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LONG TERM HEALTH EFFECTS OF EXPOSURE TO 2,4,5-T

AND/OR ITS CONTAMINANTS

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Summary of Findings

A clinical and epidemiological study of 436 subjects from a plant making 2,4,5-T from 1948-69 was carried out. The subjects in the data analysis consisted of 418 white males: 204 subjects were defined as exposed, 163 not exposed, and there were 51 subjects whose exposure was questionable. The remaining 18 who were not included in the epidemiological comparisons were 5 black males, 10 white females, 1 male American Indian, 1 male of Hispanic origin, and 1 male not employed at Monsanto but exposed to material of the runaway reaction as a child. Since the number of this group was too small for comparisons they were not included in the statistical analysis.

The data on each subject included historical information about illness from childhood to 1979, results of a comprehensive physical examination, clinical laboratory tests, organ function tests and x-rays.

The analysis of the data on 418 subjects indicates the following:

1. Chloracne is the dramatic clinical hallmark which clearly separates the exposed from the not exposed or those of questionable exposure; 86% of the 204 exposed developed chloracne and this condition persisted in 52% of the exposed. There were no cases of chloracne in the not exposed or those of questionable exposure.

2. Actinic elastosis, a degenerative condition affecting the elastic tissues in the dermis is known to be related to the degree of exposure to sunlight and is age and skin coloration dependent. In this study the frequency of actinic elastosis was 59% in the exposed as compared to 30% in the not exposed. When corrected for age there was still a significant difference between the exposed and not exposed. A biologically significant observation indicates that actinic elastosis in the exposed is found predominantly in subjects with persistent

examination among both the exposed and the not exposed groups when the ≥ 50 year old were compared to the < 50 year old. The pulmonary function examination revealed consistent differences in all parameters (FEV, FVC, FEV₁/FVC, and MMEFR) between the exposed and not exposed groups only if they are present smokers. The confounding factors were that the exposed group was 10 years older than the not exposed and that 13 of the exposed were coal miners before they started to work for Monsanto. Among current smokers with no coal mining experience however, there were significant differences in FEV₁, FVC and MMEFR between the 2,4,5-T process exposed and the not exposed.

7. When clinical laboratory data other than lipids were reviewed no differences were found between the exposed vs. the not exposed, the chloracne vs. no chloracne group.

8. Lipid findings were considered separately and in relation to exposure, age, and the presence of chloracne. When exposure alone was considered there were no significant differences in the frequency of abnormal levels in all parameters tested. Frequency of abnormal HDL levels however were somewhat greater in those with persistent chloracne than those who never had or formerly had chloracne and there was a greater frequency of abnormal LDL levels among those who only had a history of chloracne. Since the total number of abnormal values is small, the biological significance of these statistical differences is uncertain.

9. In the neurobehavioral aspects of the study no differences in neurological examination findings between the exposed and not exposed groups were found that were not age associated or with other known etiologies such as extensive burns, W.W.II injuries, cervical nerve damage, cervical spurs, etc.

In full recognition of the inherent problems associated with carrying out and interpreting nerve conduction velocity tests, a critical analysis of the qualified subjects was made. No significant differences in motor and sensory

unctions were observed between the exposed and not exposed groups, between those who had chloracne and those who did not, after adjusting for age.

In the interview, questions were asked about nervousness, decrease in libido and impotence. Nervousness was reported more frequently among the older exposed group than the younger exposed group. Recognizing the cultural considerations which influence replies to questions about sexual matters, decreased sexual desire was reported more frequently among the exposed than the not exposed. However, it should be noted that there was a 10-year difference in mean age between the exposed and not exposed. When the two groups were compared on the basis of age, the differences in sexual desire and impotence were age related not exposure related.

10. Reproduction considerations

Again recognizing the inherent defects in the method of eliciting clinical information, no significant differences in the frequency of birth defects, stillbirths and miscarriages were found when the exposed group was compared with the not exposed group.

11. An unusual problem encountered only among the exposed were three cases of Peyronie's Disease. This problem is characterized by induration and subsequently fibrous scarring of the corpus cavernosum of the penis producing distortion on erection. This problem is not known to have any relationship to toxic exposure.

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Historical Review

In 1949, at a plant of the Monsanto Chemical Company, Nitro, West Virginia, a runaway reaction occurred involving one of three autoclaves in Building 41 in which trichlorophenol was being prepared. This process accident occurred on March 8, 1949, when under abnormal temperature and pressure conditions the safety valve and pipe connection of this autoclave blew out and the contents scattered throughout the interior of the building as well as the surrounding terrain and structures.¹ The employees who were asked to clean-up the building and repair the damaged autoclave were the first to develop symptoms. They were chemical operators, pipe fitters and other maintenance personnel. The acute symptoms included irritation of the respiratory tract and eyes, headache, dizziness, nausea and a severe irritant reaction of the exposed skin. After the initial acute symptoms subsided, within 10 days to 3 weeks, an acneform eruption and other health effects were noted. The clinical findings, at that time, were acneform lesions, severe muscle pains affecting the upper and lower extremities, thorax and shoulders on exertion, easy fatigue, nervousness and irritability, the complaint of decreased libido, dyspnea, vertigo, and intolerance to cold. Of the four workers who were hospitalized in Cincinnati in 1949 for study, all had enlarged and tender livers, one manifested sensory loss in a foot. Clinical laboratory findings included severely delayed prothrombin time and a slight increase in total serum lipids. Histological examination of a nerve biopsy in the worker with pedal sensory loss demonstrated myelin degeneration. Follow-up examinations were conducted in April of 1950¹ and subsequently, in 1953.² In April of 1950 the original four and five additional subjects were seen. In 1953

a total of 36 subjects were examined, seven of those having been seen in 1949 and 1950. After four years had elapsed, those symptoms and findings observed initially which were referable to the nervous system and liver were no longer found. Those with moderate to severe chloracne had improved considerably; there was some persistence of the acne in those so affected.

In 1977-78 a mortality study³ of the group known to have been exposed to the material of the runaway reaction was conducted. This population consisted of 121 males. The follow-up was 100% complete for death certificate information. Standard mortality ratios for all causes of death were determined.

In 1957, 2,3,7,8 tetrachloro-dibenzo-p-dioxin (TCDD) was identified as a toxic and acnegenic contaminant of trichlorophenol synthesis.^{4,5} Since that time a considerable amount of scientific data has accumulated about the toxicologic and clinical effects associated with exposure to the trichlorophenol process, to 2,4,5-T synthesis and to the contaminant TCDD in man, experimental animals, and in vitro research models. The sub-acute effects which have been repeatedly observed in humans include chloracne, the most consistent clinical marker, liver function impairment, peripheral neuropathy, nervousness and irritability, sensation impairment, personality changes, porphyria cutanea tarda as well as hypertrichosis and hyperpigmentation. Elevation of serum cholesterol, triglycerides, GGPT and alkaline phosphatase have been reported.

Toxicologic examination of TCDD has revealed hepatotoxic effects and teratogenic effects in rodents, suppression of cell-mediated immunity in mice and guinea pigs and the induction of hepatic carcinoma in rats.

Some of the clinical reports appear to have been concerned with the association of cardiovascular disease and an increased risk for cancer in populations exposed to TCDD.

OBJECTIVES OF STUDY

The objectives of this clinical study were to determine and identify the possible long-term health effects of chemicals associated with the making of 2,4,5-T, including 2,3,7,8 tetrachloro-dibenzo-p-dioxin (TCDD) and to determine the increased risk for those adverse health effects which have been observed in the sub-acute phase or sub-chronic state in man as well as the effects which have been noted to occur in experimental animals including rodents, rabbits and sub-human primates. The objectives of the study, as reflected in its design, were to determine increased risks for cutaneous, pulmonary, cardiovascular, gastrointestinal, hepatic, renal, neurobehavioral problems, reproductive problems, birth defects, endocrinologic effects, effects on lipid metabolism, susceptibility to infection and the possibility of increased risk for cancer.

A most appropriate population for this study consisted of the employees and former employees of Monsanto Industrial Chemicals, Inc., Nitro, West Virginia who were involved in the manufacture of 2,4,5-T from 1948 to 1969.

STUDY DESIGN

The study design originally proposed the following three cohorts on the basis of company medical records.

- (1) Current employees, former employees and retirees who were exposed to the 2,4,5-T process during the period 1948 to 1969 and who were known to have chloracne and/or other clinical manifestations attributed to the process.

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- (2) Current employees, former employees or retirees who were exposed to 2,4,5-T process during the period 1948-1969, but who were not known to develop chloracne or other clinical manifestations attributable to the process.
- (3) Current employees, former employees and retirees who were not exposed to 2,4,5-T process and have no record of chloracne.

In the planning stage, it was anticipated that approximately 150 subjects would be available for the study in each of the above cohorts. It was estimated a total of 400-500 employees and former employees would participate in the study. Both management personnel and members of the Medical Department of the Monsanto/Nitro plant did an excellent job of recruitment of subjects for the study. However, it was recognized during review of questionnaires, including work history interviews as well as physician interviews and physical examination, that the participating subjects did not fulfill some of the criteria for these original cohort requirements. Selection bias was a factor in voluntary participation. No subject was excluded from the examination. The number in the exposed cohort exceeded the number in the non-exposed cohort. Subjects, who had never claimed or been observed to have had chloracne, were found to have history and/or residual chloracne upon examination. There was disagreement with regard to definition of exposure, i.e., exposure and intermittent exposure. On the basis of information gleaned from questionnaires and company work records three cohorts were constituted: 1) those who were clearly exposed to the 2,4,5-T process or its components; 2) those who were clearly not exposed and 3) those who were questionably exposed.

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METHODS AND MATERIALS

The design of the study and on-site survey were directed by Dr. Raymond R. Suskind. The interviews and clinical examination of 436 subjects were conducted during the week, June 11-18, 1979, in the Putnam County Health Center in Winfield, West Virginia by members of the Departments of Environmental Health and Dermatology of the University of Cincinnati Medical Center. Personnel from the above departments included physicians, nurses, graduate students in the Department of Environmental Health, technicians and interviewers. An average of 60 persons were examined per day during the study, with fewer persons seen on Sunday. The questionnaire and study program is found in Appendix I.

The complete examination included: an interview, clinical laboratory examination of blood and urine, pulmonary function tests, ECG, chest x-ray, nerve conduction velocity measurements, and a complete physical examination, including skin examination by dermatologists. During the dermatological examination, biopsies, skin scrapings, and photographs were taken as deemed appropriate by the physician and when agreed to by the participating subject. The nerve conduction velocity measurements were obtained for most subjects from two nerves: the peroneal nerve for motor function and the sural nerve for sensory function. In a relatively small number of subjects (22) nerve conduction measurements were made on the ulnar nerve.

Clinical laboratory analyses included: blood calcium, phosphorous, BUN, creatinine, BUN/creatinine ratio, uric acid, glucose (CS), total protein, albumin, globulin, albumin/globulin ratio, total bilirubin, direct bilirubin, Transaminase SGO, Transaminase SGP, alkaline phosphatase, LDH, cholesterol, iron, total lipids, sodium, potassium, chloride, G-glutamyl transpeptidase, triglycerides, CBC and differential, thyroxine R.I.A., thyroxine binding glo-

bulins. Urinalysis included routine examination as well as coproporphyrins and uroporphyrins.* Blood serum lipid fraction examination completed by the Lipid Research Division of the University of Cincinnati Medical Center included: serum cholesterol, serum triglyceride, serum HDL, and estimated serum LDL.

Chest x-rays were read by members of the staff of the Department of Radiology, University of Cincinnati Medical Center, under the supervision of Dr. Jerome Wiot. ECG's were interpreted by Dr. Te-Chuan Chou of the Cardiology Division, Department of Internal Medicine, University of Cincinnati Medical Center. Skin biopsies were prepared and read by Dr. Daniel Richfield, Dermatopathologist. Skin scrapings were cultured at the University of Cincinnati. Dr. A. Blair Smith initiated the examination of the data. Dr. Vicki Hertzberg, of the Biostatistics Division, performed all of the statistical analysis described in this report.

Data from the questionnaires were coded in the conventional manner by assigning numerical values to the responses given. Normal and abnormal findings noted by the examining physicians and salient details of each subject's medical history were coded using the SNOMED⁶ system. Chest x-rays were coded by observations and conclusions drawn by members of the Radiology Department. ECG findings were entered as interpreted by members of the Division of Cardiology, Department of Internal Medicine. ECG findings were reviewed with the subject's history, clinical findings, and chest x-rays in order to determine significant ECG findings. Laboratory data were entered as received. All data were keypunched and verified by the University of Cincinnati Computer Center. The keypunched data were then compared to the original files of the study subjects for accuracy. A computer file containing information from the questionnaires,

* Values for urinary coproporphyrin and uroporphyrin were determined from a single void sample rather than the required 10 ml. aliquot of 24 hour volume; hence levels outside of the normal range cannot be regarded as significant.

physical examinations, laboratory findings was created. All extremely large or small values for each variable were checked against the original values in the study subject's files. Following this second edit for accuracy of entered data, analysis of data began, using statistical programs available through the Statistical Analysis System (SAS) computer package. The statistical procedures used are briefly described in Appendix II. As the analysis progressed, periodic editing continued so as to insure the greatest possible accuracy.

THE SUBJECTS OF THE STUDY

The total number of subjects in the study is 436. There are 419 white males, 5 black males, 1 male of Hispanic descent, 1 male of American Indian descent, and 10 white females. One subject among the 419 white males was a 36-year old who was exposed to runaway reaction residue in 1949 at the age of 6. Since he was not an employee, he is not included in the data analysis. Since the number of women and non-white males is too small to be meaningful, they are not included in the remainder of the analysis. A brief description of these subjects, however, is contained in Appendix III. Information about sex, race, education and age range is found in Table 1.

The cohorts designated by exposure are defined as follows:

Group I - no exposure: 163 subjects

Group II - exposure, without qualification: 204 subjects

These subjects were involved in process and production and include pipefitters, electricians and all other employees working in or around the 2,4,5-T process.

Group III - questionable exposure: 51 subjects

This group includes laboratory and supervisory personnel not involved in production, but who occasionally handled 2,4,5-T or were around

the production area for short periods of time; laboratory personnel who worked in laboratories where 2,4,5-T was analyzed, but did not actually handle the material, carpenters who worked in the buildings of 2,4,5-T production occasionally, supervisory personnel whose main work efforts were in offices, but occasionally toured the buildings where 2,4,5-T was produced, and warehouse employees who described handling all types of finished products, including 2,4,5-T.

For a number of the analyses, the clearly exposed group was compared to the clearly not exposed group. Group III constituted a suspected exposure category. We report comparisons of the latter group with the clearly exposed and not exposed where pertinent.

RESULTS

A. Demographic Information

Demographic data of the 418 white males were examined to determine potentially confounding variables. Data on all subjects are found in Tables 1-4. The three groups were found to be significantly different with respect to employment status ($p < 0.0001$) (Table 2) and the highest education level achieved ($p < 0.0001$) (Table 3). 34.8% of the exposed were retirees and therefore in an older age group, whereas only 13.5% of the not exposed were retirees; 9.8% of the exposed were terminated, among them, employees who left the company in the era of 1949-1955 because of the health problem attributed to 2,4,5-T, whereas only 0.6% of the not exposed group were in the category of terminated. The mean age of the not exposed and questionably exposed groups was ten years younger than the exposed group (Table 4).

B. Alcohol and Smoking Use

History of alcohol consumption was found not to be significantly different in the three group (Table 5). When smoking status (current, former, never) was used as the criteria there was no difference between the exposed and not exposed group (Table 6). However, when the two groups were compared for pack years smoked, the exposed group was significantly higher than the not exposed group (Table 6). When present smokers were compared there was an almost 20 pack year difference between the exposed and not exposed. There was a difference of 9 pack years between the exposed and not exposed group in the former smokers. This probably reflects the 10 year age difference between the exposed and not exposed group and the heavier consumption of cigarettes in the exposed group.

C. Family History of Illness

Family history of illnesses were compared; the results are given in Table 7. All of the comparisons are made using Fisher's exact test for 2 x 2 tables. Family history of hayfever was found to be reported more often in the not exposed group ($0.01 < p < 0.05$). A family history of heart attack or angina after age 60 was reported significantly more often in the exposed group ($p < 0.01$).

D. History of Medical Problems

The comparison of the exposed and not exposed cohorts for illness elicited by medical history is given in Tables 8-9. Chloracne is reported more frequently in the exposed group: 86.27% in the exposed vs. 0.0% in the not exposed and questionable exposure group. In the not exposed group, acne vulgaris occurred more frequently than in the exposed group. A history of upper gastrointestinal ulcer occurred almost 4 times more frequently in the exposed group than in the not exposed group ($p < 0.005$). When the medical history of the exposed and not exposed are compared for two (2) age groups, less than 50 vs. 50 years and older, skin cancer and cerebrovascular accidents are only related to age not exposure; exposure, not age, appears to be a factor in the history of upper gastrointestinal ulcer. Hypertension and coronary artery disease occur more frequently in the older age group irrespective of exposure.

E. History of Medical Problems in Populations With and Without Chloracne

Since chloracne appears to be the prime clinical indicator of exposure, absorption and biological response, the study subjects were further classified into three sub-groups within the exposed populations; i.e., those who never have developed chloracne, those who had a history of chloracne but which was not observed in the 1979 examination and those whose chloracne persisted and

was noted on clinical examination (Tables 10-13). Among the 204 clearly exposed, 176 developed chloracne at some period. Of these, 107 were found to have chloracne on examination. Among the not exposed and questionably exposed, there were none who presented a history of chloracne nor were they found to have evidence of chloracne on examination. In both age groups, history of upper G.I. ulcer is more frequent in those who had chloracne than those who did not have chloracne regardless of exposure (Tables 10 and 11). As in the exposed versus not exposed group, the history of hypertension, cerebrovascular accident (CVA) and coronary artery disease are clearly age-related, not chloracne related.

When the clinical histories of the exposed population only were examined in relation to the history of and/or presence of chloracne, the same observations are made about angina, hypertension and coronary artery disease in that they are age-related not chloracne related. In the exposed group there is no association between the history of upper G.I. ulcer and chloracne (Table 12).

Although there appears to be a statistically significant relationship between exposure and history of upper G.I. ulcer, a closer examination of the temporal sequence of exposure and onset of ulcer reveals six subjects in the exposed group and one subject in the questionable group whose symptoms of ulcer occurred prior to exposure. In addition, there are four subjects among the exposed group whose ulcers were related to cortisone therapy, ASA therapy or alcoholism. If these 11 subjects are omitted from the analysis there are then 32 of 194 (16.49%) of the exposed group reporting ulcer vs. 9 of 163 (5.52%) among the not exposed subjects. This difference is statistically significant upon age adjustment ($p=0.01$). However, if the seven subjects reporting history of ulcer before exposure are considered to be not exposed subjects because of the temporal sequence, there are then 32 of 194 (16.49%) of the exposed group reporting ulcer

vs. 16 of 170 (9.41%). This difference is not statistically significant upon age adjustment.

Other potentially confounding variables were examined with the following results:

- (1) There was no relationship found between exposure category and history of ulcer among never smoking subjects.
- (2) Among formerly smoking subjects, 24.10% (20 of 83) of the exposed group report history of ulcer compared to 6.85% (5 of 73) of the not exposed group ($p < 0.005$).
- (3) There was no relationship found between exposure category and history of ulcer among presently smoking subjects.
- (4) There was no relationship found between exposure category and history of ulcer among the three categories of alcohol drinking (never, former, and present).

The following is a breakdown of data designating exposure vs. ulcer type and treatment:

<u>History of</u>	<u>Not Exposed</u> <u>(n=163)</u>		<u>Exposed</u> <u>(n=204)</u>		<u>Questionable</u> <u>Exposure</u> <u>(n=51)</u>	
	#	%	#	%	#	%
Gastrointestinal Bleeding	0		5		0	
Upper G.I. Ulcer	9	5.52	42	20.59	6	11.76
<u>Site as Reported</u>						
Peptic	3		9		1	
Stomach	4		21		3	
Duodenal	2		12		2	
<u>Treatment as Reported</u>						
Did not Report Treatment			13		3	
No Treatment	2		3			
Treated with Diet Only			4			
Medical Treatment	7		10		2	
Ulcer Symptoms Relieved with Hiatal Hernia Repair			2		1	
Gastrectomy			3			
Partial Gastrectomy			1			
Surgical Treatment of Ulcer, NOS			6			
<u>Problems Now</u>	3		8		1	

These data have the additional problem of being prevalence figures only as opposed to incidence rates.⁷ Also, report of upper gastrointestinal ulcer and designation of ulcer by site is crude at best since neither examination by gastrointestinal x-ray, nor endoscopy diagnosis was available.

Hence, while these data are suggestive, conclusions cannot be drawn due to the equivocal findings and the crude nature of the data.

F. Physical Examination Findings

The physical examination revealed a wide range of findings. These are cited in a comprehensive list in Appendix IV. The significant clinical findings were those associated with the skin and appendages, connective tissues, and sexual complaints. The discernable abnormalities referable to the cardiovascular, pulmonary, hepatic, hematopoietic systems, and nerve function were largely determined by objective tests in this study. These will be discussed under organ function tests, radiography and clinical laboratory measurements.

The significant clinical findings as related to exposure status are found in Table 14 and 15. As was indicated previously 52.45% of those who were clearly exposed still had chloracne. There is a significant difference in the frequency of acne vulgaris among the not exposed as compared to the exposed, 11.66% versus 1.96%. Among the exposed 120 or 58.82% were found to have actinic elastosis in contrast to 49 (30.06%) among the not exposed and 39.22% among the questionably exposed. There is a very significant difference in the occurrence of actinic elastosis between the exposed and not exposed in both age groups even though age is a factor in this clinical finding. There is a rather unique finding of 3 cases of Peyronie's Disease among the 204 exposed. All of them had chloracne at some time and they were all over 50 years old.

Rosacea and rhinophyma occurred in 4 exposed persons over the age of 50 and 2 questionably exposed. The complaints of loss of libido occurred more frequently in the exposed than the not exposed. When age was considered, the largest number of complaints of loss of libido or impotence occurred in the older age group but there were greater percentages of complaints about these problems in the exposed group as compared to the not exposed.

Tables 16 and 17 identify the clinical findings in the exposed population in relation to chloracne status. In these groups, actinic elastosis is found predominantly in the subjects with persistent chloracne (74.8%) but it also occurs significantly in those with a history of chloracne only (46.38%). 11 cases of malar hirsutism were found among the exposed in the persistent chloracne cases (10.28%). 9 of these 11 cases occur in the group over 50.

Table 18 lists the sites and severity of chloracne observed on physical examination. The distribution and severity of actinic elastosis is presented in Table 19.

Since there appears to be some association of actinic elastosis with chloracne further analysis of this relationship with respect to severity of chloracne and severity of actinic elastosis was carried out. These analyses are presented in Table 20 and Table 21. Of the 67 mild cases of residual chloracne, 44 had actinic elastosis. Of the 36 with moderate chloracne 33 had actinic elastosis. 33% of the actinic elastosis cases found with chloracne were severe. It would appear that the percentage of persons with severe actinic elastosis is related to presence of chloracne (Table 21). In other words, actinic elastosis appears to be more severe when found with chloracne.

Blood pressure findings at the time of examination in the exposed, questionably exposed and not exposed groups are presented in Table 22. When the criterion for abnormal systolic and diastolic use different values for the groups <59 (140/90) and ≥60 (160/95), there appears to be no significant differences between the exposed and not exposed group. These criteria are stricter than that proposed by the National Heart, Lung and Blood Institute.⁸

G. Clinical Laboratory and Other Test Findings

I) Lipids

Because of the suspected relationship of serum lipids to exposure to 2,4,5-T and its contaminants, serum lipids are analyzed in several ways. Serum cholesterol, triglyceride, LDL, and HDL were classified by age-specific percentiles as determined by the National Lipid Research Project data⁹ (See Appendix V). Serum cholesterol, triglyceride, and LDL were classified as "abnormal" if they fell above the 90th percentile, and were classified as "normal" otherwise. Serum HDL was classified as "abnormal" if it fell below the 10th percentile, and was classified as "normal" otherwise. The frequency of abnormal lipid findings are compared for exposed, not exposed workers and questionably exposed (Table 23), and no significant differences are noted in cholesterol, triglycerides, HDL and LDL levels. These relationships are further investigated within each level of smoking status (present, former and never) (Table 24). Smoking appears to have no effect on the differences between these groups. Logistic regression was used to detect associations of abnormal serum lipid findings with exposure. After adjusting for pack years smoked, no significant differences between exposed and not exposed could be found.

Multiple linear regression was also used to detect possible "shifts" in the mean serum lipids for the two exposure groups after correcting for the effects of age, tobacco, and alcohol. In all cases (cholesterol, triglycerides, HDL, LDL) there are no significant differences between the two exposure groups after adjusting for the effects of age, tobacco and alcohol.

When the lipid levels of the exposed group only are examined in relation to the occurrence of chloracne, the HDL levels in those with persistent chloracne are found to be somewhat lower than of the group with a history only of

chloracne or those who never had chloracne (Table 25). Those who had a history only of chloracne have somewhat higher LDL levels than those who had chloracne currently or never. Among former smokers in the exposed group there is a greater frequency of abnormal triglyceride and HDL levels among those with residual chloracne than those with no history or only history of chloracne (Table 26). Among those who smoke presently there is a higher frequency of abnormal or high LDL levels for those who have a history only of chloracne.

2) Pulmonary Function Status

Both FEV_1 and FVC were considered abnormal if the observed value was less than 80% of the predicted value of Morris. A FEV_1/FVC ratio of less than 0.70 (70%) was considered to be abnormal. The FEF_{25-75} was considered abnormal if the observed value was less than 70% of the predicted value of Morris.

Consistent differences in abnormal pulmonary function values (FEV_1 , FVC, FEV_1/FVC and FEF_{25-75}) are found between the exposed and not exposed groups (Table 27). Among present smokers there was a significant difference in pulmonary function values between the exposed and not exposed group. There are no differences in pulmonary function values among persons who have formerly smoked or among persons who have never smoked when the exposed were compared to the not exposed (Table 28).

Logistic regression was used to detect associations of abnormal pulmonary function parameters with exposure. The model and coefficients are given in Appendix VI. The significance and odds ratios for abnormal pulmonary function for exposed vs. not exposed after adjusting for pack years smoked are

as follows:

<u>Pulmonary Function Parameter</u>	<u>Significance of Exposure</u>	<u>Odds Ratio</u>
FEV ₁	0.02	2.71
FVC	0.05	2.10
FEV ₁ /FVC	0.01	2.89
FEF ₂₅₋₇₅	0.06	1.82

The association of exposure and abnormal pulmonary function among current smokers was investigated further for the confounding by prior coal mining exposure. None of the not exposed subjects who currently smoke had prior coal mining exposure. Of the exposed subjects who currently smoke, 13 individuals had prior coal mining exposure. Among the current smokers who had no coal mining exposure the exposed were found to have a higher frequency of abnormal FEV₁, FVC and MMEFR than the not exposed as follows:

Non-Miners-Current Smokers						Miners Current Smokers	
Pulmonary Function Parameter	Exposed (n=60)		Not Exposed (n=44)		Probability	Exposed (n=13)	
	#	%Abnormal	#	%Abnormal		#	%Abnormal
FEV ₁	12	20.00	2	4.55	0.039	7	53.85
FVC	14	23.33	1	2.27	0.003	4	30.77
FEV ₁ /FVC	10	16.67	3	6.82	N.S.	8	61.54
FEF ₂₅₋₇₅	18	30.00	5	11.36	0.039	8	61.54

3) ECG and X-ray Findings

Tables 29 and 30 list the occurrence of ECG findings in the exposed and not exposed as well as the differences in occurrence when the exposed and not exposed are separated into <50 and ≥50 year groups. Number of persons with PVC's and PAC's appear to be only age-related. Tables 31 and 32 list the x-ray findings in the exposed as compared to the not exposed as well as the comparison between the <50 and ≥50 year old groups. Significant x-ray findings appear to be age-related but not exposure related.

4) Clinical Laboratory Findings

In Table 33 the relative frequency of out of normal range values is compared in the exposed vs. not exposed group. In Table 34 the relative frequency of out of normal range values of clinical laboratory findings for the exposed group in relation to chloracne status is presented.

Analysis of covariance was used to compare the laboratory findings in the exposed group to the not exposed group after adjusting for the effects of age, smoking status and alcohol status. The same procedure was used to compare the laboratory findings for three chloracne groups after adjusting for the effects of age, smoking and alcohol status.

Fisher's exact test was used to determine the existence of differences between the exposed and not exposed with respect to the relative frequency of out-of-reference-range laboratory findings. The χ^2 test for independence in contingency or two way tables was used to determine differences in the three (3) chloracne groups with respect to the relative frequency of out-of-reference-range laboratory findings (Appendix VII).

In several other incidents involving the synthesis of trichlorophenol and 2,4,5-T, porphyria cutanea tarda was observed with uroporphyrinuria. The exposed, not exposed and questionably exposed in this study were compared for percentage of abnormal levels of coproporphyrin and uroporphyrins. No significant differences were found (Table 35).

5) Nerve Conduction Velocity Data

Prior to the beginning of the data analysis, all subjects reporting diabetes or reporting a high weekly alcohol intake were eliminated from the analysis (high weekly alcohol intake was defined as greater than or equal to 35 oz. alcohol per week). After these deletions, there were 319 with complete sural examinations and 336 with complete peroneal examinations remaining. The data from the nerve conduction velocity study were adjusted according to the deJesus method (Appendix VIII). Tables 36-38 lists all nerve conduction parameters considered. The following methods of analysis were then used:

a) Analysis of covariance was used to compare the nerve conduction parameters after adjusting for age differences in the groups. No significant differences were found between the exposed and not exposed. Also within the exposed only, no relation to chloracne status was found after adjusting for age.

b) Using the normal ranges provided by NIOSH (see Appendix IX) the exposed group has a significantly higher proportion with abnormally low peroneal nerve conduction velocities than the not exposed group (74% vs. 49%, $p < 0.0001$). No differences were found with respect to abnormal sural nerve conduction velocity or peroneal distal latency (not enough ulnars were tested to say anything about them). However, Boyd points out that these ranges are not age-adjusted. Moreover, temperature has a great effect on velocity and latency, and

temperatures were not reported with these ranges. (Ranges and appropriate references are attached). Among young men (age < 50) no difference between exposed and not exposed with respect to any of the parameters was observed. Among the older men (age ≥ 50) a significantly higher proportion of the exposed group have abnormally low peroneal nerve conduction velocities as compared to the not exposed group (79% vs. 57%, $0.01 < p < 0.05$).

c) The z-score method (as used by Selikoff) was also used to detect differences in the nerve conduction parameters between exposed and not exposed. No significant differences were found using this method.

H. Reproduction and Birth Defect Data

In presenting this information it should be understood that all of the data depended on personal recall of such information as number of pregnancies, miscarriages, live births, stillbirths, infant mortality, etc. Confounding factors which effect accuracy are obvious - e.g. interviewed male subject only, age of subject, and change in cultural attitudes about reproductive matters. It was also confounded by the fact that the pregnancies, miscarriages, stillbirths were not designated in a time period related to exposure e.g., pre, during, post exposure.

Fisher's exact test was used to compare the reported occurrence of reproductive anomalies in the exposed group to the not exposed group. The data on reproductive and birth defect findings are found in Tables 39 and 40. No significant differences were found in miscarriages, in rate of birth defects or stillbirths.

The TCP Runaway Reaction Group

Among the 204 persons who were known to be exposed to the 2,4,5-T synthesis and preparation, there were 55 who were involved in a trichlorophenol process runaway reaction, which occurred on March 8, 1949, described in the historical introduction. There were a total of 122 persons known to have been involved in this accident and with its products.

This group of 55 was matched as closely as possible in age with an unexposed group and a third group of exposed but not to the accident material. Since the range of the runaway reaction group was 49 years and older, the matched unexposed control group consisted of persons in the same age range, e.g., ≥ 49 years. There were 65 persons in the unexposed group. Because of the percentage of aging retirees in the TCP runaway group there was still a four year age difference between the accident group and controls. There was no difference in age between the TCP accident group and the other exposed. In the other exposed group the age range was ≥ 55 . There were 77 subjects in that group. Data comparisons were made between the accident group and the unexposed group as well as the accident group and the other exposed group.

The following were the significant findings elicited:

- 1) When smoking status was considered, the average number of pack years was significantly greater in the TCP runaway group than the not exposed group by 10 pack years. The difference between the TCP group and other exposed group was 9.5 pack years.

- 2) All of the TCP exposed had a history of chloracne; none of the not exposed had chloracne; 83% of the other exposed group had a history of chloracne. In the TCP group examined, 58.2% still had chloracne; in the other exposed 55.8% still had chloracne.

3) There were no significant differences in the current findings of chlor-acne and actinic elastosis between the TCP and the other exposed group.

4) The percentage of persons with actinic elastosis was significantly larger in the TCP group than the not exposed group (75% vs. 49%). The difference in the occurrence of actinic elastosis between the TCP and other exposed group (75% vs. 68%) was not significant.

5) There was a significantly larger number of systolic murmurs found in the TCP as compared to the other exposed group (12.7% vs. 1.3%, $0.01 < p < 0.05$). Although there appears to be a statistical difference between the percentage of abnormal BUN's found in the TCP group (9.43% or 5/55) as compared to the other exposed (1/73 or 1.37%) there was no difference between the TCP group and not exposed group (9.43% vs. 6.35%).

6) The complaints of nervousness were greater in the TCP runaway group than the not exposed controls (21.8% vs. 7.7%). In the other exposed controls the occurrence of nervousness was 16.9%.

7) Logistic regression was used to compare TCP runaway group to the not exposed and to the other exposed group, for abnormal serum lipid findings, while simultaneously adjusting for pack years smoked. No significant differences were found in either comparison.

8) In the pulmonary function tests, the number of persons with abnormal FEV_1/FVC test findings in the TCP group was significantly greater than among the not exposed. This same difference was seen among current smokers in the two groups. There was also a slightly greater percentage of persons with abnormal $\dot{V}_{MEFR}$ among the TCP group who are currently smoking than the not exposed who are currently smoking.

9) Logistic regression was used to compare TCP runaway exposed to not exposed for abnormal pulmonary function while adjusting for pack years smoked. No relationship was found between TCP runaway exposure and abnormal FEV₁, FVC or MMEFR. However, a significant relationship was found between this exposure classification and abnormal FEV₁/FVC, with a greater frequency seen in the TCP runaway group (p=0.01). The odds ratio is 4.02 for abnormal FEV₁/FVC, given pack years smoked, for TCP runaway exposure vs. not exposed.

Logistic regression was used to compare TCP runaway exposed to other exposed for abnormal pulmonary function while adjusting for differences in pack years smoked. No significant differences were found.

Other Work Exposures of the Study Population

Other than 2,4,5-T, its intermediates, and/or contaminants, all of the workers in the study were exposed to multiple chemicals and industrial processes under varying working conditions, at Monsanto/Nitro and/or other industries, in a period roughly between the late 1930's and 1979 (a few subjects were born before 1900).

Chemicals identified by the subjects as other Monsanto/Nitro exposures include: rubber processing chemicals, i.e., diphenylguanidine; a group of benzothiazolesulfenamide accelerators (Santocures[®]); antioxidants, i.e., derivatives of m-cresol; antiozonants (Flectols[®] and Santoflexes[®]), most of the Santoflexes are p phenylenediamine derivatives; vulcanizing agents such as 4,4-dithiodimorpholine; insecticides, i.e., ethyl and methyl parathion. Additional chemicals named were phthalic anhydride, o and p dichlorobenzene, o and p nitrophenol, toluene and mercaptobenzothiazole, derivatives of TMTD and trimethylquinoline components. One of the products, Sopanax[®], an antioxidant, was alleged by the study subjects to be associated with the occurrence of kidney stones. The reporting of the H/O kidney stones is not statistically different among the three groups.

One of the chemical hazards to which the study population was exposed was p aminobiphenyl, a known bladder carcinogen. Ninety-five subjects, or 22.72% of the 418, reported exposure to p aminobiphenyl during the course of their employment. To the best of our knowledge these subjects are periodically examined in a monitoring program established by the Medical Department of the Company. Subjects reported the exposure and the monitoring program during the interview and to the examining physician. The data analyses relating to this exposure and

exposure to 2,4,5-T is as follows:

	<u>2,4,5-T Exposure</u>					
	<u>Not Exposed</u> <u>(n=163)</u>		<u>Exposed</u> <u>(n=204)</u>		<u>Questionable Exposure</u> <u>(n=51)</u>	
	#	%	#	%	#	%
p aminobiphenyl Exposure	8	4.91	71	34.80	16	31.37
H/O Bladder Tumor*	1	0.61	7	3.43	0	0.00
H/O Bladder Cancer*	1	0.61	2	0.98	2	3.92

* Figures are mutually exclusive

The degree to which each subject may have been exposed to other chemicals both at Monsanto/Nitro and other places of employment varied markedly. This fact must be considered in attempting to interpret biological significance to statistical differences based on small occurrences.

Discussion and Conclusions

The strengths and weaknesses of this study must be considered critically in any appraisal of the medical significance of the data elicited from it.

Data gleaned from interviews and clinical examinations are useful but have some inherent weaknesses. Medical, work and personal histories depend on memory; findings of physical examinations carried out by different physicians are based on judgments which may differ somewhat with the examiner. Nevertheless these constitute an important data base for making clinical judgments.

Since quantitative TCDD levels for any period during the manufacture of 2,4,5-T were not available, it was only possible to relate effects found, to the history of definite exposure, no exposure, and questionable exposure to any aspect of 2,4,5-T production.

Since chloracne is the hallmark of biological activity of TCDD it was possible in this study to relate all systemic and other cutaneous effects to the presence or absence of chloracne in the exposed group as well to exposure itself.

From this study one can conclude that for those exposed to TCDD the dramatic long term effects are cutaneous and involve the pilosebaceous apparatus and the elastic tissues of the dermis. Chloracne may persist for as long as 30 years after onset and is associated with elastic tissue changes in the dermis and some persistence of malar hirsutism.

There are suggestive but inconclusive data indicating that for the exposed there was an increased risk for upper gastrointestinal ulcer; that among those in whom chloracne persists there is (although numbers are small) an association with low HDL levels and further for those with only a history of chloracne there is an association with higher LDL levels.

In those who currently smoke and have been exposed to the 2,4,5-T process

materials there appears to be an association of abnormal pulmonary function findings when compared to those who smoke but who were never exposed.

There is no evidence from this study that there are identifiable long term effects on the cardiovascular system, on the liver, on the gastrointestinal tract, on peripheral nerve function. There is no indication, on the basis of historical information (recognizing all of its weaknesses) that there was a greater risk for miscarriages, stillbirths, or birth defects among the families in which the male parent was exposed.

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TABLES

TABLE 1
DEMOGRAPHIC DATA ALL STUDY SUBJECTS

	<u>Total</u>	<u>White</u>	<u>Black</u>	<u>Other</u>
Males	426	419	5	2
Females	<u>10</u>	10	0	0
Total	436			

HIGHEST EDUCATION LEVEL ACHIEVED

0 - 8th Grade	25
9 - 12th Grade	266
> 12th Grade	<u>122</u>
Total	416

TABLE 2
PRESENT EMPLOYMENT STATUS

	<u>Active</u>	<u>Retired</u>	<u>Terminated</u>	<u>Total</u>
Not Exposed				
Number	140	22	1	163
Percent	85.89	13.50	0.61	
Questionable Exposure				
Number	38	11	2	51
Percent	74.51	21.57	3.92	
Exposed				
Number	113	71	20	204
Percent	55.39	34.80	9.80	
Total	291	104	23	418

$p < .0.0001$

TABLE 3
EDUCATION LEVEL

	<u>0 - 8th Grade</u>	<u>9 - 12th Grade</u>	<u>>12th Grade</u>	<u>Total</u>
Not Exposed				
Number	2	92	68	162
Percent	1.23	56.79	41.98	
Questionable Exposure				
Number	4	27	20	51
Percent	7.84	52.94	39.22	
Exposed				
Number	19	147	37	203
Percent	9.36	72.41	18.23	
Total	25	266	125	416

$p < 0.0001$