MCA

MANUFACTURING CHEMISTS ASSOCIATION

1825 CONNECTICUT AVENUE, N.W. WASHINGTON, D.C. 20009 (202) 483-6126

July 20, 1977

TO: Technical Panel on Vinyl Chloride Research

SUBJECT: Research Proposal from the University of Louisville Covering the Second (1977-78) and Third Years (1978-79)

Gentlemen:

The Vinyl Chloride Research Coordinators have approved the subject document and recommend that it be sent to the entire Technical Panel.

As may be observed,Dr. Tamburro has submitted the proposals in two parts: Part A, a summary of the research projects, and Part B, a summary of the budgets. In addition, there are appendices of information which the investigators included in an effort to assist in your evaluation of the proposals.

August 31, 1977, is the expiration date of the agreement covering the initial year of research (1976-77). Should you wish that this overall research effort have the optimum degree of continuity (subject, of course, to the approval of the Management Contacts), the enclosed ballot should be completed at the earliest possible time, certainly no later than August 3, 1977.

Sincerely,

J. T. Seawell
Project Manager
Vinyl Chloride Research

JTS:ec
Enclosure

Manufacturing Chemists Association

Technical Panel on Vinyl Chloride Research

July 20, 1977 Washington, D.C.

Ballot on Research Proposal from University of Louisville

"Research Techniques and Methods for Detection and Prevention of Carcinogenesis in Industrial Workers"

☐ I approve of the research proposal as it has been presented by Dr. Tamburro and recommend continuation of this research project through the second year, 1977-78.

☐ I approve of the research proposal as it has been presented by Dr. Tamburro and recommend continuation of this research project through the second (1977-78) and third (1978-79) years.

☐ I do not approve of the research proposal as presented and recommend changes and/or modifications as given in the attached letter.

Name (Signed)_____

Name (Typed)_____

Company_____

Address_____

_____ Date

Telephone: Area Code_____Number_____Ext._____

Prior to August 3, 1977, please return to:

Mr. J. T. Seawell
Manufacturing Chemists Association
1825 Connecticut Avenue, N.W.
Washington, D.C. 20009

Manufacturing Chemists Association

Technical Panel on Vinyl Chloride Research

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Prior to August 3, 1977, please return to:

Mr. J. T. Seawell
Manufacturing Chemists Association
1825 Connecticut Avenue, N.W.
Washington, D.C. 20009

P A R T A

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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TECHNICAL PROPOSAL A

IMMUNOLOGICAL SYSTEMS FOR THE DETECTION OF VINYL CHLORIDE AND OTHER CHEMICAL INJURY

H. PHILIP FORTWENGLER, M.S.

TECHNICAL PROPOSAL A

IMMUNOLOGICAL SYSTEMS FOR THE DETECTION OF
VINYL CHLORIDE AND OTHER CHEMICAL INJURY

The continuation of testing of lymphocytes from individuals with varying degrees of vinyl chloride exposure is planned to determine if the defects in the immunological system precede or accompany the initiation of vinyl chloride disease. Individuals with a history of vinyl chloride exposure, those with no history of vinyl chloride exposure but other chemical exposures, and those individuals with histologically diagnosed liver disease will have their lymphocytes tested against a panel of 10 immunological evaluations. Previous investigations have demonstrated that the lymphoid cells of thymus origin (T cells) can kill human tumor cells. Our own preliminary studies have shown that lymphocytes from workers heavily exposed to vinyl chloride react to angiosarcoma extracts, as did one worker with angiosarcoma. One tantalizing explanation to this observation is that the workers with high exposure to vinyl chloride in the past, but without the development of the tumor, have had some of the endothelial sinusoidal cells transform into cancer and have been destroyed by the individual's own T cells, thereby leaving behind sensitized lymphocytes in an individual whose own immunological systems have eradicated a potentially developing tumor.

A second vinyl chloride-exposed employee who developed angiosarcoma and was tested very late in the disease process did not demonstrate reactivity by his lymphocytes to the angiosarcoma extract. This may represent an inability of his lymphocytic system to respond to the developing tumor, or this may be a reflection of the immunological system's being overwhelmed by the tumor.

Lymphocytes in future studies will continue to be isolated from various groups of workers as noted above, and the T cells will be enumerated and tested for their functional activity and capability. Dr. Herberman, from the National Cancer Institute, has suggested that since cancer patients often have a decreased number or an impaired function of lymphocytes, assessment of the immunocompetence may be an early indicator of cancer development.

The second immunological approach under investigation will be the tissue-typing of individuals for histocompatibility antigens as a means of determining possible markers for genetic predisposition. This tissue-typing, one-third completed, includes individuals with and without vinyl chloride exposure and injury. Those individuals with vinyl chloride injury will be matched as closely as possible for age, sex, race, and degree of chemical exposure with individuals having no evidences of

hepatic or other organ injury. The frequency of each of the 25 separate HLA-A and HLA-B factors is being continually tabulated and will be analyzed for comparison when 250 have been completed. Chi square tests will be performed by Dr. Richard Greenberg, our biostatistician and epidemiologist, to determine the potential statistical intergroup variations. These statistical variations will be used to determine the increased risk of individuals who carry a particular genetically determined antigen. Results of these analyses, hopefully, will enable the prediction of those HLA types which are genetically predisposed to vinyl chloride disease or other chemical injuries.

The third immunological component development is a specific immunological assay for the detection of angiosarcoma. Individuals from the vinyl chloride plant with negligible vinyl chloride exposure, individuals from outside the plant with no vinyl chloride exposure, those with high vinyl chloride exposure, and those with histologically proven liver and other parenchymal injury are continuing to be tested. These results are in continuous tabulation and analysis. Individuals whose lymphocytes have given or give elevated test values on initial screening will be studied in greater depth to determine the specificity of the reaction and the cause of reactivity. The specificities of the reactions will be tested, using a panel of tissue reagents prepared from three separate angiosarcomatous and three normal livers, as well as the matched respective non-involved kidney. This system will allow the separation of cross reactivity due to histocompatibility reactions versus those reactions due to specific angiosarcoma antigens.

The rationale for the development of this test for angiosarcoma lies in the established fact that the body mounts an immune response (becomes "hypersensitive") to the cancer. Thus, the detection of the body's specific immune response to the angiosarcoma will, hopefully, prove to be useful in indicating the presence or development of angiosarcoma tissue antigens in specific individuals and may assist in determining at which levels or degrees of exposure these antigens begin to emerge. This might further add to data supportive of a threshold level for vinyl chloride exposure and subsequent development of angiosarcoma or other cancers.

TECHNICAL PROPOSAL B

BIOCHEMICAL ENZYMATIC SYSTEMS FOR DETECTION OF VINYL CHLORIDE AND OTHER CHEMICAL INJURY AS A POTENTIAL MEANS OF DETECTION OF CANCER IN HUMANS

JULIE T. DU, PH.D.

TECHNICAL PROPOSAL B

BIOCHEMICAL ENZYMATIC SYSTEMS FOR DETECTION OF VINYL CHLORIDE AND OTHER CHEMICAL INJURY AS A POTENTIAL MEANS OF DETECTION OF CANCER IN HUMANS

Characterization of the biochemical and enzymatic changes occurring in progressive exposure in animal experiments will be continued. This includes the following studies:

(1) The determination of microsomal P-450 content, the cytochrome protein that is responsible for the oxidation of vinyl chloride;

(2) The activity of mixed function oxidase; i.e., the capacity for the microsomes to metabolize drugs (N-demethylation);

(3) The activity of glucose-6-Pase, a gluconeogenic enzyme and a marker for preneoplastic change;

(4) The activity of glucose-6-P dehydrogenase, a pentose shunt enzyme (an increase of this can lead to more ribose and nucleic acid biosynthesis;

(5) The level of reduced glutathione in liver for detoxification of carcinogenic metabolites; and

(6) The activity of glutathione reductase. This enzyme activity indicates the capacity to regenerate and maintain the level of reduced glutathione. An increase can be an early alarm of the animal for overexposure to the toxic agent, indicating possible early hepatocellular injury.

These studies will enable us to identify the sequential cellular enzymatic changes occurring from early injury to tumor development. As has already been reported, the changes in gluconeogenesis and in the pentosephosphate shunt already parallel the alteration seen in primary hepatocellular tumors. The present working hypothesis for the non-development of primary cell tumors in the adult animal is the ability of the adult hepatocyte to adequately detoxify the carcinogenic metabolites of vinyl chloride, chlorooxirane and chloroacetaldehyde. The adjacent cells, sinusoidal lining cells, as well as newborn liver cells, most likely have a decreased ability of detoxification. In this or similar manner, these cells have a greater susceptibility to DNA injury

due to the prolonged exposure of chloroacetaldehyde to the DNA molecule. This would explain why angiosarcomas occur in adult rats and primary hepatomas occur in newborn rats, as has been recently shown by Maltoni.

In our next stage of animal exposure experiments, we will add weanling rats to the vinyl chloride exposure animal group. In these animals, enzymatic assays to document this hypothesis will be performed in a manner similar to the adult rats. Both weanling and adult rats will be utilized for verification and further elucidation of the immunological and tissue antigen test systems by Espinosa's group. Urines from these same rats will be utilized to further verify the urinary glycosaminoglycan studies by Kupchello's group and the biological end-product analysis by Wong and Taylor.

In addition, we plan to investigate two more enzymes related to glutathione and detoxification:

(A) Gamma-glutamyl transpeptidase (GGTP) - This enzyme is responsible for the breakdown of glutathione. It has also been used as a circulating enzyme for the detection of early hepatocellular injury. It has, under recent years of clinical evaluation, lost some of its clinical importance due to the high frequency of an elevation of its activity in circulating blood in the absence of any documentable hepatocellular injury. The activity of this enzyme in the liver has been found to increase considerably during several cases of chemical carcinogenesis. Higher levels of the enzyme have also been seen in patients with either primary or secondary neoplasms. The possibility that the high enzyme activity in blood might reflect more breakdown of glutathione rather than hepatocellular injury is under evaluation. It may well be that the "falsely positive" elevated GGTP levels in vinyl chloride-exposed individuals without evidence of hepatocellular injury may really reflect a disturbance in the glutathione levels and indicate an impairment in the detoxification of the intermediate metabolite, chloroacetaldehyde, which subsequently leads to hepatocellular injury.

(B) Aralkyl glutathione-S-transferase - The activity of this enzyme can be used to indicate the capacity for detoxification of vinyl chloride metabolites in the rat. The possibility that this might be a better predictor of whether the animal is more or less or equally susceptible to vinyl chloride injury at various exposure levels is the goal of this investigation. These studies in parallel with circulating glutathione reductase will give us a better understanding of the detoxifying capability of the

animal and, hopefully, we may be able to extrapolate the findings to man to predict better about the worker's detoxifying capacity. There has been some thought that manipulation of the diet may add to the increased ability to form these enzymes for better capacity of detoxification. This potentiality is, at present, most speculative; and, if the previously stated investigations warrant, it would be followed by subsequent studies.

The final portion of this study will include the electron and light microscopic studies of these animals exposed to determine the graded morphological changes due to vinyl chloride exposure and to compare these to the enzymatic results already determined. These tissues have already been prepared for analysis, and the first set is now being studied. These include selected groups from 28, 71, and 103 hours of exposure with their paired controls. Similar studies will be performed with the next group of more prolonged exposures. We feel strongly from our human studies that these animal electron microscopic evaluations will greatly assist us in understanding the changes we see in our human studies being performed by Drs. Schrodt and Tamburro.

TECHNICAL PROPOSAL C

TISSUE AND URINARY GLYCOSAMINOGLYCAN CHANGES RELATED TO VINYL CHLORIDE INJURY: USE AND EARLY DETECTION AND DIAGNOSIS

CHARLES E. KUPCHELLA, PH.D.

TECHNICAL PROPOSAL C

TISSUE AND URINARY GLYCOSAMINOGLYCAN CHANGES RELATED TO VINYL CHLORIDE INJURY: USE AND EARLY DETECTION AND DIAGNOSIS

During the second year of this proposal, work will continue to complete studies undertaken during the first year. These will include a completion of our evaluation of the effects of vinyl chloride exposure with and without liver injury on the urinary glycosaminoglycan excretion patterns. We have begun and, hopefully, will complete during the middle of the second year the evaluation of 24-hour urine sample collections from workers who fall into high, medium, and low exposure categories (exposure data coming from the Vinyl Chloride Medical Surveillance Data Bank). In addition, individuals already demonstrated to have liver injury, with both high vinyl chloride exposures as well as low, will also be studied.

There will be two control groups involved in this study. One will be those derived from individuals with other fibrogenic liver diseases, such as cirrhosis, hepatitis, and alcoholic liver injury. Since the preliminary data suggests the possibility that the urinary excretion patterns for vinyl chloride injury are distinctly different from these other frequently found non-occupational illnesses, this work will involve determination of the specific GAG excretion fractions and their complete biochemical characterization. This is needed in order to determine the usefulness of specific GAGs in the assessment of vinyl chloride injury as well as other chemical liver injury. Such data will have great value in future assessments of the effects of many types of hepatic toxic chemicals and exposure to them in the work place. These studies are expected to result in the development of specific tests for early liver damage and would, hopefully, become part of the routine medical screening of workers with potential exposure to hepatic toxic chemicals. Methods to determine the chemical characterization and identification of these specific GAGs are cited in the original grant application.

Animal studies to be conducted during the second year will be the experiments, described in the original application, designed to determine the direct effects of vinyl chloride exposure on the experimental animals on the GAG changes in tissue and urine. These studies will involve both animals exposed to relatively high (10 to 20 thousand parts per million) and relatively low (25 to 50 parts per million) levels of vinyl chloride. These studies will enable us to identify the specific testing parameters that would lend themselves best to routine screening

in the working population, as well as to assess the specific role of the GAG in fibrogenesis both in their reversible and irreversible stages. Depending on these observations, those specific GAG changes in the urine that appear to be valuable as indicators of liver injury will be further developed into a "final" procedural modification for the determination of these urinary GAG fractions by quick simply methodology--spot tests, perhaps--that can be used as a routine medical screening.

In the third year, studies will be made with other animal models; and we will conclude our evaluation of the human population. The animal model studies will include:

(1) A study of the effects of partial hepatectomy on urinary and liver GAG levels - These studies will be done to assess the effect of cell division and cell regeneration on the liver and urinary GAG levels. This is of vital importance since many individuals working in the industrial environment also have non-occupationally related liver injury--such as viral hepatitis and alcoholic injury--which stimulate liver cell division and liver regeneration.

(2) Studies of the role of GAG in fibrogenesis, generally - One set of studies will involve the effect on urinary and tissue GAG after stopping chemical liver injury (carbon tetrachloride) before and after the stage of irreversibility.

(3) Studies of the incorporation of radio sulfur labeled GAG subunits into liver tissue during fibrogenesis induced by both carbon tetrachloride and vinyl chloride exposure.

(4) Studies of prolonged exposure to determine the specific effects of the presence of developing cancer on the GAG levels - This will be conducted both in the animals with vinyl chloride-induced angiosarcoma and/or primary hapatoma, as well as in experimental animals carrying transplantable hepatomas (Novikoff).

SUMMARY

Preliminary results, both in the human material and in the preliminary animal experiment studies, indicate that the GAG changes in both urine and tissue may well be in the earliest demonstrable change to occur with liver injury. Work in the second and third years will help confirm the usefulness of these parameters in early detection, identify the significant leads for the design of future studies of the role of GAGs and, hopefully, give further information that may permit interference

with and/or modification of GAG metabolism's leading to fibroses of the liver. Finally, these studies will help produce the data concerning the relative specific effects of cancer development and how this may differ from non-occupational hepatocellular injury, such as that due to viruses or alcohol.

TECHNICAL PROPOSAL D

ELECTRON MICROSCOPIC EVALUATION OF LIVER INJURY FROM CHEMICAL WORKERS

G. RANDOLPH SCHRODT, M.D.

CARLO H. TAMBURRO, M.D.

TECHNICAL PROPOSAL D

ELECTRON MICROSCOPIC EVALUATION OF LIVER INJURY FROM CHEMICAL WORKERS

Recent studies in this and other laboratories have suggested that in individuals exposed to vinyl chloride there is an increase in intralobular collagen (fibrous tissue) deposition. The more obvious increase in portal and subcapsular fibrosis is now well known. Our own preliminary data indicates that this collagen deposition occurs much earlier in the parenchyma of the liver rather than in the portal or subcapsular area.

The collagen deposits apparent in light microscopic studies only with special staining are more readily visualized with the electron microscope. Most of this collagen appears confined to the space of Disse along the surface of the hepatocyte. This occurs more frequently between the sinusoidal lining cell and the hepatocyte surface rather than between hepatocyte to hepatocyte junctions; not infrequently deep invaginations of the space of Disse creates the impression that collagen bundles are found within the hepatocyte. This gives the impression that the fibrous strands are actually compressing the hepatocyte.

As part of the total patient evaluation of individuals exposed to vinyl chloride, electron microscopic studies of the liver biopsies of a selected number of individuals have already been made. Following these initial observations, evaluation of the ultrastructural findings, with both the light microscope as well as the electron microscope, has been underway.

Observations in individuals who have developed angiosarcoma following extensive exposure to vinyl chloride have demonstrated a marked increase in the collagen deposition in the hepatic perisinusoidal spaces. This appears to eventually lead to the destruction of the hepatic cell cords and is associated with the activation of the sinusoidal lining cells with focal sinusoidal widening and sinusoidal cell atypia. This fibrosis and widening of the sinusoids with the sinusoidal atypia appear to be a premalignant indicator of hepatic injury due to vinyl chloride exposure. These studies are supportive of those findings by Kupchella et al. in which there are increased glucosaminoglycans in both tissue and urine samples of vinyl chloride-exposed individuals.

The importance of recognizing this early increase in collagen deposition in the perisinusoidal space and its lobular distribution may well characterize the vinyl chloride-exposed injury from other types of

collagen deposits, such as those associated with alcohol, drugs and viral hepatitis. These other non-occupational diseases are associated with the hepatocellular injury and necrosis and subsequently have increases in collagen deposition. In contrast, it appears that the vinyl chloride injury is associated with earlier deposition of collagen in an unusual pattern (parenchymal distribution) and occurs before any significant hepatocellular injury.

Systematic comparison of the sinusoidal cell structures and the collagen content of the perisinusoidal spaces in the various zones of the hepatic lobule in individuals with vinyl chloride exposure, as well as exposure to other chemicals, will be continued during the second and third years of this grant proposal.

The use of a Hewlitt-Packard 9815 desk-top calculator with surface digitizer with interface, which has recently arrived, will increase both the capability of studying larger numbers of individuals as well as increase the accuracy and rapid reproducibility of the manual methods. The calculator is now being programmed to evaluate in a semi-quantitative manner the various ranges of collagen deposition seen in vinyl chloride and other chemically exposed workers. This data will later be analyzed using the work exposure histories of these individuals for comparison of the collagen disposition to the other 19 chemicals to which they had potential exposure.

TECHNICAL PROPOSAL E

MULTIVARIANT ANALYSIS OF BIOLOGICAL END-PRODUCTS AND VINYL CHLORIDE METABOLITE SYNTHESIS

JOHN L. WONG, PH.D.

K. GRANT TAYLOR, PH.D.

J. T. JOSEPH, PH.D.

TECHNICAL PROPOSAL E

MULTIVARIANT ANALYSIS OF BIOLOGICAL END-PRODUCTS AND VINYL CHLORIDE METABOLITE SYNTHESIS

The methodology for the analysis of vinyl chloride end-products in body fluids and tissues will be developed by the end of the first year of this proposal. With the completion of the methodology, the quantitation of the metabolic end-products of vinyl chloride and the establishment of an exposure level-product concentration relationship will begin, first in animals' tissue and urine. Animal tissue and urines will be collected during the next phase of animal exposure studies and stored for future analysis. The first target metabolite for analysis is chloroacetic acid. The methodology will include gas chromotography and mass spectrometry (GC/MS), as well as high pressure liquid chromatography and Fourier Transform Nucleur Magnetic Resonance (HPLC/FT-NMR). We have just received a $152,000 FT-NMR spectrometer and plan to couple a gas chromatograph to our present mass spectrometer to make the GC/MS system for use in the developed methodology. Dr. Grant Taylor is the co-principal investigator to this aspect of the project.

Since previous animal studies have not demonstrated chloroacetic acid in the urines of animals at low exposure levels but have been reported in humans at higher exposure levels, these methodologies will help to verify this observation and can lead to the determination of specific threshold exposure levels and could greatly aid in the determination of what constitutes a safe environmental exposure for humans, as well as being used as an indicator of excessive exposure for individual employees. The second part of this research proposal involves the metabolic activation and inactivation of vinyl chloride. The identification of urinary metabolites is still incomplete. There is 35 percent of the vinyl chloride carbon-14 activity in the urine unaccounted for. We will continue to investigate the reactions of nonprotein sulfhydryls (e.g., cysteine and glutathione) with chloroacetaldehyde (CAA) to determine the sequence of conversions. In addition to our recent observations of the double reaction (bis-alkylation) of CAA with cysteine to form the cyclic product mentioned in our first progress report, we have made additional preliminary observations that glutathione (GSH) yielded GS-CHOH-CH_2-SG with CAA. These intermediates or their subsequent oxidation/hydrolysis products may account for the elusive urinary metabolites. Furthermore, we have had some initial success with the isolation of nucleic acid derivatives from the putative metabolite reactions; viz., with chloro-oxirane and chloroacetaldehyde. Our future studies will continue to elucidate their structures, properties, and reactivities. These metabolic activation and inactivation data will form part of the basic picture which will facilitate the interpretation of the cellular and clinical symptoms,

as well as help us in the early determination of damage induced by vinyl chloride. This information is also essential in identifying these urinary metabolites and in developing the specificity of the methodology for detecting these end-products in biological fluids and tissues.

The third portion of this proposal will deal with the prediction of vinyl fluoride as a noncarcinogen and vinyl bromide, iodide, and chloride as carcinogens. On the basis of the physicochemical properties of chlorooxirane in chloroacetaldehyde which we have found, we can make the following reasonable predictions:

(1) Fluorooxirane will also be the obligatory intermediate in the metabolic process; and it will behave like ethylene oxide, which is not carcinogenic. It will not have diradical character; hence, the fluoroethanol will not be formed from vinyl fluoride.

(2) The fluoroacetaldehyde can be formed from fluoro-oxirane via the NIH shift. It will have similar activities as acetaldehyde, which is not known to be a carcinogen.

(3) The major urinary metabolite would be fluoroacetic acid.

(4) Vinyl bromide and vinyl iodide will be very much like vinyl chloride in the metabolic conversions, and they have carcinogenic potential.

It would be very important to confirm these predictions by actual studies of the chemical and biological behavior of the respective metabolic intermediates. If confirmed, the practical implications of the above predictions for the chemical industry would be far-reaching. A graduate student has already expressed an interest in working in this project. Thus, the support of a research assistant can rapidly get this project underway.

TECHNICAL PROPOSAL F

ANALYSIS AND IDENTIFICATION OF CARCINOGENIC POTENTIAL OF INDUSTRIAL CHEMICALS

ULDIS N. STREIPS, PH.D.

TECHNICAL PROPOSAL F

ANALYSIS AND IDENTIFICATION OF

CARCINOGENIC POTENTIAL OF INDUSTRIAL CHEMICALS

During the next two successive years of this proposal, this group will continue its mutation screening, part of which has already been completed and detailed in the first progress report. These mutation screening capabilities will include both the salmonella reversion assays (which measure the base substitutions and deletions) and the bacillus repair assays (which measure susceptibility of repair deficient strains to chemicals). Using these tests, we examined several samples for mutagenic activity. (See the attached table.) The forward mutation rate assay will be added; it measures the ability of a chemical to cause cell mutations directly. Similar assays can be performed in mammalian cells. In the forthcoming years of this program, we plan to implement a mammalian tissue culture assay for mutagenesis. The chemicals to be assayed and tested for mutagenicity include the 19 chemicals for which exposure indices are already available within the medical surveillance data bank and their metabolites, which are to be provided by Dr. Wong. The metabolites will be purified samples of industrially relevant chemicals. Dr. Yankeelov in the Department of Biochemistry and Dr. Taylor in the Department of Chemistry will synthesize various derivatives of both biologically and environmentally occurring molecules; these will also be tested for mutagenicity.

In addition, we will continue the carcinogenesis model study of the molecular mechanism for chloroacetaldehyde-mediated mutagenesis/carcinogenesis. This, hopefully, will result in the isolation of specific cell products which may participate in the mutagenesis process, as well as the identification of other components which may block this mutagenesis. Agents which may shift a cell's predisposition to chloroacetaldehyde-caused mutagenesis and thus develop resistance to this chemical will also be examined. The carcinogenesis model would then be applicable for use in the study of other potential chemical carcinogens or chemicals whose intermediates are carcinogenic.

These studies have great practical application since the understanding of the mechanism by which chemicals or their intermediates induce mutagenesis or carcinogenesis will be of enormous assistance in characterizing which chemicals have the practical potential for cancer development. It will also demonstrate those whose mutagenic capabilities are only of biological importance. In addition, these studies will identify those chemicals and/or their metabolites to which priority attention should be given.

TABLE

SUMMARY OF PERTINENT SUBSTANCES TESTED FOR MUTAGENICITY

		Salmonella	Bacillus
1.	Meta-chlorobenzoyl-cyclobutanecarbonyl peroxide	±	NR
2.	Benzoyl peroxide	NR	NR
3.	N-acetoxy-N-phenylacetamide	NR	NR
4.	N-cyclobutanecarboxyloxy-N-phenylacetamide	NR	NR
5.	bis-cyclobutane carboxyl peroxide	NR	NR
6.	Aflatoxin	+++	++
7.	Chloroethanol	NR	NR
8.	Benzo(a)pyrene	++	+
9.	4-nitro quinoline-1-oxide	+++	++
10.	Chloroacetaldehyde	+++	++
11.	Epichlorohydrin	++	+
12.	Chlorooxirane	+++	++
13.	Butane diepoxide	++	+
14.	Styrene oxide	++	+
15.	3,4 epoxide butene	±	NR
16.	bis(Beta-chloroethyl)phenyl phosphate	NR	NR
17.	Beta chloroethyl phosphate (bis cyclohexylamine salt)	NR	NR
18.	Lung aspirates from smokers with cancer	±	NR
19.	Lung aspirates from smokers without cancer	±	NR

NR - no reaction + - mutagenic +++ - extremely mutagenic

± - borderline mutagenicity ++ - strongly mutagenic

TECHNICAL PROPOSAL G

TISSUE ANTIGENIC SYSTEMS FOR DETECTION OF CHEMICAL-INDUCED CARCINOGENESIS

ENRIQUE ESPINOSA, M.D.

TECHNICAL PROPOSAL G

TISSUE ANTIGENIC SYSTEMS FOR DETECTION OF CHEMICAL-INDUCED CARCINOGENESIS

Tissue antigenic studies during the next two years of this proposal will be addressed to two aspects: (1) Those related to experiments on human vinyl chloride-associated liver injury and (2) those to experiments on rats exposed to vinyl chloride and other related potential chemical carcinogens.

A. Experiments on Human Vinyl Chloride-Associated Liver Injury

(1) The antigen (S) involved in the stimulation of antibody bound to the angiosarcoma tumor will be further investigated. In parallel studies with Fortwengler et al. we will study the antigen (S) stimulating the cell-mediated immune response to angiosarcoma extract described by this latter investigator. We will study the role in these phenomena of the angiosarcoma-associated antigen described by us and of the other antigenic constituents of the angiosarcomatous liver tissue. These antigens will be prepared from liver angiosarcoma tumor which is now stored frozen. Isolation of the antigen will involve fractionation of the extract by ammonium sulfate, ion exchange chromotography, Sephadex filtration, gel electrofocusing, and acrylamide electrophoresis. Antigen purification will be monitored by double diffusion tests measuring the highest dilution of test fractions giving precipitin reactions; or, if feasible, a single radial immunodiffusion procedure will be used. Immunoelectrophoresis and disc electrophoresis will be used to control homogeneity of the antigen preparations. Molecular parameters to be estimated will include molecular weight, sedimentation and diffusion coefficients, molecular radius and frictional ratios. These will be measured by sucrose gradient ultracentrifugation and Sephadex filtration. The antigens in these procedures will be monitored by double immunodiffusion tests. The specific antisera to be used are stored frozen.

(2) A radioimmunoassay for the detection of the angiosarcoma-associated antigen will be developed. This will be used for the assay of this antigen in serum samples from the B. F. Goodrich Company workers with history of vinyl chloride exposure. The value of this method for the diagnosis of vinyl chloride-associated liver disease will thus be determined. Following

isolation of the antigen by the procedures described above, this will be labeled with I125 by a method described by McCammom and Yohn. The radioimmunoassay will be used for the detection of the tumor-associated antigen in patients' sera and will be done according to the Egan et al. procedure.

B. Experiments on Rats Exposed to Vinyl Chloride

The changes in liver antigens and antibodies described in workers in the vinyl chloride industry will also be studied in rats chronically exposed to vinyl chloride in order to determine, under well-controlled experimental conditions, the time required for these changes to occur. This is of obvious great importance in order to determine the value of these alterations in the early detection of the disease. Sprague Dawley rats will be exposed to vinyl chloride monomer for up to 52 weeks and used for both the biochemical (Dr. Du) and the present studies. Some rats will be sacrificed on a weekly interval during the first six weeks of exposure and on a monthly interval thereafter. Non-exposed animals kept in similar conditions to the experimental ones will be used as controls. Abnormalities found at sacrifice will be recorded, and the livers will be examined histologically. Immunoglobulins bound to the angiosarcomas or the fibrotic livers identified at sacrificing will be examined by indirect immunofluorescence using frozen tissue sections. Serum prepared from the blood obtained from these rats will be examined for presence of anti-tumor antibody by the immunofluorescence procedure using frozen sections of the tumor as substrate. Liver abstracts will be prepared in saline solutions and examined for antigenic changes. These will be tested by immunodiffusion using both anti-human and anti-rat angiosarcoma sera prepared in rabbits.

The practical value of these studies is now of even greater importance since Fortwengler et al. have demonstrated by the lymphocyte transformation test cell-mediated reactivity against an angiosarcomanous liver extract in one of two individuals with liver angiosarcoma. This cell-mediated reactivity was also in two individuals of a group that had extended exposure to elevated quantities of vinyl chloride. The identification and purification of the angiosarcoma antigens will make the specificity and sensitivity of the angiosarcoma lymphocyte transformation test and the development of immunological methods of early detection of greater clinical use.

P A R T B

BUDGET PROPOSAL

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

UNIVERSITY OF LOUISVILLE

CANCER CENTER

LOUISVILLE, KENTUCKY 40201

BUDGET SUMMARY

	Year 2	Year 3
I. CLINICAL IMMUNOLOGY		
Personnel	30,605	31,523
Supplies and Expenses	19,441	21,371
II. CLINICAL BIOCHEMISTRY		
Personnel	23,225	23,922
Supplies and Equipment	13,075	14,373
III. CLINICAL BIOCHEMISTRY		
Personnel	8,900	9,476
Supplies and Equipment	8,800	8,690
IV. CLINICAL PATHOLOGY		
Personnel	—	—
Supplies and Expenses	7,735	8,508
V. MICROBIOLOGY		
Personnel	15,160	16,747
Supplies and Expenses	12,400	11,500
VI. CHEMISTRY		
Personnel	11,100	11,845
Supplies and Expenses	16,452	17,657
VII. PATHOLOGY		
Personnel	13,990	14,719
Supplies and Expenses	7,200	7,590

		Year 2	Year 3
VIII.	FRINGE BENEFITS	(11,033)	(11,596)
IX.	TOTAL (Personnel and Expenses include fringe benefits)	188,083	197,921
X.	INDIRECT COSTS	59,765	62,813
	TOTAL	247,849	260,734

BUDGET

M.C.A. PROPOSAL

I. CLINICAL IMMUNOLOGY H. Philip Fortwengler, Investigator	Year 2	Year 3
A. Toxicity/Tumorgenecity Study		
1. Personnel		
a. H. Philip Fortwengler, MS	15,500	15,965
b. Michael Dever, BS Research Technician	9,305	9,584
c. Linda Smith, BS Research Assistant	5,800	5,974
	30,605	31,523
2. Supplies and Expenses		
a. Animals, 300 per year	1,100	1,210
b. Animal Maintenance	1,195	1,315
c. Cell Culture Reagents	1,300	1,330
d. Sterile Culture Flasks	1,000	1,100
e. Chemical Reagents	500	550
f. Needles, Syringes	300	330
g. Sterile Cell Culture, Disposable Plasticware	900	990
h. Miscellaneous Expendables	300	330
SUBTOTALS	6,595	7,155
B. Immunocompetence Study		
1. Personnel (No additional cost)		
2. Supplies and Expenses		
a. Lymphocyte isolation reagents	1,500	1,650
b. Cell culture media	500	550
c. 3H isotopes	450	495
d. Scintillation reagents	1,100	1,210
e. Miscellaneous reagents, glassware	250	275
SUBTOTALS	3,800	4,180

	Year 2	Year 3
C. HL-A Tissue-Typing		
1. Personnel (No additional cost)		
2. Supplies/Expenses		
a. Hamilton syringes	500	550
b. Tissue-typing antisera and plates	5,300	5,840
c. Miscellaneous reagents, glassware	300	330
SUBTOTALS	6,100	6,720
D. LAI Test for Angiosarcoma		
1. Personnel (No cost)		
2. Supplies and Expenses		
a. Cell cultures	250	266
b. Cell maintenance	1,346	1,590
c. Miscellaneous reagents, glassware	1,100	1,210
SUBTOTALS	2,696	3,066
E. Xeroxing and publication costs	250	250
YEARLY TOTALS	50,046	52,894

II. CLINICAL BIOCHEMISTRY - ENZYME
Julie T. Du, Ph.D., Investigator

A. Personnel		
1. Julie T. Du, Ph.D.	13,225	13,622
2. Ruth Shelton, MS	10,000	10,300
SUBTOTALS	23,225	23,922
B. Supplies and Expenses		
1. Laboratory Supplies		
a. Enzymes	1,100	1,200
b. Radioactive	979	1,078
SUBTOTALS	2,079	2,278

	Year 2	Year 3
2. Animal Expenses		
a. Animals	1,350	1,485
b. Animal care		
1. Short term (200)	1,920	2,112
2. Long term (300)	7,726	8,498
SUBTOTALS	10,996	12,095
YEARLY TOTALS	36,300	38,295

III. CLINICAL BIOCHEMISTRY
Charles E. Kupchella, Ph.D., Investigator

A. Personnel		
1. Charles E. Kupchella, Ph.D. (No cost)		
2. Leslie Caine, Procurrent Technician	8,900	9,476
SUBTOTALS	8,900	9,476
B. Supplies and Expenses		
1. Lab/supplies/chemicals	2,900	3,590
2. Enzyme preparations	600	600
3. Glassware	1,550	1,200
4. Equipment maintenance	350	350
5. High pressure liquid chromatography columns	1,500	1,050
SUBTOTALS	6,900	6,790
C. Computer	1,000	1,000
D. Travel	900	900
YEARLY TOTALS	17,700	18,166

IV. CLINICAL PATHOLOGY
G. Randolph Schrodt, M.D., Investigator

A. Personnel		
1. G. Randolph Schrodt, M.D. (No cost)		
B. Supplies and Expenses		
1. Lab materials	2,550	2,700
2. Chemicals	650	800
3. EM preparation and tissue storage material	1,435	2,508
4. Photography equipment and supplies	3,100	2,500
YEARLY TOTALS	7,735	8,508

	Year 2	Year 3
V. MICROBIOLOGY Uldis N. Streips, Ph.D., Investigator		
A. Personnel		
1. U. N. Streips, Ph.D.	2,000	2,000
2. Research Technician	7,500	8,087
3. Research Assistant	5,660	6,660
SUBTOTALS	15,160	16,747
B. Supplies and Expenses		
1. Media	4,500	5,000
2. Glassware	2,000	1,000
3. Isotopes	3,000	2,100
4. Equipment maintenance	500	600
5. Publishing costs	1,000	1,200
SUBTOTALS	11,000	9,900
C. Travel to national meetings to consult with experts in the field	1,400	1,600
YEARLY TOTALS	27,560	28,247
VI. CHEMISTRY John L. Wong, Ph.D., Investigator		
A. Personnel		
1. John L. Wong, Ph.D. (No cost)		
2. Joseph P. Joseph, Ph.D. Post-doctoral	8,600	9,000
3. Research Assistant	2,500	2,845
SUBTOTALS	11,100	11,845
B. Supplies and Expenses		
1. Chemicals, VPC, HPLC supplies	3,500	3,600
2. Glassware	3,800	3,700
3. Lab supplies	3,400	3,605
4. Publication costs	2,800	3,000
5. Equipment maintenance	552	752
SUBTOTALS	14,052	14,657
C. Travel to meetings to consult (for 2)	2,400	3,000
YEARLY TOTALS	16,452	17,657

	Year 2	Year 3
VII. PATHOLOGY Enrique Espinosa, M.D., Investigator		
A. Personnel		
1. Enrique Espinosa, M.D. (No cost)		
2. Virginia Ford, B.A. Research Assistant	9,330	9,980
3. Janice Gettelfinger Part-time Clerk-Typist	4,660	4,739
SUBTOTALS	13,990	14,719
B. Expenses and Supplies		
1. Supplies/expendable	3,300	3,490
2. Rabbits, purchase and maintenance	2,200	2,300
3. Equipment maintenance	500	500
4. Publication costs	600	650
SUBTOTALS	6,600	6,940
C. Travel to other laboratories for consultation (2 trips)	600	650
YEARLY TOTALS	21,190	22,309

ADDITIONAL BUDGETARY JUSTIFICATIONS

In the individual budget summaries of each proposal, there will be noted an 18 percent increase in budgetary personnel costs and a 12 percent increase in the cost of supplies and materials. This constitutes an over-all increase in the budget of approximately 16 percent. This increase in both personnel and supplies budgets is totally a reflection of inflationary costs and is not an increase in actual fund requests.

The major increase in the budget is in indirect costs. The University overhead cost is based on a fixed value of 65 percent of wages and salaries. From these indirect costs comes the physical and structural support from the University to the investigative projects. In addition, this year this includes the availability of a Type 3 exposure facility and its safety and operational personnel. This facility is under construction and was funded by the University through general University funds, and its operation and maintenance will be supported by general University funds. The indirect costs reflect the depreciation costs for the availability of these facilities. In the absence of such indirect costs, the direct charges to various investigators and projects for housing hereof and exposure of experimental animals would be substantially and significantly increased.