Vinyl chloride

MANUFACTURING CHEMISTS ASSOCIATION

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January 26, 1976

To: Technical Panel on Vinyl Chloride Research

Subject: Enclosures with Minutes of Meeting, December 11, 1975

Gentlemen:

The following are enclosed with the minutes of the above captioned meeting:

1. Abstracts supplied by Dr. Rinehart from the recent International Congress on Occupational Health in Brighton, England. Papers on (a) Evidence of an Immune Complex Disorder in Vinyl Chloride Workers, by Dr. Ward et al., and (b) Chromosomes - Clinical Aspects of Vinyl Chloride Disease, by Dr. Purchase et al.

2. Key sections from University of Louisville Proposal transmitted by Dr. Tamburro, October 31, 1975. These include (1) Table of Contents (2) Introduction (3) Overview, and (4) Budget Summary.

3. Translation of current German regulations entitled "Decrees of the Committee for Dangerous Materials" supplied by Mr. R. N. Wheeler.

4. Translation of German article entitled "VC/PVC: An Example of a Problem Resolved" supplied by Mr. Wheeler.

5. Summary of European VC feeding studies supplied by Dr. Maye Smith.

Sincerely,

Milton Freifeld

Milton Freifeld
Project Manager

MF/mj
Enclosures

Reproduced by MCA for the Technical Panel on Vinyl Chloride. Abstracts of papers presented at the 18th International Congress on Occupational Health, Brighton, England, September 1975.

Evidence of an immune complex disorder in vinyl chloride workers

Dr. A. Milford Ward, Department of Immunology, Hallamshire Hospital, Sheffield.

Recent investigation of 52 workers from vinyl chloride plant has revealed evidence of a circulating immune complex disorder in 28 individuals. The immunological and immunochenical features include polyclonal hyperimmunoglobulinaemia, cryoglobulinaemia and cryofibrinogenaemia, and in vivo complement activation and conversion. There is also evidence of a reduced T cell population and a moderate B cell proliferation. Some of the patients showed low levels of nonorganspecific autoantibodies of varied specificities.

Immunofluorescent examination of biopsy material from skin, muscle andlung have shown the presence of circulating immune complexes with depostion on vascular endothelium and incorporation into the subintimal proliferation.

The results obtained from the immunological profile have allowed the construction of a theoretical model of the disease process. The final phase of the process follows the formation of the immune complex. Complement activation and cryoprecipitation precipitate the observed vascular anomalies and the secondary clinical manifestations. The initial phase of the process remains speculative, but experimental metabolic data suggest that vinyl chloride metabolites may be incorporated into protein. The structually altered or antigenically foreign protein would escape tolerance and promote an immune response. The resultant 'antigen' and antibody complex would then be able to initiate the final phase of the process. The presence of non-organspecific antitissue antibodies may represent a tissue damage response or simply an aberrant immune mechanism.

Chromosomes - clinical aspects of vinyl chloride disease

Dr. I. F. H. Purchase, Dr. C. Richardson and Dr. Anderson, Imperial Chemical Industries Limited, Central Toxicology Laboratory, Alderley Park, near Macclesfield, Cheshire.

Vinyl chloride, in common with several other carcinogenic agents, produces in exposed workmen, abnormalities in the chromosomes of lymphocytes. The examination of lymphocyte chromosomes has the great advantage that it offers a clinical method of assessing damage to genetic material. It may, thus, be possible to determine the exposure level which produces no chromosomal genetic damage relatively rapidly.

We have carried out a study of chromosome abnormalities occurring in exposed workmen and related these to their occupation and hence to their relative level of exposure. Eighty peripheral blood samples were collected including those from 56 exposed workmen and non-exposed controls from the same and another site. Preparations of lymphocytes in metaphase were made using standard techniques. One hundred cells from each sample were examined without the examineer being aware of their origin. The cells were scored for abnormalities and classified either as A cells (normal), B cells or C cells (Buckton and Pike, Int. J. Rad. Biol. 1964, 8 439) and the results were expressed as the percentage of each abnormal cell type.

The percentage of abnormal cells (B and C cells) seen in the exposed group was significantly higher than that in the control groups. A general trend

VCM Feedings

Laboratory	No. Animals M	No. Animals F	Time	Levels Mg/Kg/Day	Medium	Method	Status
CIVO	--	--	28 days	0, 90, 300 - 6/week	Oil	Gavage	No tumors or toxicity
	15	15	90 days	0, 30, 100, 300 - 6/week	Soya Oil	Gavage	No tumors
	60	60	2 years	0, 1, 3, 9, 300	PVC and Oil	Gavage	Started late 1974 No report
Maltoni	40	40	1 year to death	0, 3.3, 16.6, 50	Olive Oil	Stomach Tube	No report since March (2 tumors)
	?	?		0, 0.03, 0.3, 1.0	Olive Oil	Stomach Tube	No tumors after 21 weeks
ICI	18	18	2 years	0, 3, 30, 300	Corn Oil	Stomach Tube	Started June 1974
	18	18	90 days	0, 3, 10, 30, 100, 300	Corn Oil	Stomach Tube	Started June 1974 No tumors to date
BIBRA	?	?	15 weeks	0, 2.5, 25, 250*	Drinking Water	--	Started September 1974 - no results
	48	48	2 years	0, 0.37, 3.7, 37	Drinking Water	--	In progress
DSM	- - - - - - - - - - - - - - - - No Knowledge -- Will Try To Find Out -						

* ppm

Revised 11/25/75
/df

TECHNICAL PROPOSAL

RESEARCH TECHNIQUES AND METHODS FOR DETECTION AND PREVENTION OF CARCINOGENESIS IN INDUSTRIAL WORKERS

UNIVERSITY OF LOUISVILLE
CANCER CENTER
LOUISVILLE, KENTUCKY 40201

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I. Introduction

A. Prologue

Most industries have been very cognizant of the occurrence of industrial related disease and have made continuing efforts to identify as well as prevent the possible development of industrially related illnesses. The recent discovery of angiosarcomas of the liver related to the vinyl chloride polymerization has led to a refocusing of attention on industrial related diseases, especially so in the petroleum and plastics industry. Most efforts in the past have been directed toward identifying the cause of disease after its development and overt clinical presentation. Methods are then developed for its prevention, and implementation of these preventive measures is then made a requirement. Frequently, these preventive measures are based on limited information concerning the etiological cause and are based on limited clinical data as to their relative value and effectiveness.

In the past few years, due to the discovery of angiosarcomas of liver's relationship to vinyl chloride polymerization workers of the B. F. Goodrich Louisville Plant, there has developed a screening program begun by the combined efforts of the B. F. Goodrich Louisville managerial and medical staff led by Dr. John Creech, the Plant Physician. Following this, a starter grant from the B. F. Goodrich Company was given to the University of Louisville's Cancer Center to design a comprehensive program of continuing medical surveillance and consultation, to establish a data bank and to develop a clinical research unit which would lead to the development of a health maintenance and prevention system. The University, utilizing this initial grant, developed a prototype medical surveillance program for industrial workers which has received support in the form of an $2.8 million contract from the National Cancer Institute's Cancer Control Division for the specific purpose of further development and demonstration of this prototype program's implementability in an industrial environment. Its objectives are to design methods for early detection, prevention, diagnosis, and treatment ofthose individuals exposed to potentially toxic chemicals in an economical and safe manner with the aim of preventing any serious injury, precancerous lesions or the development of cancer either on a short or long-term basis. The program is designed to identify, at the earliest possible time, potentially harmful chemicals and to lead to a reduction in their exposure based on objective chemical data. The program is also designed for continued updating ofmethods of detection, prevention and their applicability and for the storage of this data for ongoing continous analysis and study. Furthermore, biological materials, such as urine, blood and tissue, obtained via medical screening purposes, are being retained for the purposes of further study by newer, more accurate testing methods presently being developed.

Simultaneously, efforts are being made at the University of Louisville to study the problem of vinyl chloride toxicity and disease developed in animal models. Studies of such nature are of key importance in testing our potentially more effective methods of detection and/or treatment. One of the major shortcomings, however, in any animal

experimentation concerning human disease, is the extrapolation of animal results and their applicability to humans.

At present, there is a national shift in emphasis from the purely toxicological investigations to one more directed at developing methods of early diagnosis and clinical management of potentially toxic industrial chemicals. Vinyl chloride provides such an example which can be used as a model for evaluation of other potentially toxic materials. The need for developing methods which can validate the sensitivity and specificity of new testing methods for early detection of organ involvements is of key importance to any medical surveillance program. Accurate documentation of the relative values of certain screening procedures will lead to elimination of those of limited values, and information obtained will aid in determining what, if any, industrial changes need to be made.

The uniqueness of this medical surveillance system and research program at the University of Louisville is that these experimental results can be applied and tested rapidly in a population of industrial workers whose medical data and biological material are available for testing without having to restudy these individuals or begin a new program to evaluate them.

B. Purpose

Our clinical research group has developed, as indicated in this proposal, a combined intradisciplinary approach for the evaluation of chemical agents for their potential toxicity, identification of biological abnormalities produced by these chemicals or their metabolites, determining which biological systems are involved and which would require surveillance; and finally, selecting the most sensitive methods for detection that could be applied to human medical surveillance systems. This unique, intradisciplinary effort coupled with an ongoing medical surveillance program in a cohort population under study will allow us to clearly demonstrate the effectiveness of such a program in accurately identifying which industrial agents may or may not be of medical danger, the levels of exposure which may be harmful, the duration of exposure needed and methods of preventing normally nontoxic agents from becoming toxic by their change in the biological system.

Our proposal here describes the ongoing clinical investigative program being conducted at the University of Louisville for which we are submitting a formal proposal for sponsorship by the Manufacturing Chemists Association.

The clinical investigative programs are listed in the following order: first are those programs which are now in existence and functioning; secondly those programs which are about to begin and have such unique importance related to the industrial surveillance system that they warrant your consideration.

II. Overview

Section A describes the proposals for the immunological systems of detection presently performed under the direction of Mr. Philip Fortwengler, our Immunochemist. These will include the genetic identification of individual susceptibility to vinyl chloride injury based on the HL-A histocompatibility system. This histocompatible identification needs only to be performed once and can be used to determine susceptibility to multiple agents. Evaluation of the immunocompetence systems of individuals with both short and long term exposure to vinyl chloride and other chemicals will greatly aid in identifying defective or reduced immune surveillance prior to the development of malignancy. This is being done on those individuals who are found by the screening program to have developed liver lesions which are thought to precede the development of cancer. Also, the recent development of the lymphocyte adherence inhibition test for tumors is in the process of being developed for angiosarcoma specifically and other primary liver tumors. This system utilizes actual tumor tissue to develop an antigen which can be used to test a suspected individual of the earliest indications of tumor development (i.e., productions of specific tissue antigens which will interfere with leukocyte adherence). This system has already been shown to be specific and sensitive in other tumors and may yet be the earliest indication of cancer formation. Finally, the determination of toxicity and/or transformation of human cells when exposed to chemicals or their metabolites is of key importance. An immunological system for such detection is being developed utilizing human fibroblasts and other mammalian cells which will be grown with specific chemicals (i.e., vinyl chloride) and their metabolites supplied by Dr. Wong's group. This will predetermine which of the mammalian cells is first involved in physical and biochemical changes and will assist in determining which methods should be utilized for detection of early evidences of transformation.

Section B presents the proposals for developing biochemical enzymatic systems for the detection of liver injury due to vinyl chloride or its metabolites. These studies are being conducted under the direction of Dr. Julie Du, our Biochemist. These include studies to determine the activity of coupling enzymes in the hepatic cytosol fraction of rats exposed to different levels of vinyl chloride for various lengths of time. Methods for determining this activity in the bloods of animals is also being developed for use in the human biological system. These studies would then be extended for implementation in the medical surveillance program and could serve as an early method of hepatocellular injury. The problem of a liver cell metabolizing a chemical and converting it into a potentially carcinogenic agent can be increased by inducing microsomal drug metabolizing enzymes in the liver cell. These studies will be extended from the rat liver microsomal fraction to human lymphocytes so that inducibility studies might be carried on in vitro. Subcellular enzyme assays have been conducted in experimental animals under various exposures to vinyl chloride without evidences of hepatocellular injury. Similar studies using vinyl chloride metabolites before and after induction are under way. These will be coupled with a newly developing microsomal

mutagenecity assays for vinyl chloride and its metabolites. The system of determining non-protein sulfhydryl content in liver and in blood is being developed for possible utilization as a screening process for workers as an indicator of early induction by potential chemicals. Bile acid determination and clearances, as a means of general screening, are under way to determine their increased sensitivity and specificity to the presently used anionic dye clearances.

Finally, morphological studies by both light and electron microscopy will be conducted on the same vinyl chloride exposed animal tissue that are to be used for the various biochemical and enzymatic studies. These will be performed on animals of different ages, one month, two months, etc., thereby allowing us, in addition, to determine the effect of aging in response to vinyl chloride and/or its metabolites.

Section C presents a proposal for a biochemical protein system which has already demonstrated evidence of its usability. This work is being conducted by Dr. Charles Kupchella and utilizes the study of acid mucopolysaccharides both in serum and urine as a means of monitoring the development of early fibrosis and carcinogenesis in vinyl chloride and other chemically induced liver or tissue injury. These acid mucopolysaccharides have already been shown to be increased in certain cancer patients, and our own preliminary studies indicate that there may be a significant increase in some of the specific components of acid mucopolysaccharides which appears to have some predictive or diagnostic value in identifying significant exposure to vinyl chloride. The initial data suggests strongly that there is a characteristic urinary acid mucopolysaccharide secretion pattern associated with vinyl chloride induced liver injury. This assay method could be made suitable for hospital clinical laboratories.

Section D describes the proposal for the continuation of the study of human histology presently being carried out by Dr. Randolph Schrodt and the medical surveillance team. A system of systematic, objective identification and semi-quantitation of the histological and the electron-microscopic evaluation of abnormal findings in workers found to have medical screening abnormalities is under way. This is of vital importance since there is no data available concerning the normal limits and normal distribution of collagen formation (scar tissue - fibrosis) in the hepatic lobules of humans; and before correct assessment can be made of the possible significance of vinyl chloride exposure to hepatic fibrosis, a standard must be set and an objective comparison must be able to be made between normals and individuals having hepatic lesions of other etiologies. This has special clinical importance since our present studies indicate that the increased amounts of collagen (fibrosis) may not necessarily be a specific indication of industrial chemical injury.

Section E describes proposals for the chemical systems for detection. These, being developed under the direction of Dr. John Wong and Dr. Grant Taylor, are aimed at quantitation of the metabolic end products of vinyl chloride and other industrial monomers in human body fluids and tissues. The establishment of a "chemical profile" of non-enzymatic biochemicals in body fluids and tissues associated with various pathological states can greatly aid in determining both the etiology as well as the degree of exposure needed for verification of the diagnosis and allow the early

institution of appropriate preventive or treatment measures. Equally important is the capability of synthesizing and manufacturing not only the metabolites of potential chemical toxic agents but also their variance. Under the direction of Dr. John Wong, potentially mutagenic and carcinogenic industrial chemicals and their metabolites will be synthesized independently and provided for both immunological and biological studies. Study models for the detoxification and biological oxidation reactions in the absence or presence of blocking agents and the effect on DNA will be provided in collaboration with the biochemical, immunological and microbiological groups. This system will allow the identification and will illustrate the activity of the "ultimate" carcinogenic forms of industrial monomers and determine the efficacy of various trapping agents for blocking such reactions thereby sparing the target site in the host from chemical insults.

Section F presents the proposal for the microbiological systems of detection which are being performed under the supervision and direction of Dr. Uldis Streips. The need to develop a system of rapid identification of the potential toxic mutagenic or carcinogenic properties of chemical agents or their derivatives is of enormous importance. Tri-collaborative efforts between Dr. Streips, Dr. Wong and Dr. Tamburro will allow for the synthesis of the potential metabolic derivatives of vinyl chloride and other chemicals and the study of their mutagenic properties in a bacteriological system similar to that expected to occur in the human. This system will identify potentially mutagenic chemicals or metabolites in a far more rapid, reproducible system which has tremendous advantages over the total animal model system usually used for toxicological studies. This system is capable of mass screening and will provide a rapid alerting system for the medical surveillance screening program.

Section G describes the proposal for a tissue antigenic system under study for purposes of early detection. These investigations, performed under the direction of Dr. Enrique Espinosa, are studying the changes of blood antigens and the presence of anti-tumor antibodies in angiosarcoma patients as a possible means of early diagnosis of vinyl chloride injury. These studies will look for significant differences in the number of levels of antigenic constituents of liver angiosarcoma tissue and those of normal livers. They will include a further characterization of the antigens found to be abnormal in angiosarcoma tissue and the identification of antigenic components which will stimulate anti-tumor antibody response in patients. Comparison studies between antigens of angiosarcoma tissue and those of embryonic antigen will also be carried out. Once these have been developed, our immediate search for the possible presence of these angiosarcoma associated antigens and antigen antibody immune complexes will be carried out in the stored seras and urines of the employees undergoing surveillance.

Section H describes a proposal for the pathogenic models for determining carcinogenesis after exposure to chemical agents. These series of studies will be conducted under the direction of Dr. Curtis Sigdestad and will hopefully provide information concerning the mechanism of early development of angiosarcoma and the possible means of protection. Studies will be conducted to demonstrate whether the liver, as the detoxification center of the body, will produce toxic materials which

will be incorporated into various liver cells but remain dormant until there is a wave of mitosis in hepatocytes or supporting structure cells due to an external stimulus or other cause of injury. This would lead to a period of restorative hyperplasia associated with an increased incidence of primary liver cancer development. This study, of the role of proliferation in chemical carcinogenesis formation, will greatly increase our understanding of the importance of multiple exposures in the development of hepatic or other organ lesions, (i.e., does the exposure to one chemical, with the development of its metabolites, become capable of carcinogenic transformation in the presence of a secondary stimulus from another chemical or agent causing regeneration).

The Section I proposal describes biochemical studies into the effect of chemical carcinogens on protein metabolism. This study, under the direction of Dr. Jerald Hoffman has the objective of determining the impact of chemical carcinogens on sulphur amino acid metabolism and to develop a better understanding of carcinogenesis to the degree that biochemical and nutritional principles may be applied to cancer prevention. These studies will aid greatly in determining by what mechanism the metabolites may interfere with sulphur amino acid metabolism and how one might be able to then develop and apply sound biochemical or nutritional principles in the treatment of those inadvertently exposed to carcinogens and invading toxins, whether they be biological or chemical.

BUDGET SUMMARY

	Year 1	Year 2	Year 3	Total
I. CLINICAL IMMUNOLOGY				
Personnel	34,100	36,500	39,000	109,600
Supplies and Expenses	16,795	15,895	15,895	49,485
				$159,085
II. CLINICAL BIOCHEMISTRY				
Enzyme				
Personnel	34,300	37,184	40,159	111,643
Supplies and Equipment	21,796	22,876	23,956	68,628
				$180.271
III. CLINICAL BIOCHEMISTRY				
Protein				
Personnel	14,000	14,980	16,030.	45,010
Supplies and Equipment	12,500	10,225	10,357	19,657
				$64,667
IV. CLINICAL PATHOLOGY				
Personnel	3,700	4,000	4,300	12,000
Supplies and Expenses	3,400	3,600	3,800	10,800
				$22,800
V. MICROBIOLOGY				
Personnel	26,000	28,520	31,342	85,862
Supplies and Expenses	9,450	11,300	9,500	30,250
				$116,112
VI. CHEMISTRY				
Personnel	34,500	36,700	39,284	110,484
Supplies and Expenses	30,500	7,100	7,700	45,300
				$155,784
VII. PATHOLOGY				
Personnel	11,720	12,530	13,480	37,730
Supplies and Expenses	5,512	2,950	3,200	11,662
				$49,392

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BUDGET SUMMARY
(continued)

	Year 1	Year 2	Year 3	Total
VIII. RADIOLOGY				
Personnel	31,000	33,410	35,835	100,245
Supplies and Expenses	37,350	25,050	26,325	88,725
				$188,970
IX. BIOCHEMISTRY				
Personnel	12,400	13,300	14,300	40,000
Supplies and Expenses	36,490	10,717	19,677	66,884
				$106,883
X. FRINGE BENEFITS	25,526	27,453	29,524	82,503
XI. INDIRECT COSTS	131,118	141,131	151,924	424,173
SUBTOTAL FRINGE BENEFITS AND INDIRECT COSTS	156,644	168,584	181,448	506,676
TOTAL				$1,550,640