingestion of breast milk with a higher total TEQ may alter thyroid function, but the study is limited by the small size and a short observation period (Pluim et al., 1992). Because the products of the thyroid control many aspects of metabolism, a better understanding of the relationship between 2,3,7,8-TCDD and thyroid function is important, especially regarding the developing human.

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