MANUFACTURING CHEMISTS ASSOCIATION

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February 13, 1979

Carlo H. Tamburro, M.D.
Professor of Medicine
Chief, Division of Digestive
Diseases and Nutrition
University of Louisville
P. O. Box 35260
Louisville, Kentucky 40232

Dear Dr. Tamburro:

Your letter of February 2nd with the plan for "Chemical Monomer Research Program" has been received. I have discussed this with Mr. Milton Freifeld, Secretary of our Occupational Safety and Health Committee and he is investigating what further action MCA might take based on the assumption that the participants in the vinyl chloride research program will not fund your work beyond the currently authorized third year of study ending in March of 1980. Either Mr. Freifeld or I will be in communication with you prior to the end of March 1979.

Sincerely,

John C. Van Horn
Manager
Engineering and Services
Division

JCV:ep
cc: Mr. M. Freifeld
Vinyl Chloride Technical Panel

CMA 002675

THE WRITER'S DIRECT DIAL NUMBER IS (202) 328- 4248

University of Louisville
Health Sciences Center

School of Medicine
Department of Medicine
Division of Digestive Diseases and Nutrition

Louisville, Ky 40232
P.O. Box 35260
Walnut & Preston Streets

February 2, 1979

Mr. John Van Horn
Manufacturing Chemist Association
1825 Connecticut Avenue, N.W.
Washington, D.C. 20009

Dear Mr. Van Horn:

I appreciate the time you took in our discussion and for your interest in our proposal.

Enclosed is the proposal for the Manufacturing Chemist Association's consideration and includes a technical summary as well as a budget for the first year.

I appreciate your forwarding it to the suitable individuals for proper consideration.

With appreciation and thanks, I remain

Sincerely yours,

Carlo H. Tamburro, M.D.
Professor of Medicine
Chief, Division of Digestive
Diseases and Nutrition

CHT:nlh

Enclosure

cc: Mr. Milton Freifeld

Chemical Monomer Research Program

Plan for the Manufacturing Chemist's Association

University of Louisville: Chemical Monomer Research Group (CMRG)

Period Covered 1979-1982

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University of Louisville

Chemical Monomer Research Program

I. Background

Chemical industries have been very cognizant of the occurrence of chemically-related job injury and have made continuing efforts to identify, as well as prevent, the possible development of these occupationally-related illnesses. The discovery of liver angiosarcoma related to the vinyl chloride polymerization process has directed new attention to occupationally-related diseases, especially cancer. In the past, most efforts had been directed toward identifying the cause of these disorders after their development and overt clinical presentation. Methods are then designed for prevention, and implementation of these preventive measures is often made a regulatory requirement. Frequently, these preventive measures are based on limited information concerning cause or pathogenesis and are based on very limited human clinical data as to their value and effectiveness.

The discovery in 1974 of vinyl chloride-related liver angiosarcoma at B.F. Goodrich's Louisville plant led to the development of a prospective medical surveillance program by the University of Louisville in collaboration with the B.F. Goodrich Company. This medical surveillance program applied all existing methods of early detection, diagnosis of disease and the treatment of those individuals exposed to potentially toxic chemicals.

Early in the application of the medical surveillance program three factors became very evident. First, it was clearly evident that chemical related injury was present among the chemical workers ranging from minor disorder to most severe form of injury--cancer. The second factor was that the extent and occurrence of this occupational injury was far less than expected considering what was then believed to be extensive exposure. Finally, the scientific knowledge about the pathogenicity of this chemical led to federal requirements for medical tests which we found to be inadequate and often misleading in detecting occupationally-related chemical injury.

Faced with this task, the University of Louisville brought together investigators to form a chemical research group. This group's research objectives are the systematic, prospective study of the toxicity and carcinogenicity of vinyl monomers which have potential risk but are of high socio-economic benefit. Our prototype for study has been the toxicity and carcinogenicity of vinyl chloride. The other vinyl monomers under study include ethylene, propylene, styrene, acrylonitrile, chloroprene, and vinyl bromide. The techniques and findings by this research group have led to the development of a systematic approach toward understanding the toxicity and carcinogenicity of vinyl monomers.

II. Rationale

Most information concerning chemical toxicity in humans has been obtained on the basis of accidental exposure to chemicals. Limited studies of workers have been conducted to determine the actual toxic potential of chemicals used within the industrial environment. The concern for the

possible carcinogenicity of many chemicals has led to increased regulatory requirements based on limited human data and a variety of extrapolated information from studies of various animal species. Despite great concern for the potential danger due to the large number of chemicals in use and the large population at risk from exposure, the occurrence of known chemically-induced toxicity, including carcinogenicity, is relatively small in comparison to the number and exposure of chemical agents within our environment. This would suggest that some chemicals are not biologically active or that there are some biological protective mechanisms or thresholds for most low dose chronic exposures. Unlike drugs, chemicals do not undergo human clinical trials for determining toxicity. Therefore, most studies on humans are performed retrospectively and on symptomatic or ill individuals. However, the University of Louisville's integrated research group now has the ability to apply the results of our laboratory studies to field trails in exposed worker populations.

III. Program Objective

The objective of this scientific research group is a better understanding of the toxicological problems that may arise through the production, handling, use and disposal of vinyl monomer commodity chemicals. To determine what potential risks, if any, are associated with human contact with these chemicals and how this information can be applied for early, specific detection in humans. This work is being pursued by a multidisciplinary group interacting in a coordinated collaborative manner with a common goal. This group's expertise ranges from chemistry, toxicology, molecular biology, immunology, biochemistry, and pathology to clinical application of the group's findings to the worker populations at risk. The overall research program goals are 1) to identify and characterize both the toxicity and potential carcinogenicity of vinyl commodity chemicals, 2) to identify the biological manner in which they are handled, and 3) to test out newly developed techniques for detection and screening of toxicity to humans exposed in an actual industrial environment. This research group proposes a prospective investigative approach to commodity chemicals (such as ethylene, propylene, styrene, vinyl chloride, acrylonitrile, chloroprene, vinyl bromide, and butadiene) of high socio-economic importance for which there is inadequate or incomplete human data. By designing basic experimental research to answer questions about metabolite formation and distribution, mutagenicity and potential carcinogenicity, we will provide the information needed to conduct clinically relevant detection and screening studies for industrial workers. These will include biochemical, immunological, histological, pathological, electron microscopic, and physiological studies, which are being tested in appropriate animal models. Screening tests would then be applied to human populations at high risk by properly designed protocols to determine whether there is any evidence of toxicity and/or carcinogenicity.

Our overall approach is unique and is directly aimed at providing information on humans. These integrated studies address the following: (a) is this chemical or its metabolite(s) toxic; (b) is this chemical or its metabolite(s) carcinogenic; (c) where are they distributed and how soon are they discharged from the body; (d) at what level is the toxicity present; (e) at what level is the carcinogenicity identifiable; (f) how significant are the host factors, nutrition or otherwise, in the induction of chemical injury; (g) what are the organ/tissue sites of injury; (h) can it be reversed?

(i) are there markers in the form of a metabolite or a cell biochemical to identify human exposure to the chemical; (j) what are the sensitivities and specificities of screen tests for chemically-induced injuries; and (k) how can these tests be applied to a prospective clinical screening program to determine the epidemiological significance of human exposure.

IV. Component Laboratories

The University of Louisville Chemical Research Program has three major types of laboratory resources available within the University. One entails the basic academic research laboratories conducting chemical, molecular and animal research studies related to chemical toxicity and carcinogenicity. The second includes the clinical research laboratories conducting human investigative and diagnostic research in the detection, evaluation and prevention of chemical toxicity including carcinogenicity. The third is composed of the epidemiological research facilities which have developed methods to facilitate future links of research development in the laboratory to the application to populations at high risk. Our prototype populations have been those who have had exposure to a variety of chemical vinyl monomers and polymers. These research facilities are supported by a newly constructed animal exposure facility and an industrial surveillance data bank.

The laboratories available at the University of Louisville may be categorized into six areas of research strategy. Some of these areas are composed of several investigators with different approaches toward a common goal. The six divisions are:

A. Synthesis and detection of metabolites
   Drs. Wong and Hurst

B. Organ specific metabolism of monomers
   Drs. Feldhoff, Nerland and Du

C. Tissue disposition of monomers and metabolites
   Dr. Waddell

D. Rapid screening for carcinogenicity of monomers
   Drs. Streips and Sonnenfeld

E. Detection of changes in biological tissue due to monomers
   Drs. Espinosa, Kupchella and Tseng

F. Clinical applications
   Drs. Tamburro, Fortwengler, Chan, Greenberg and Schrodt

These laboratories each employ different procedures to answer questions about the mechanism of toxic action of vinyl monomers and their ultimate effect on humans. While they function independently, they have a common collaborative goal(s) and their cooperation and sharing of information engender a concerted action which enhances the effort over that conducted in isolation. The activities in each of the laboratories is summarized in the following outline.

A. Synthesis and detection of metabolites

Dr. J. L. Wong, Chemical Structure Laboratory
Department of Chemistry

The extensive experience of this laboratory in the synthesis of reactive compounds provides an extremely valuable foundation to the entire project. Since all current evidence indicates that the reaction of oxidized monomers with biological tissues is the proximal cause of the toxicity, this unit provides the capability of synthesizing each of the putative intermediates between monomer and proximal toxin and their radiolabeled derivatives. Dr. Wong's laboratory is synthesizing each of the potential and proven intermediates/metabolites for study in the other laboratories in this group. In addition, the analytical capabilities of Dr. Wong's laboratory are able to investigate or determine unknown metabolites in various biological materials which are provided from the other studies.

Dr. Harrell Hurst, Analytical Toxicology Laboratory
Department of Pharmacology and Toxicology

The analytical capabilities of this laboratory are being focused on the detection and quantitation of metabolites in biological samples from the exposed individuals. Samples of expired air, urine and biological tissues will be analyzed in collaboration with Dr. Wong's group to correlate the effect of the intact individual on alteration of the monomer. Dr. Hurst, a new faculty member, brings toxicological expertise to the group.

B. Organ specific metabolism of monomers

Dr. Richard C. Feldhoff, Cellular Biochemistry Laboratory
Department of Biochemistry

Metabolism of chemical monomers in isolated mammalian liver cells is the primary investigative work of this laboratory. Emphasis is placed on the metabolism of the chemicals by isolated liver cells. Since the hepatocyte, quantitatively, is responsible for most metabolic conversions of chemicals, isolated hepatocytes will help identify the specific metabolic conversions which occur in this cell type. Since most of the toxic effects of chemicals are in cells other than the hepatocyte, it is essential to know whether the reaction occurred in the hepatocyte or in the cell where the toxic action is seen. These studies will delineate the sequence of events following acute or chronic chemical monomer exposure.

Dr. Donald E. Nerland, Drug Metabolism and
Biochemical Toxicology Laboratory
Department of Pharmacology and Toxicology

Tissues other than liver are being studied with regard to their metabolism of vinyl monomers. Many tissues such as lung, kidney and intestinal epithelium have been shown to have higher rates of xenobiotic metabolism, per cell, than hepatocytes. In many tissues, the metabolic route of conversion is entirely different from that which occurs in the hepatocyte. Toxic effects from monomers may, very likely, be due to metabolic conversion within the affected cell. These studies will define the metabolic activities of monomer conversion in lung, kidney, intestinal epithelium and any other tissue that

is shown to accumulate the monomer and its metabolites.

Dr. Julie T. Du, Biochemical Liver Research Laboratory
Department of Medicine

Specific enzyme activities in intact liver tissue, isolated hepatocytes and other types of isolated liver cells-Kupffer cells, fibroblasts, and sinusoidal cells-are being studied after exposure to various vinyl monomers. The biochemical changes that are detected in liver tissue may be a reflection either of the metabolism of the monomer in the tissue or a toxic action of the monomer on that tissue. Enzyme studies are selected which allow discernment between these two types of action. These studies are being done in concert with those of Drs. Feldhoff and Nerland to make the correct inferences.

C. Tissue disposition of monomers and metabolites

Dr. W. J. Waddell, Drug Disposition Laboratory
Department of Pharmacology and Toxicology

Whole-body autoradiography for soluble substances is being conducted in this laboratory for the localization of radiolabeled monomers and their metabolites. Mice are administered the labeled compounds by the route through which humans are ordinarily exposed, i.e., inhalation, oral, skin, etc. This technique samples every tissue in the body and identifies the specific tissues and cells which accumulate the chemical. The accumulation may be due to metabolism in those cells or due to specific cellular affinities. The laboratory has had extensive experience in clarifying the nature of accumulation mechanisms in tissues and correlating these with toxic or non-toxic effects.

D. Rapid screening for carcinogenicity of monomers

Drs. U. N. Streips and G. Sonnenfeld
Chemical Screening Laboratory
Department of Microbiology and Immunology

Two novel rapid screening procedures to identify the carcinogenicity of chemicals are being implemented and verified in these laboratories. In addition, standard microbiological tests such as the Ames Salmonella test, are performed in parallel with assays for mechanisms of DNA repair. These studies not only will identify potential carcinogens by the new screening techniques but also allow correlations with more basic and descriptive biochemical events in the cell growth and division processes.

E. Detection of changes in biological tissue due to monomers

Dr. Enrique Espinosa, Immunopathology Laboratory
Department of Pathology

Changes in tissue antigens and antibodies are under study in this laboratory in animal models of human exposure to monomers. Liver is the primary tissue of analysis because early antigenic changes have previously been detected in this laboratory using this procedure with vinyl chloride. Immunochemical methods identify the specific alterations that are produced in these cells from the exposure to such monomers. Also, samples of serum are analyzed for the presence of specific antibodies to tissue constituents of the exposed animal. These studies provide evidence of preneoplastic, antigenic changes in host tissues.

Dr. Charles Kupchella, Glycosaminoglycan Laboratory
Cancer Center and Department of Physiology

Characterization of the tissue and urinary glycosaminoglycans (mucopolysaccharides) following exposure to chemical monomers are being evaluated as a measure of the early toxic injury. These mucopolysaccharides have been shown by this laboratory to be altered following exposure to vinyl chloride and also to be altered in patients who have angiosarcoma from vinyl chloride exposure. The chemical structure of the altered glycosaminoglycans (GAG) has not been fully defined and is still under study. The identification of these compounds and the parameters of their production will hopefully provide a rapid screening test for use in the industrial environment field trials. Preliminary field trials have already begun.

Dr. Michael Tseng, Electron Microscopy Laboratory
Department of Anatomy

Electron microscopy studies are being used to identify early changes in cells following exposure to chemical monomers. Subcellular organelles which show consistent alterations to each of the vinyl monomers can then be implicated as sites of reaction. Correlations are being studied between the effects seen by Dr. Tseng and the results found by the other investigators in the group from the same experimental animals and tissues.

F. Clinical application

Dr. C. Chan and P. Fortwengler, Clinical Immunological Liver
Research Laboratory, Department of Medicine

Histochemical staining of tissue, for identification of the various types of liver cells are used to help understand the different responses by these cells to monomer exposure. Specific enzymes, which have been shown clinically to be elevated, are also studied by histochemical techniques. These experiments are used as indices for the toxic effect of chemicals on the various liver cells. Correlation of the histochemical studies with new clinical clearance tests now being used for determining liver integrity will provide new, more sensitive means for the detection of early toxic effects from the monomers.

Drs. Schrodt, Barrows, and Tamburro, Liver Pathology Laboratory
Departments of Pathology and Medicine.

This laboratory has been studying by light microscopy the sequential histological changes occurring in humans exposed to various vinyl monomers (vinyl chloride and acrylonitrile). Specific attention is presently being directed toward the characterization of early specific histological changes characteristic of chemical injury. These studies are being correlated with the animal liver pathology studies of Drs. Tseng and Du.

Drs. Greenberg and Tamburro, Epidemiological Industrial Data Bank
Departments of Preventive Medicine, Medicine, Cancer Center

This facility has collected, stored, and is actively analyzing four years of comprehensive medical data obtained from its ongoing medical surveillance program conducted at a large (1200 employees) vinyl monomer industrial facility and one smaller polymer industrial facility (400 employees).

The data bank includes work history, chemical exposure estimates, and complete medical information on approximately 90% of all workers. This medical and exposure data provides for human verification of the results and information developed from laboratory studies. It also provides clinical leads for further animal investigation.

V. Research Program Components

A. Title: Chemical Methodologies in Monomer Toxicity Study

Investigator: John L. Wong, Ph.D., Chemical Structure Laboratory

Background: Our current study of vinyl chloride toxicity/carcinogenicity has been concerned with (1) the chemistry in vinyl chloride metabolism, i.e. the structure of the intermediates and their reaction with cytoplasmic chemicals, and (2) the putative actions of the primary metabolites on nuclear materials. We have synthesized all plausible oxygenated derivatives at progressive levels of catabolism of the vinyl monomer and screened them for mutagenicity. Two putative metabolites, chloroxirane and chloroacetaldehyde, have been shown to react with sulfhydryls (detoxification study) and nucleic acid constituents (mutagenesis and carcinogenesis study). Radiolabeled derivatives are being prepared to observe disposition and conversions in animals and isolated liver cells.

Objective: We propose to extend the above chemical methodologies to investigate the toxicity/carcinogenicity of other industrial vinyl monomers, viz. ethylene, propylene, styrene, acrylonitrile, chloroprene, and vinyl bromide.

Specific Aims: As part of the multidisciplinary team study of vinyl monomer carcinogenesis, our contributions will involve the following:

1. Synthesis and purification of compounds in Table 1 for (a) mutagenicity/carcinogenicity screening, and (b) transformation and cell function study of different types of liver cells.

Table 1. Vinyl Monomers and their Potential Primary Metabolites

Monomers		Oxiranes	Carbonyls
Ethylene	$H_2C{=}CH_2$		
Propylene	$H_2C{=}CH{-}CH_3$		
Styrene	$H_2C{=}CH{-}Ph$		
Acrylonitrile	$H_2C{=}CH{-}CN$		
Vinyl Chloride	$H_2C{=}CH{-}Cl$		
Vinyl Bromide	$H_2C{=}CH{-}Br$		
Chloroprene			

2. Synthesis of the above compounds with radioactive labels (carbon-14, tritium) for (a) tissue disposition study, (b) bacteria cell and isolated liver cell distribution study, (c) animal and liver cell metabolic study, and (d) enzymatic detoxification study.

3. Synthesis of metabolite-sulfur conjugates and ^{13}C-monomers and ^{13}C-metabolites for structural identification of metabolites and detoxification products.

4. Analysis of the above compounds in various biological and chemical samples by gas chromatography, high pressure liquid chromatography, scintillation counting, and spectroscopic methods (Fourier Transform-Nuclear Magnetic Resonance, Fourier Transform-Infrared Red, and Mass).

5. Reaction of oxirane and carbonyl metabolites with DNA constituents to study their putative actions and to relate the induced DNA modifications with bacteria assays.

Significance: Broadly speaking, this team study will lead to early detection and prevention of industrial cancers. Our chemical methodologies (synthesis, structure, and analysis), applied as an integral part of the multidisciplinary approach, will elucidate specific molecular events in the effects of vinyl monomers on industrial workers. This information will form a rational basis for safe use of chemicals and design of preventive measures, e.g. setting exposure threshold, diet supplement recommendations, therapeutic intervention, exonerating or suggesting substitute for a certain chemical. Our molecular studies also provide the opportunity to develop a useful marker in the form of a metabolite or cell biochemical in the pathogenesis of chemical injury.

B. Title: Novel Rapid Screens for Chemical Monomer Carcinogenicity

Investigators: Uldis N. Streips, Ph.D. and Gerald Sonnenfeld, Ph.D., Chemical Screening Laboratories

Background: Rapid screen methodology (i.e. Ames test, DNA repair assays, mammalian cell reversion tests, transformation assays) has for the past several years served well as the initial indicator of the biological activity of important industrial chemicals. Ames has correlated mutagenesis to carcinogenesis. However, to date, no rapid test has been demonstrated to detect the carcinogenic potential of suspected chemicals. The reason for this is that most of the current tests examine direct damage to DNA. While such damage contributes to the mutagenic event, carcinogenesis may be elicited by other physiological and morphological alterations in addition to DNA damage. Recently assays for "SOS repair" have been reported and also have been developed in our laboratories. The tests measure the production of several substances, notably protease, by a cell which has received major insult to its DNA. Protease production activates many cell systems which are normally silent. Also, some normal processes, possibly interferon production, may be destroyed by protease. Therefore, SOS induction may be preliminary to morphological and immunological alterations observed in neoplastic transformation. Our two novel assays (Bacillus SOS Induction Test, and Inhibition of Mammalian Interferon Induction Test) will identify the induction of the diverse events constituting SOS repair and associated morphological alterations. Since we can add activation systems (microsomal preparations) as a component of these assays, we feel these tests can serve as comprehensive screens for chemical carcinogens.

Objective: To refine and implement two new rapid screening assays - the Bacillus SOS Induction Test, and the Inhibition of Mammalian Interferon Test - which will allow us to judge the carcinogenic potential of industrial chemicals. These two new assays, along with our standard battery of tests (Ames Salmonella reversion, Bacillus repair, Bacillus forward mutation, mammalian cell line sister chromatid exchange) will allow us to construct a composite evaluation on the toxicity, and on the mutagenic and carcinogenic potential of important chemical monomers.

Specific Aims:

A. We will calibrate our two new assays by examining the following groups of chemicals.

1) Known carcinogens - B- napthylamine, 2-acetylaminoflourene, chloroacetaldehyde, methylmethanesulfonate (MMS).
2) Suggested carcinogens - Benzene, trichloroethylene, acrylonitrile, ethylenedichloride, metal oxides (nickel, beryllium, selenium), ethylene, vinyl bromide.
3) Suggested noncarcinogens - ethylethanesulfonate (EMS), toluene, n-hexane, methylene chloride.

This will establish two new carcinogen-specific screens for use in the testing of chemicals by both industry and University-related laboratories.

B. Apply our calibrated new assays, and our standard battery of assays to other important chemical monomers such as styrene, choloroprene, and propylene.

C. Correlate our findings to interferon induction in human cell lines with potential of results within 5 days.

D. Correlate all our findings with active chemicals to transformation of mammalian and human cell lines, and, ultimately to tumor induction in mice.

E. Distribute active chemicals to other investigators in the University of

Significance: Two new reliable, calibrated, carcinogen-specific screens will be made available to screening components, in industry, University, and government laboratories. Chemical monomers important in many industrial processes will be thoroughly tested for toxicity, mutagenicity, and carcinogenicity.

C. Title: Chemical Monomer Metabolism in Isolated Liver Cells

Investigator: Richard C. Feldhoff, Ph.D., Cellular Biochemistry Laboratory

Background: The mammalian liver is the principal organ responsible for the metabolism and/or detoxification of many organic metabolites including potential carcinogens. The exposure of the liver to an hepatotoxin such as ethanol can interfere with its ability to detoxify potential carcinogens. In addition, nutritional deficiencies in vitamin intake or amino acid supply to the liver are often interrelated with alcohol consumption and therefore with the metabolic capacity of the liver. In order to better protect industrial workers and the general public from chemically-induced cancers, it is desirable to define pathways for the metabolism and detoxification of chemical monomers such as styrene and acrylonitrile by the liver and to assess the role of alcohol exposure and nutrition on these pathways. Studies of chemical monomers and their metabolites, for example the epoxides, can be facilitated by reducing the complexities associated with whole animal experiments to the level of individual liver cells. Techniques have recently been developed which allow for the preparation of isolated liver cells that retain normal liver-specific functions.

Objective: To apply our isolated mammalian liver cell system to studies of chemical monomers and their metabolites so that the mechanisms can be determined by which they are a) detoxified or b) metabolized to potential carcinogens. Such a system reflects the normal metabolism of chemicals in intact mammals and for biochemical investigations is superior to most whole animal experiments or cell-free studies.

Specific Aims: Isolated mammalian liver cells will be used:

(1) to determine the uptake, intracellular transport and metabolism by various cell fractions of radioactively-labeled chemical monomers and their epoxides.

(2) to identify and quantitate metabolites released into the medium

(3) to determine the effects of individual metabolites on normal liver cell functions and to assess the mutagenic potential of newly indentified compounds.

(4) to determine the effects of ethanol, vitamins and essential amino acids on the ability of the liver to metabolize/detoxify vinyl monomers and their epoxides.

Significance: The exposure of the liver to an hepatotoxin such as ethanol is likely to interfere with its ability to detoxify organic chemicals or potential carcinogens. Many of the metabolic and hepatic effects of alcohol have been the subject of extensive investigations, however, the concept of the potentiation of carcinogenesis by ethanol is relatively new and has not been adequately investigated. Animal experiments suggest that alcohol intake increases the incidence of cancer when the animals are also exposed to a carcinogen. The proposed experiments should help define levels of alcohol consumption and nutritional deficiencies which can be tolerated by industrial chemical workers.

D. Title: Tissue Disposition of Monomers and Metabolites

Investigator: William J. Waddell, M.D., Drug Disposition Laboratory

Background: Identification of the specific sites in the body which have an affinity for an industrial compound will allow inferences regarding the toxicity of that chemical. Chemicals that react with specific tissues and cells often produce their toxic effect through that reaction. Experience and analysis are required to discern accumulations which are toxic from those which are not.

Objectives: (1) Localization of the specific tissues and cells in the body which accumulate and retain monomers and their metabolites.

(2) Calculation of the rate of elimination of these compounds from each tissue in the body.

Specific Aims: Small laboratory animals, such as mice, will be used as models of human exposure. The technique to be used is whole-body autoradiography. The chemical of interest will be labelled with radioactive carbon, tritium, or other isotope. The radioactively-labelled chemical will be administered to mice by the route through which humans are ordinarily exposed, e.g. by inhalation, orally, application to skin, etc. At carefully selected time intervals after exposure, the mice will be anesthetized and frozen rapidly by immersion in a dry-ice bath. Thin sections (20 microns thick) will be taken lengthwise through the entire body of the mouse. This allows every tissue and cell in the body to be exposed for analysis. The frozen sections will be placed against x-ray film for exposure. The radioisotope will expose the x-ray film and therefore reveal sites of accumulation in the body. If the chemical is completely eliminated, then one can be certain that no tissue has been overlooked.

Significance: The technique is one, par excellence, for locating chemicals and their metabolites in the body. Federal regulatory agencies in Japan, Sweden, Germany, France and Switzerland now require all new drugs to be studied by this technique before release. It is only a matter of time before agencies in the U.S. will require this type of study.

It is important to point out that if a chemical is shown by this study to be promptly and completely eliminated, then it is largely exonerated from potential toxicity. The widely used plasticizer DEHP was shown in this laboratory, by the technique proposed, to be free from potential chronic toxicity. Similar studies on other chemicals could quickly and effectively remove unfounded concern for potential toxicity.

E. Title: Biochemical Systems for the Detection of Chronic Vinyl Monomer Injury.

Investigator: Julie T. Du, Ph.D., Biochemical Liver Research Lab

Background: Characterization of the metabolic pathway and changes undergoing cumulative chemical exposure comparable to those of humans has helped in the clinical diagnostic process. Our laboratory's past experience with rats exposed to vinyl chloride has shown a lower glucose-6-phosphatase, increased glucose-6-phosphate dehydrogenase, glutathione reductase, and glutathione transferase in liver prior to any changes in the conventional clinical liver function tests demonstrating that metabolic changes may predict impending physical changes. Histological and electron microscopy studies verified this concept and provide information as to time and dose relationship to exposure. These studies indicate when and where (which cells or subcellular organelle) these changes are occurring. They allow us to better design tests to detect these chemically-induced changes, identify when in the time course to apply the tests and how to better interpret them.

Objectives: To investigate the enzymatic alterations in animal with progressive chemical exposure and the tissue adapation to the exposure.

Specific Aims:

1. To study the sequential enzymatic changes related to the metabolism of vinyl monomers (oxidation and detoxification) in various hepatic cell types and in subcellular fractions of whole liver homogenate from animals progressively exposed to various doses of vinyl monomers. To study the sequential enzymatic patterns of carbohydrate metabolism and nucleic acid synthesis resulting from progressive chemically (vinyl monomer) induced liver injury in animals.

2. To assess the capability of various isolated liver cell types (hepatocyte, Kupffer cell and endothelial cell) to metabolically handle vinyl monomers and their metabolites.

3. To correlate the sequential morphological changes (light and electron microscopy) with the enzymatic findings.

4. To evaluate the use of glutathione transferase activity in serum in animals exposed to chemicals as a potential clinical test for early liver injury.

Significance: Our limited understanding of chronic or prolonged low grade exposure to chemical agents has repeatedly led to use of inadequate medical and biological screen assessments. Standard clinical tests (even federal O.S.H.A. requirements) are too non-specific an insensitive an indicator of chemical injury. They are mainly markers of cell death rather than adaptation or injury. Delineating oxidation and detoxification routes for chemical monomers will indicate which enzyme evaluations will be useful in clinical diagnosis and when and where these evaluations should be applied and how they should be interpreted.

F. Title: Ultrastructural Diagnosis of Tissue Responses to Chemical Monomers.

Investigator: Michael T. Tseng, Ph.D.
Electron Microscopy Laboratory

Background: Conventional light microscopy is useful in diagnosing overt pathologic changes but its ability to assess subcellular changes is limited by resolving power. Electron microscopy offers resolving power of a few Angstroms and thus can probe subcellular pathology. The combination of morphometry and autoradiography with electron microscopy further enhance the value of this research tool. Morphometry furnishes quantitative data on subtle changes in cell organelles which should corroborate biochemical data. Whole body autoradiography in toxicological studies has shown its value at the light microscopic level. The distribution of labelled monomers and the specific organs that concentrate them can be revealed. However, since the cell populations of most organs are heterogeneous, the identification of the specific cell types which sequester particular compounds is a problem. Electron microscopic autoradiography can pinpoint specific cell types and reveal the subcellular distribution of given chemicals and their metabolites. Moreover, this technique may be applied to intact tissues, enzymatically dispersed cells, or clinical specimens.

Objectives:

1. To define ultrastructurally and morphometrically the sequential changes in cell organelles of liver, kidney, and lung after acute and chronic exposure to toxic monomers.

2. To identify by electron microscopic autoradiography the specific cell types that sequester and metabolize these monomers.

3. To define the pathway of labelled monomers and their metabolites within tissues and within enzymatically dispersed cells of animals and humans by pulse-chase electron microscopic autoradiography.

Significance: The initial effects of chronic exposure to toxic monomers must be manifested by relatively subtle changes in the body. Electron microscopy alone or in combination with morphometry and autoradiography constitutes a powerful tool in assessing early cellular and subcellular damage, and in identifying the intracellular pathway of a given monomer and its metabolites. Data on ultrastructural changes in cells may suggest new means for the detection, prevention, or treatment of monomer-induced injury.

G. Title: Clinical Use of Clearance Studies and Histochemical Changes in Identification of Chemical Monomer Exposure.

Investigators: H. Phillip Fortwengler and Chao.H. Chan, M.D., Clinical/ Immunological Liver Research Laboratory.

Background: A number of recent investigations have provided data to support the concept that chemically-induced carcinogenesis in the liver is initiated by alteration of a few cells at random which become resistant to the cytotoxic action of that chemical. The proliferation of these resistant "initiated" cells into "foci" may ultimately determine the number of tumors developed. The morphologic changes, such as focal hyperplasia, in the liver are accompanied by other changes such as an increase in collagen deposition, an increase in gamma glutamyl transpeptidase, glucose-6-phosphate dehydrogenase, and increased incorporation of radioactive thymidine, most of which can be demonstrated histochemically.

Clinically, hepatic toxicity and necrosis is indicated by markedly elevated transaminase values (SGOT, SGPT). More recently, indocyanine green (ICG) clearance values, bile acid clearance, and in certain instances, ^{13}C-aminopyrine values, have been demonstrated to be more sensitive indicators of early latent liver damage. We are presently testing whether these newer, more sensitive clinical tests can identify the presence of hepatic foci and if the increased numbers of foci formed are reflected by increasingly abnormal "clinical" values in circulating serum enzyme levels or ICG clearance values.

Objectives: 1. To use histochemical techniques to assess the development of early chemical injury including premalignant "foci" following chemical monomer exposure.

2. To evaluate the usefulness of clinically applicable serum and blood clearance tests in the early detection of chemical injury.

Specific Aims: 1. To study the histochemical changes in laboratory animals following exposure to known chemical toxins/carcinogens as a means of "calibrating" the system.

2. To run parallel clinical assays such as serum transaminases and blood clearance tests to determine whether the histochemical changes following exposure to chemical toxins/carcinogens are reflected in these tests.

3. To quantify "foci" and determine the ability of the histochemical techniques to assess the degree of toxicity/carcinogenicity.

Significance: These studies will help determine which chemicals in which quantities will produce hepatocellular "foci", the lesion thought to precede frank malignancy. Correlations between clinical clearance tests/blood assays and hepatic injury will broaden and better our interpretation of those same tests when performed on humans with suspected chemically-related liver injury. It will also provide the opportunity to modify the clinical clearance test so as to be least invasive as possible (e.g. ^{13}C aminopyrine and bile acid clearance may be performed via oral route without any, or at least less, blood drawing requirements).

H. Title: Clinical Use of Urinary and Tissue Glycosaminoglycan in the Detection of Chemically-induced Liver Injury.

Investigators: Charles E. Kupchella, Ph.D.; Raya Warick, Ph.D.
Glycosaminoglycans Laboratory

Background: It is well established that glycosaminoglycans (GAGs) are involved in wound healing and scar formation. Although it is less certain what role they play, it is also known that certain GAGs are elevated in malignant tumors including hepatic tumors, and it has been postulated that GAGs may be important determinants of tumor-cell properties. A number of laboratories, including ours, have established that urinary GAGs may serve as markers in the pathogenesis of chemical injury, fibrosis, and cancer. Despite the fact that urinary GAG analyses have long been used clinically to detect and diagnose genetically-determined metabolic disorders of GAG metabolism, a systematic evaluation of the usefulness of urinary GAG patterns in the detection and diagnosis of acute and/or chronic necrotic and/or fibrotic liver injury -- or cancer -- has never been made.

Objectives: Determination of the practical utility of urinary and tissue glycosaminoglycan measurements in the detection of chemically-induced liver injury.

Specific Aims: Animal Studies. 1. Determine the sequence of GAG changes (in tissue, urine and blood) associated with the onset of experimentally-induced fibrotic injury with special attention to any change coinciding with the transition from reversible to irreversible fibrosis.

2. Determine the degree to which chemically-induced necrosis of the liver results in altered urinary GAG excretion and confirm changes seen in tissue in earlier studies.

3. Determine the relationship between GAG patterns in tumor tissue (and urine) in animals with fast vs. slow growing and in metastasizing vs. non-metastasizing, chemically-induced, transplantable, hepatocellular tumors. (As an initial means of exploring the functional role of GAGs known to be elevated in hepatic cancer).

Human Studies. 1. Repeat the double-blind clinical trail in which we evaluate the ability of urinary GAG analysis to correctly identify active liver disease.

2. Evaluate urinary GAG patterns in groups of alcoholics with active liver injury over time as a comparison group to those with chemical injury.

3. Compare liver tissue and urinary GAG patterns in patients with alcohol-injured livers vs. liver angiosarcomas, vs. livers carrying hepatocellular tumors and livers with metastatic cancer vs. chemically-induced hepatitis and cirrhosis.

4. Improve further the present methods of evaluating urinary GAG's as to cost and time while maintaining good specificity and sensitivity.

Significance: These studies: 1. can provide useful screening tests for liver injury -- urine testing fits the ideal for screening tests: a) are non-invasive, b) have no morbidity/mortality and c) require minimum down time.

2. can provide a means for not only identifying early injury but for distinguishing inactive disease.

3. can provide tests to identify individuals at risk of chemical injury. Characteristic GAG changes may be able to reflect chronic alcohol injury or other types of non-occupational liver injury prior to employment or assignment to jobs utilizing potential hepatotoxins.

I. Title: Tissue Antigenic Changes and Antibody Response in Chemical — Monomer Exposure.

Investigator: Enrique Espinosa, M.D., Immunopathology Laboratory.

Background: Chemicals soon after entering the body combine with tissue or plasma proteins and their presence often results in the stimulation, inhibition or modification of certain antigenic constituents and of the antibodies they elicit. These are early events that often precede subsequent pathologic effects. Because of this, when studying means of detection and prevention of pathologic effects of industrial chemicals the measurement of these early changes is of importance. In the case of chemical carcinogenesis of the liver, preneoplastic changes consisting in the appearance of foci of liver cells showing enzymatic and antigenic changes have been identified. Alterations shown include decreased glucose-6-phosphatase and ATPase activity and the presence of gammaglutamyl transpeptidase not normally observed in mature hepatocytes. Early appearance in liver hyperplastic nodules of a liver microsomal protein, termed preneoplastic antigen has also been reported. Appearance of tumor-associated antigens and loss of certain normal tissue antigens later develop in the course of tumor formation.

Another immunologic approach to screening and prevention of the effects of industrial chemicals involves the use of antibodies produced in animals injected with the chemical linked to macromolecular carrier. Such antibodies can then be used in the sensitive determination of the level of the chemical in the tissues or blood stream.

Objectives: To determine antigenic changes in liver and other tissues including tumors from animals or man exposed to chemical monomers. Antibodies stimulated by these antigenic changes and with specificity for the chemicals will also be studied.

Specific Aims: The following research work will be undertaken using rats and mice exposed, acutely or chronically, to the selected chemicals. Human material from individuals with work exposure to these chemicals will be included in these studies.

a) Liver, tumors and other tissues will be examined for quantitative or qualitative antigenic changes by means of immunochemical methods alone and in combination with histologic and tissue culture techniques.

b) Serum will be collected and examined for presence of antibodies reacting with tissue constituents of both exposed and unexposed animals. Immunochemical and immunofluorescent procedures will be employed.

c) Antibodies with specificity for the chemical under study may be produced in rabbits by injecting them with the chemical linked to macromolecular carriers. The use of such antibodies in the development of radioimmunoassay to detect chemica levels in tissues and body fluids will be investigated.

Significance: Demonstration of antigenic and antibody changes resulting from industrial chemical exposure offers an opportunity to develop sensitive methods for the early detection of chemical-associated disease including cancer.

J. Title: Identification of Metabolites in Expired Air and Urine, in Vivo

Investigator: Harrell E. Hurst, Ph.D.
Analytical Toxicology Laboratory

Background: Exposure of industrial workers to certain foreign chemicals often results in direct toxicity due to the adverse effects of these agents on sensitive components within the biological systems. Alternatively, such toxicity can be mediated through metabolic transformations which may produce reactive or toxic metabolites from compounds which are relatively innocuous per se. Identification and quantitation of the agents within the exposure situations will enable minimization of the associated hazards. For this purpose and for the elucidation of the role of innate metabolic processes in chemical-mediated toxicity, a versatile analytical laboratory is an invaluable asset.

The aforementioned analytical capability requires a variety of sampling, separation, and chemical detection instruments and expertise to encompass the spectrum of agents employed in industry. Certain techniques available within this laboratory include absorbent trapping of airborne organics, gas and liquid chromatography, and definitive analysis by the combined technique of gas chromatograph/mass spectrometry (GC/MS).

Objectives:

1. The identification of metabolites of monomers in expired air, urine and tissues of animal models and humans.

2. Correlation of these metabolites with those found in vitro.

Specific Aims: The analytical capabilities available within this laboratory will be deployed toward solution of the above objectives. Ambient air sampling will be conducted followed by thermal desorption and GC/MS analysis of trapped materials. Additionally, methodology is available for identification and quantitation of foreign compounds and their metabolites in biological media from human or experimental animal exposure to vinyl monomers or other industrial chemicals as needed.

Significance: Definition of exposure situations and toxicity-mediating metabolic processes through analytical techniques can help provide the necessary knowledge to explain and alleviate hazards associated with industrial chemical exposure.

K. Title: Extra-Hepatic Metabolism of Chemical Monomers

Investigator: Donald E. Nerland, Ph.D.
Drug Metabolism and Biochemical Toxicology Laboratory

Background: The major routes of exposure to industrial chemicals include ingestion, topical and inhalation. Of these, inhalation represents the major route of entry to the body. Not only are the lungs exposed to high concentrations of the parent compounds but they also represent a site for metabolism of these compounds. The lungs possess enzymes similar to those located in the liver which are known to convert these compounds to toxic metabolites. While the lungs are the most frequent example of an extra-hepatic site involved in xenobiotic metabolism, other organs such as the kidney are also capable of metabolizing foreign chemicals. The rate of metabolism is often significant if the chemicals tend to concentrate in these extrahepatic organs.

Objective: To study the metabolism of industrial chemicals by lung and tissue shown to concentrate these compounds. Other tissues will be examined for their ability to metabolize selected chemicals only if they are shown to accumulate at a specific extrahepatic organ.

Specific Aims: 1. To determine the qualitative and quantitative nature of metabolites produced by lung tissue homogenates. The test compound will be incubated with a lung homogenate and the metabolites isolated from the incubation medium will be identified and quantitated. Other tissues will be analyzed for their ability to metabolize the test compounds only if they are shown to accumulate within specific tissue or exert their toxicity at a specific tissue.

2. To determine the effect of acute and chronic exposure to industrial chemicals on the rate of metabolism by lung tissue. The enzymes responsible for xenobiotic metabolism are unique in that their activity may be induced by exposure to foreign compounds. Induction may not only change the rate of metabolism of chemical compound but also alter the spectrum of metabolites produced, possibly leading to more toxic products.

3. To determine the effect of industrial chemicals on marker enzymes and compounds in the lung. Exposure to industrial compounds is known to alter certain liver enzymes and substrates. For example, after chronic exposure to bromobenzene, the level of glutathione is reduced in the liver. Glutathione is required for the detoxification of bromobenzene. It is not known, however, if these marker enzymes are affected in a similar manner in the lung.

Significance: Currently there is very little information available on the extrahepatic metabolism of industrial chemicals. The purpose of this study is to determine if toxic metabolites are produced outside the liver and to determine to what extent extrahepatic metabolism contributes to a compound's "overall" toxicity.

L. Title: Liver Collagen Changes With Age vs. Chemical Injury

Investigators: G. Randolph Schrodt, G. Barrows, and C. H. Tamburro
Liver Pathology Laboratory

Background: Comparatively little is known about the sinusoidal lining cells and the space of Disse in the liver. There has been a great deal of research controversy over the quantity and distribution of collagen or scar tissue in chemical injury vs. aging. Collagen (scar tissue) formation is a common finding in a variety of illnesses independent of chemicals. Adequate knowledge of the natural course of collagen deposition is therefore very important.

It should be emphasized that the changes in the liver involving increased deposition of collagen are of themselves not this specific for vinyl chloride exposure. Increased collagen deposition in the liver is a fairly regular response to persistant injury of the hepatocytes. Additionally there is some evidence that there is increased collagen deposition with age in the liver but there is no clearcut data regarding this point.

Objective: The purpose of the present investigation is to analyze on an age related basis the collagen present in the space of Disse of normal human livers and those exposed to vinyl monomers and also to determine what constitutes the normal "sinusoidal lining cells" (cell of origin for angiosarcoma) of the human liver.

Specific Aims: 1) To identify the sinusoidal and perisinusoidal cells of the liver with the use of the electron microscope, using refined methods of differentiation include the peroxidase stains and injection of latex particles.

2) Study the sinusoidal lining cells for abnormalities observed in the liver biopsies of patients exposed to vinyl monomers

3) Determine if other vinyl monomers have similar changes in sinusoidal lining cells as seen with vinyl chloride.

4) Determine if individuals exposed to other vinyl monomers have some observed increases in collagen in the space of Disse, corrected for age.

Significance: The origin of collage in the liver is a matter of some debate. If the natural aging process induces increased collagen then more accurate standards need to be documented. This would prevent incorrect associations between the amount of collagen as evidence of chemical injury and time (or age) exposures to a chemical.

VI. Vinyl Chloride Research Progress

University of Louisville's Chemical Monomer Research Group has received generous support from the Manufacturing Chemist's Association's Vinyl Chloride Research Committee, B. F. Goodrich Company, the American Cancer Society, and private foundations within the Louisville area to conduct research on vinyl chloride and its clinical application to the industrial worker population.

Dr. John Wong has synthesized the potential active intermediates and metabolites of vinyl chloride and has provided these to the other members of the study group. He has also provided the means and chemical models for identifying these substances in the various experimental studies. Dr. Streips has carefully studied vinyl chloride and all its potential metabolites utilizing the standard microbiological tests for mutagenicity and has further studied vinyl chloride and its metabolites' effect on the repair mechanisms for DNA injury. In addition, he and Dr. Sonnenfeld have developed and are applying two new tests for carcinogenicity which will more correctly identify the biological activity of chemical monomers in mammalian cell lines as well as in bacteria. Dr. Feldhoff's addition to the research group has expanded the group's ability to study the metabolic activities of vinyl chloride in isolated mammalian liver cells. The methods will be applied to continuous human liver cell lines. Dr. Du's laboratory has systematically studied the metabolic and enzymatic changes occurring in the intact animal having progressive chronic chemical exposure similar to human experience. These studies have shown changes in gluconeogenesis consistent with the histological findings of focal hyperplasia seen in both animals and humans. Her group is further characterizing the various types of liver cells ability to oxidize and detoxify vinyl chloride and its active metabolites, chloroacetaldehyde and chloroxirane.

Concomitant studies with Fortwengler and Chan are being conducted to utilize histochemical markers for these enzymatic changes so that they might be adaptable for clinical detection. They have developed methods for identifying the various liver cell types by the use of histochemical fluorescent antifactor VIII, peroxidase staining, and latex/iron particle uptake for the identification of sinusoidal lining cells, fibroblasts, and Kupffer cells. These techniques help to morphologically identify the various cells biochemically under study. These methods are being adapted for study of human cells obtained from liver biopsies. In addition, Fortwengler has determined, by study of the immune system in exposed workers, that vinyl chloride does induce a depression of the immune system. This depression, however, appears insufficient to account for individual susceptibility to the development of hepatic angiosarcoma.

Dr. Kupchella's studies on mucopolysaccharides (GAG's) in both humans and animals have shown that hepatic angiosarcoma and fibrotic liver diseases are accompanied by elevated tissue GAG levels and that these GAG values in tumor tissue differ from those of fibrotic tissue adjacent to the tumor. He has also demonstrated that the characteristic urinary GAG patterns of angiosarcoma and hepatoma patients are not found in normal controls. In addition, he has shown that hepatic necrosis (injury) is accompanied by significant tissue GAG elevations and that this is not seen in regeneration or repair. Preliminary clinical trials using urinary GAG determinations as a screening procedure demonstrated that it was a better indicator of liver disease than

ultrasound analysis or radioisotopic scanning of liver and provided information not available through the standard clinical biochemical tests. A second clinical trial to further assess how urinary GAG levels might be further applied is now being prepared for field study.

Dr. Schrodt's studies have demonstrated a morphometric increase in collagen deposition in the sinusoidal space of Disse as one of the earliest associated findings with vinyl chloride exposure. He and Dr. Tamburro have been studying the normal distribution of hepatic collagen in adult humans. Preliminary results demonstrate that there exists, independent of vinyl chloride exposure, a progressive increase in the collagen deposition with increase in age. These histological findings are further supported by the age dependent increase in abnormalities by ICG clearance which is distinct from ICG clearance abnormalities of vinyl chloride exposure. This adds biochemical evidence to support histological findings.

Dr. Espinosa's group has demonstrated antigens and antibody modifications occurring in vinyl chloride associated liver angiosarcoma. These changes have led to the further study of antigenic changes in chemically-induced transplantable liver tumors in animals. Work is in progress to study the applicability of these observations to the diagnosis and prognosis of liver tumors for possible use in clinical detection.

Dr. Hurst's and Dr. Nerland's addition to the group is now providing further analytical capability. Specifically, gas chromatography, liquid chromatography and a combined gas chromatograph/mass spectrometer system. This will provide further capability to expand our present studies of metabolic end product identification in biological tissues and fluids.

Dr. Waddell has established whole-body autoradiography for purposes of identification of the specific sites in the body and various organ cells which have affinity for vinyl chloride and its metabolites. These results will be correlated with biochemical, clinical and electron microscopic findings of other investigators.

Finally, Dr. Tseng's electron microscopic (EM) study of early changes occurring in liver parenchyma after exposure to vinyl chloride has illustrated ultrastructural changes in the hepatocyte especially in the smooth endoplasmic reticulum. These structural changes further corroborate the biochemical sequential changes identified by Dr. Du and supports the concept that sublethal damage exists in the hepatocyte after early exposure to vinyl chloride without clinical or biochemical manifestations. This data further supports our clinical finding that present federally-required screening tests are inappropriate for purposes of identifying early exposure related injury. Similar EM studies are now being planned for other vinyl monomers.

This extensive experience with the vinyl chloride model, both in the laboratory and in field, has provided the group with the opportunity to develop a multidisciplinary approach to the study of chemical toxicity. This research group now plans to apply these developed techniques to the study of other important vinyl monomers for which we have very limited understanding and experience.

VII. Program Overview

The human consequences of chemical exposure, such as asbestos and vinyl chloride, may result in the appearance of cancer as late as 30 years, and even then in only a very small percentage of the exposed population. Thus, commodity vinyl monomers such as ethylene, propylene, styrene, acrylonitrile, chloroprene, and vinyl bromide in addition to vinyl chloride (whose polymers have sales of over 12 million tons in 1978) are a legitimate concern. These chemicals are probably not carcinogenic in their native state, but once having entered the body, may become so after metabolic activation. Oxiranes and aldehydes, e.g., ethylene oxide, styrene oxide, and chloroacetaldehyde are potential metabolites of ethylene, styrene and vinyl chloride respectively and are current suspects as carcinogen-mutagen. In this proposal, the Louisville chemical research group proposes coordinated, multidisciplinary experiments to answer the pragmatic questions of (a) is this chemical or its metabolites toxic; (b) is this chemical or its metabolites carcinogenic; (c) where are they distributed and how soon are they discharged from the body; (d) at what level is the toxicity present; (e) at what level is the carcinogenicity identifiable; (f) how significant are the host factors, nutrition or otherwise, in the induction of chemical injury; (g) what are the organ/tissue sites of injury; (h) can it be reversed; (i) are there markers in the form of a metabolite or a cell biochemical changes to identify human exposure to the chemical; (j) what are the sensitivities and specificities of screen tests for chemically induced injuries; and (k) how can these tests be applied to a prospective clinical screening program to determine the epidemiological significance of human exposure.

Thus, experiments are designed to determine the chemical's structure-reactivity-biological activity relationship, metabolite formation in a specific tissue type, mutagenicity and carcinogenicity of the parent agent and its metabolites as revealed by rapid screens, the distribution of the chemical and its metabolites, radiolabeled, in various animal organs using autoradiography and microscopy, the effect of the parent chemical and its metabolites on cell functions and enzyme levels of various cell types with ethanol and vitamins as variables, the effect of chronic low dose exposure of these chemicals to the primary organs of metabolism or organ site of injury, liver toxicity tests based on hepatic foci, glycosaminoglycan patterns, antigenic changes and antibody response, histological and ultrastructural pathology.

We believe that this is a unifying approach directly aimed at providing human information for the benefit of the chemical industry. The experimental results will provide the needed data to assess risk to workers, suggest realistic preventive measures, prevent unnecessary regulations, and recommend effective health maintenance protocols. Our track record in the vinyl chloride study so far allows us to predict that early detection and prevention of industrial cancers are realizable goals.

VIII. Budget

First Year Budget
of the
Chemical Monomer Research Program Plan

A. Synthesis and detection of metabolites	
Personnel and fringe benefits	$26,554
Supplies and expenses	13,050
B. Organ specific metabolism of monomers	
Personnel and fringe benefits	$33,110
Supplies and expenses	19,850
C. Tissue disposition of monomers and metabolites	
Personnel and fringe benefits	$12,430
Supplies and expenses	5,500
D. Rapid screening for carcinogenicity of monomers	
Personnel and fringe benefits	$24,860
Supplies and expenses	11,500
E. Detection of changes in biological tissue due to monomers	
Personnel and fringe benefits	$37,290
Supplies and expenses	17,610
F. Clinical application	
Personnel and fringe benefits	$52,633
Supplies and expenses	10,000
Direct Costs	$264,387
Indirect Costs	$118,922
TOTAL COST	$383,309