Bull. Environ. Contam. Toxicol. (1984) 33:662–672

Environmental Contamination and Toxicology

The Pilot Missouri Health Effect Study

Karen B. Webb

Division of Occupational and Environmental Medicine, St. Louis University School of Medicine, 1325 S. Grand Blvd., St. Louis, MO 63104

St. Louis University School of Medicine was pleased to assist in a pilot epidemiologic study of the health effects of TCDD contamination in Missouri.[1] We worked directly with the Centers for Disease Control and the Missouri Division of Health plus St. Joseph Hospital to design and execute this study of the health effects of dioxin victims in Missouri.

Figure 1 demonstrates that the various dioxin sites are rather widespread throughout the state. On the small inset map nearer to St. Louis, Times Beach is number 21. Moscow Mills is the site of the

A report of work done in collaboration with Paul Stehr, Gary Stein, and Henry Falk (Centers for Disease Control, 1600 Clifton Road NE, Atlanta, GA 30333).

horse stables that have been referred to so often. The plant that produced most of this chemical is located in the southwest.

The potential health effects considered in this study were based primarily on animal toxicology of dioxin and results from studies of long-term industrial and acute human exposures. The results of most of these were discussed by Dr. Kimbrough. The organ systems most prominently affected are the liver, the immune system and the skin (Anaiz and Cohen 1979; Gupta et al. 1973; Hook et al. 1975; Hook et al. 1978; Kimmig and Schulz 1957; Kociba et al. 1978; McNulty 1982; Thigpen et al. 1975; Van Miller et al. 1977). Some reproductive effects have been reported in animals (Allen et al. 1979; Murray et al. 1979; Smith et al. 1976; Kimbrough 1980). Most of our direct knowledge of human health effects has been obtained from workers who were exposed to dioxin during the production or subsequent handling of trichlorophenol or 2,4,5-T. In some plants exposed workers developed chloracne, but no systemic illness (May 1973). Other authors have reported weight loss, easy fatigability, myalgias, insomnia, irritablity and decreased libido. The liver may become tender and enlarged and sensory changes, particularly in the lower extremities, have occurred. Total serum lipids may be increased and the prothrombin time has been reported to be prolonged (Bauer et al. 1961; Bleiberg et al. 1964; W.H.O. 1977; Jensen and Walker 1972; Oliver 1975). Porphyria cutanea tarda has also been observed (Bleiberg et al. 1964; Jirasek et al. 1967; Poland et al. 1971). Chloracne is regarded as the most specific of these findings; however, it can also occur after exposure to related compounds, such as PCBs and chlorinated napthalene. Studies addressing the association of TCDD exposure to soft tissue sarcoma have been conducted in the industrial setting and the results are, as yet, inconclusive (Eriksson et al. 1981; Hardell and Sandstrom 1979; Honchar and Halperin 1981).

Information on the health effects involving a non-occupational environmental exposure of this type is very sparse. After an explosion in 1976 in Seveso, Italy, exposed children developed chloracne and liver function abnormalities, and the incidence of abnormal nerve conduction studies was elevated in subjects with chloracne (Fillipini et al. 1981; Hay 1976; Reggiani 1980). A child in Missouri who played in the dirt in the riding arena contaminated to 33 parts per million of TCDD developed hemorrhagic cystitis (Carter et al. 1975).

The Times Beach health screening study was intended by the Missouri Division of Health to be a service to those exposed in that it was meant to provide them with some information on the actual effects of their exposure. From an epidemiologic point of view, the study was to provide a perspective on what types of problems might be observed in a group of individuals with a high risk of environmental TCDD exposure. This preliminary information would then be used to generate hypotheses for further, more rigorous epidemiologic studies. The study, therefore, was not meant to resolve all or even a majority of the questions pertaining to environmental effects of TCDD exposure, but mainly to direct future and more definitive research.

There were several uncertainties in the Missouri dioxin situation that made a quick, simple design of a more definitive health study impossible, and Dr. Hoffman has referred to many of these mitigating circumstances. First, there was very limited data on what type of health effects should be expected in an environmental exposure of this type. There was also limited environmental data at the time of the planning of the study. As you might recall, during the times that the study was executed, new levels of TCDD were being discovered almost daily. Another problem that caused uncertainty was the unavailability of a direct manner of measuring exposure, such as a dioxin blood level, for example. Lastly, another problem that was encountered was that some of the highly exposed individuals were distributed at a number of different sites and settings.

We demonstrated potential health effects related to the dioxin exposure by three means. The first was through a health effects survey. This was a questionnaire that was constructed to elicit information on exposure risk, medical history for each individual, and potentially confounding influences, such as life style and occupation. The initial group of approximately 800 completed questionnaires was reviewed and a group of individuals for inclusion in a pilot medical study was selected. The study cohort was selected so as to enhance the contrast and risk of exposure status between a high and a low risk group. The high risk group consisted of individuals living or working in TCDD contaminated areas or participating more than once a week in a high soil contact activity in TCDD contaminated areas, such as working in a stable. We also sponsored a dermatology screening clinic available to anyone in the general population who had a current skin problem and reason to suspect that he or she might have been exposed to environmental TCDD. The individuals selected for the study as high risk were believed to have been exposed to between 20 and 100 parts per billion for a period of at least two years, or greater than 100 parts per billion for at least six months.

The high risk group consisted of a total of 82 participants of which 56 were exposed at primarily residential areas and 26 were exposed at non-residential areas. The final group consisted of 68 in the high risk group and 36 in the low risk group, for a study population of 104. The low risk group was selected, again, from the 800 people who completed the questionnaires. They were matched on type of exposure site, age, sex, race and socioeconomic characteristics.

In addition to the data from the health effects survey questionnaire, these 104 individuals were assessed by a clinical protocol which included several elements, namely, a physical examination, neurologic and dermatologic examinations and laboratory analyses. These labs focused mainly on liver function tests, urinary porphyrins as well as a variety of other testing (Table 1).

Table 1. Clinical protocol

1) Physical examination

2) Neurologic examination

3) Dermatologic examination

4) Laboratory analysis
   - SMA liver function profile II plus beta-glucuronidase in serum
   - Serum T4, TSH and cortisol
   - HBsAg titers
   - HDL-C
   - Urinary porphyrins (total and pattern) and D-glucaric acid
   - CBC
   - Routine urinalysis

5) Immune response tests
   - Standard skin tine tests reaction to candida, old tuberculin, streptococcus, proteus, diphtheria, tetanus, tricophyton and a glycerine control (marked by Mileau Instituto under FDA approval)
   - T-cell subset assays
   - Lymphocyte proliferation response to PHA, concanavalin A, pokeweed mitogen, and tetanus toxoid

6) Frozen serum kept for long-term storage to be used for TCDD serum analysis when such tests become available

An extensive battery of immunologic tests to investigate T cell function was also administered based on data showing that this system was abnormal in TCDD exposed animals. Dr. Knutsen will detail the results of this investigation in his presentation. Serum was also drawn and frozen to be kept for long-term storage until such time when TCDD serum analysis became available. Examining physicians did not know each individual's risk of exposure status. Further, the identification code was revealed only after all examinations, laboratory analysis and data encoding had been completed.

The data was analyzed independently by St. Louis University, Centers for Disease Control and the Missouri Division of Health by a variety of different approaches and the same results were obtained by each group. The quality of the data was examined by a contingency table approach and chi square analysis to determine if there were categorical differences existing between the high and low risk groups. For the continuous data, analysis of variance and T-test for means were used to compare group and stratified means. A variety of other multivariable techniques were used to examine interactive or confounding associations.

Results. The results of this analysis showed the defined high and low risk groups were comparable in terms of age, race, sex, education

of head of household and participation in a variety of outdoor activities. Table 2 is an abbreviated list of some of the different attributes that we looked at when comparing the two different groups.

Table 2. Comparison of demographic characteristics

Item	N	% High risk	N	% Low risk	P-value (Fisher's 2-tail)
Employment in hazardous occupations	68	5.9	36	5.6	1.00
Relationship of respondent to subject		No difference			0.11(X^2)
Sex (proportion female)	68	45.6	36	52.8	0.54
Ever drink alcohol	68	63.2	36	47.2	0.15
Ever smoke	68	57.3	36	47.2	0.41
Exercise regularly	67	74.6	36	41.7	0.001*
Consumption of locally-caught wild game or fish	68	44.1	35	54.3	0.41
Vietnam military service	68	0.0	36	2.8	0.35
Education of head of household		No difference			0.44(X^2)
Pesticide use					
Pest control services	68	19.1	35	11.4	0.41
Lawn care services	68	4.4	35	8.6	0.41
Indoor bug sprays	68	79.4	35	88.6	0.29
Outdoor bug sprays	68	66.2	35	65.7	1.00
Use of prescription medicines					
Blood pressure medicines	66	4.6	36	5.6	1.00
Other heart medicines	66	1.5	36	2.8	1.00
Antihistimes	66	9.1	36	11.1	0.74
Anticoagulants	66	0.0	36	0.0	--
Long-term antibiotics	65	6.2	36	8.3	0.70
Cortisone and related drugs	66	3.0	36	5.6	0.61
Diabetes pills or insulin	66	1.5	36	0.0	1.00
Sleeping pills	66	0.0	36	0.0	--
Birth control pills	30	6.7	19	10.5	0.61
Multiple vitamin supplements	66	16.7	36	30.6	0.13
Thyroid replacement hormones	65	4.6	36	5.6	1.00
Muscle relaxants	66	6.1	36	8.3	0.70
Sedatives or tranquilizers	66	13.6	36	2.8	0.09
Radiation therapy	66	0.0	36	0.0	--
Other medications	59	23.7	30	16.7	0.59

* indicates significance at the 0.05 level

It did appear that the high risk group exercised more regularly than the low risk group and consumed more sedatives and tranquilizers. There were no significant differences or trends between the two groups in terms of consumption of locally caught wild game or fish and the use of pest control or lawn care services or the use of any other type of pesticide. In regards to other potentially confounding variables, there was no difference in the proportion of workers in hazardous occupations. Similarly, there was only one low risk and no high risk person who served in the armed forces in Vietnam. The high risk group evidenced higher proportions of people who had ever consumed alcohol or tobacco products, but this difference was non-significant. Five cases of cancer were reported. Three were in the high risk group and two were in the low risk group, and this did not represent a significant difference. None of the cancers were soft tissue sarcoma. The amount of weight loss in the two different groups was also not statistically significantly different.

On the general physical examination, we also compared a variety of different attritubes and found no consistent differences between the two groups. There was a very weak trend of diminished peripheral pulses in the high risk group. Approximately 3%-6% of the high risk group had decreased dorsalis pedis and posterior tibial pulses where there was no decrease in the low risk group, although this result was not significant.

No consistent overall trends or statistically significant differences were detected for reproductive outcome. Table 3 shows some of our results from the questionnaire regarding reproductive outcomes.

Table 3. Comparison of reproductive health findings

Condition	N	High Risk % with Abnormality	N	Low Risk % with Abnormality	P-value Fisher's 2-tail
Male					
Infertility	31	3.2	15	0.0	1.00
Impotence	32	6.3	14	7.1	1.00
Libido loss	32	15.6	14	20.0	0.65
Female					
Irregular menses	24	12.5	15	20.0	0.66
Chronic excessive menstrual flow	24	20.8	15	13.3	0.69
No menstrual flow	23	17.4	14	7.1	0.63
Infertility	23	4.4	11	9.1	1.00

Measurement	Means (Standard Error) High Risk	Low Risk	P-value (t-test)
Age at menarche (total group)	12.7 (0.4)	11.5 (0.4)	0.06
Age at menarche (if possibly exposed)	12.7 (0.6)	11.0 (0.6)	0.17
Age at menopause	49.1 (6.4)	40.0 (9.0)	0.51

Females in the high risk group reported a later age at menarche. When the analysis was restricted only to those women who had reached menarche during the time that they would have potentially been exposed to environmental dioxin, the significance diminished to a p value of .17. Only 33 births were reported in the entire study population since 1972, and there were no trends noted by the questionnaire. Only one birth defect was reported among children born to women in both the high and low risk groups after the time that exposures would have occurred.

We carefully analyzed the results of the dermatologic exam and history by questionnaire. In the screening process, no cases of chloracne were seen. For the 104 individuals in the study population, there was no significant difference in the dermatologic findings demonstrated either by medical history or by physical examination. Likewise, the data on the central nervous system showed no significant differences between the two groups. Other than a diminished vibratory sense in the high risk group with a p value of .13, none of the differences in the neurological examination approached statistical significance.

Routine hematology tests showed no consistent differences, with the exception that the mean platelet count was elevated in the high risk group, with a p value of .01 (Table 4). The mean platelet count for the high risk group was 281,927 and for the low risk was 249,061.

Table 4. Lab analytes

Measurement	Mean High Risk	Mean Low Risk	P-value (t-test)
CBC: HCT	42.9	43.2	0.72
Platelets	281,927	249,061	0.01*
WBC	7.1	7.2	0.85
Serum: Glucose	92.6	89.3	0.12
Total urinary porphyrins	82.4	92.2	0.27
Heptacarboxylporphyrin	0.8	1.8	0.04*
Copro/Uroporphyrin ratio	6.3	6.4	0.95
Serum: Bile acid	1.4	1.5	0.61
Total protein	7.1	7.1	0.22
Albumin	4.5	4.5	0.40
Globulin	2.6	2.6	0.52
SGOT	20.4	19.0	0.39
Alkaline phosphatase	80.8	84.1	0.73
GGTP	14.8	15.8	0.81
Total bilirubin	0.6	0.7	0.20
Bilirubin (direct)	0.2	0.3	0.11
SGPT	16.9	17.3	0.92
HDL-C	52.4	52.2	0.94
Triglycerides	134.6	93.8	0.37
Cholesterol	203.2	190.2	0.27
LDH	180.1	188.7	0.28
Beta-glucuronidase	1.9	1.6	0.08

* Indicates significance at the 0.05 level

The urinary heptocarboxyl porphyrins was elevated in the low risk group; however, the two groups showed no differences in the characteristic urinary porphyrin pattern and no cases or porphyria cutanea tarda or any precursor condition were diagnosed.

There were no statistically significant differences between the two groups in regard to liver function test results. The serum beta glucuronidase level was elevated in the high risk cohort, but this failed to reach significance. In regards to the hepatic system, there was an increased prevalence of reported nonspecific liver diseases in the low risk cohort. On physical examination, there was a greater prevalence in the high risk group of hepatomegaly, although this finding, as well as all the others in the hepatic system, did not reach statistical significance.

There appeared to be a trend of increased urinary tract problems among the high risk cohort as reported from the medical history section of the questionnaire. The urinalysis showed an increase in urinary white blood cells, red blood cells and a positive dipstick for blood. None of these results were stastically significant but the trend is a very interesting one (Table 5).

Discussion. Our analyses did not produce any firm indications of increased disease prevalence related to exposure. Of significant note is that no cases of chloracne or overt porphyria cutanea tarda were seen in either group that was examined. It seems plausible to suggest that such dermatologic findings may be more compatible with acute high dose than chronic low dose exposure, such as that which occurred in the Times Beach population. The findings of no cases of soft tissue sarcoma is of questionable importance when one considers the small sample size that was examined and the relatively short latency period available for this study cohort. These results, however, do offer some interesting leads for further studies. It is unclear how to interpret an elevated mean platelet count in the high risk cohort since this runs counter to previous findings from animal toxicologic work. Of greater interest is the apparent trend indicating urinary abnormalities among high risk individuals. There was a greater prevalence of microscopic hematuria in the high risk cohort. Four of the six of these cases were women between the ages of 14 and 44 for whom no detailed records of menstrual cycle were available; however, because of the previous findings of hemorrhagic cystitis in exposed individuals we feel that these associations require further and more detailed investigation. The finding of no significant differences in standard liver function tests is important because the previous investigative work detected such abnormalities in both animals and humans. Previous investigators have also reported an elevation in serum lipids in association with TCDD exposure, but our results showed no such abnormalities. Neither of the two individuals from the high risk group who were considered to have hepatomegaly reported any disease that might explain this finding. Because of the extensive animal and high dose human data which suggests hepatotoxic effects of TCDD, it is important that hepatic function continue to be examined in further studies.

Table 5. Comparison of renal/urinary tract findings

Condition	N	High Risk % with Abnormality	N	Low Risk % with Abnormality	P-value Fisher's 2-tail
Questionnaire					
Nephritis	68	10.3	35	0.0	0.09
Urinary tract infection	67	14.9	35	5.7	0.21
Cystitis	67	10.4	35	5.7	0.71
Kidney stones	68	1.5	35	0.0	1.00
Hematuria	68	10.3	35	2.9	0.26
Proteinuria	68	0.0	36	0.0	--
Other kidney disease	66	4.6	35	2.9	1.00
Lab Analyses					
Proteinuria	67	7.5	33	6.1	0.58
Urinary WBC (hpf)	68	10.3	34	0.0	0.09
Urinary RBC (hpf)	68	8.8	34	0.0	0.17
Hematuria (dipstick)	67	19.4	33	15.2	0.78
Urinary eithelial cells	68	0.0	36	0.0	--
Bacteria in urine	67	3.0	33	0.0	0.45
Crystals in urine	67	6.0	33	3.0	0.47
Urinary glucose	67	6.0	33	0.0	0.15
Urinary acetone	67	6.0	33	6.1	1.00
Urinary nitrite	67	7.5	33	3.0	0.66

Measurement	Means (Standard Error) High Risk	Low Risk	P-value (t-test)
BUN (mg/dL)	13.4 (0.43)	14.3 (0.69)	0.28
Urobilinogen (mg/dL)	0.46(0.05)	0.53(0.09)	0.52

With regard to future studies, the CDC and the Missouri Division of Health are currently developing and refining protocols for further evaluation of groups throught to be exposed to TCDD in an attempt to provide more definitive conclusions. Efforts are also underway to develop laboratory techniques to provide a more direct measure of TCDD exposure, which would certainly be helpful in providing accurate classificaiton of exposure status. It is my hope and belief that St. Louis Univeristy will continue to be involved in assisting victims of this disaster.

REFERENCES

Allen JR, Barsotti DA, Lambrecht LK, Van Miller JP (1979) Reproductive effects of halogenated aromatic hydrocarbons on non-human primates. Ann NY Acad Sci 320:419-425.

Anaiz NH, Cohen J (1979) Effects of TCDD on the renal tubular secretion of phenosulfonphthalein. J Pharmacol Exp Ther 207:748-755.

Bauer H, Schulz KH, Spiegelberg U (1961) Berufliche Vergiftungen bei der herstellung von chlorphenol-verbindungen. Arch Gewerbepathol Gewer Behyg 18:538-555.

Bleiberg J, Wallen M, Bradkin R, Applebaum I (1964) Industrially acquired prophyria. Arch Dermatol 89:793-797.

Carter CD, Kimbrough RD, Liddle JA, et al. (1975) Tetrachlorodibenzodioxin: an accidental poisoning episode in horse arenas. Science 188:738-740.

Eriksson M, Hardell L, Berg NO, Moller T, Axelson O (1981) Soft tissue sarcomas and exposure to chemical substances: a case-fererent study. Br J Med 38:27-33.

Fillipini G, Bordo B, Crenna P, et al. (1981) Relationship between clinical and electrophysiological findings and indicators of heavy exposure to 2,3,7,8-TCDD. Scand J Work Environ Health 7:257-262.

Gupta BN, Vos JG, Moore JA, Zinkl JG, Bullock BC (1973) Pathologic effects of 2,3,7,8-TCDD in laboratory animals. Environ Health Per 5:125-140.

Hardell L, Sandstrom A (1979) Case-referrent study: soft tissue sarcomas and exposure to phenoxyacetic acids and chlorophenols. Brit J Cancer 39:711-717.

Hay A (1976) Toxic cloud over Seveso. Nature 262:636-638.

Honchar P, Halperin W (1981) 2,4,5-TCP and soft tissue sarcomas. Lancet 1:268-269.

Hook GER, Haseman JK, Lucier GW (1975) Induction and suppression of hepatic and extrahepatic microsomal foreign-compound-metabolizing emzyme systems by 2,3,7,8-TCDD. Chem Biol Interact 10:199-214.

Hook JB, McCormack KM, Kluwe WM (1978) Renal effects of 2,3,7,8-TCDD. Environ Sci Res 12:381-214.

International Agency for Research on Cancer (1977) Chlorinated dibenzodioxins. In: Monographs on the evaulation of the carcinogenic risk of chemicals to man. Lyon: World Health Organization 15:41-102.

Jensen NE, Walker AE (1972) Chloracne: three cases. Proc R Soc Med 65:687-688.

Jirasek L, Kalensky J, Kubic K, Pazderova J, Lukas E (1967) Chlorakne, porphyria cutanea tarda und andere intoxikationen durch herbizide. Hautarze 27:328-333

Kimbrough RD (ed.) (1980) Halogenated biphenyls, terphenyls, naphthalenes, dibenzodioxins and related products. Top Environ Health 4:406.

Kimmig J, Schulz KH (1957) Occupational acne (chloracne) caused by chlorinated aromatic cyclic ethers. Dermatologica 115:540.

Kociba RJ, Keyes DG, Beyer JE, et al. (1978) Results of a two year chronic toxicity and oncogenecity study of 2,3,7,8-TCDD in rats. Toxicol Appl Pharmacol 46:279-303.

May G (1973) Chloracne from the accidental production of tetrachlorodibenzodioxin. Br J Ind Med 30:276.

McNulty WP (1982) Rhesus macaques: pertinence for studies on the toxicity of chlorinated hydrocarbon environmental pollutants. In: Chiarelli AB, Carruccini RS (eds) Advanced views in primate biology. Springer-Verlag, Berlin, pp 111-113.

Murray FJ, Smith FF, Nitschke KD, et al. (1979) Three generation reproduction study of rats given 2,3,7,8-TCDD in the diet. Toxicol Appl Pharmacol 50:241-252.
Oliver RM (1975) Toxic effects of 2,3,7,8 tetrachlorodibenzo 1,4 dioxin in laboratory workers. Br J Ind Med 32:49-53.
Poland AP, Smith D, Metter G, Possick P (1971) A health survey of workers in a 2,4,-D and 2,4,5-T plant. Arch Environ Health 22:316-327.
Reggiani G (1980) Acute human exposure to TCDD in Seveso, Italy. J Toxicol Environ Health 6:27-43.
Smith FA, Schwetz BA, Nitschke KD (1976) Teratogenicity of 2,3,7,8-TCDD in CF-1 mice. Toxicol Appl Pharmacol 38:517-523.
Thigpen JE, Faith RE, et al. (1975) Increased susceptibility to bacterial infection as a sequela of exposure to 2,3,7,8-TCDD. Infec Immunol 12:1319-1324.
Van Miller JP, Lalich JJ, Allen JR (1977) Increased incidence of neoplasms in rats exposed to low levels of 2,3,7,8-TCDD. Chemosphere 10:625-632.

Received July 5, 1984; accepted September 2, 1984.