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Toxicologic Investigation of Vinyl Chloride with Relevance to Non-worker Populations: With Emphasis on Transplacental Carcinogenesis and Co-factors

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APPENDIX F

Toxicologic Investigation of Vinyl Chloride with Relevance to Non-worker Populations: with emphasis on transplacental carcinogenesis and co-factors.

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Background

Vinyl chloride monomer (VCM) is a chemical of increasing industrial and environmental importance. VCM has many uses, e.g. the production of polyvinyl chloride (PVC) resin, as a co-polymer in saran and other plastics, as a solvent, as a propellant for pesticides and hair-sprays, as a refrigerant, and, in the past, as an anaesthetic.

Despite the apparent usefulness of this chemical, recent findings implicate VCM as the agent responsible for induction of a rare liver cancer, angiosarcoma, among workers employed in the production of PVC. Toxicological experiments with various levels of VCM performed by Maltoni and in Italy and by Biotest Laboratories, Northbrook, Illinois have demonstrated that similar rare neoplastic lesions can be induced in experimental animals.

A great need exists for data on toxicity, persistence and for the assessment of risks associated with low level concentrations of VCM, including those levels that are likely to exist beyond the fence line of the manufacturing plant. Materials loss in PVC production processes has been found to be approximately 6%, but this varies with type of process, the age of the plant, the level of technology employed and the manufacturing processes. However, there is no doubt that substantial amounts of VCM have been discharged into the environment during PVC production processes. These PVC and VCM losses occur as air emissions and as components of water effluent and solid wastes.

A recent report indicates that a woman, living downwind from a PVC plant and having no occupational exposure to this substance, developed hepatic angiosarcoma. Russian scientists have determined that exposure to VCM may be very widespread. It has been found, in a study performed in a newly constructed

building for child care, that VCM is continuously released from PVC flooring for at least two months after installation. They have recommended excessive ventilation of a newly constructed building be mandatory before occupancy. The use of vinyl chloride as a propellent in aerosol containers has raised a concern for individuals exposed in the general population who may have been exposed before removal of this propellent from the market.

It appears, therefore, that segments of the population, in addition to the industrial workers, may be at risk from exposure to VCM. The risk of such exposures to the various elements of the population must be determined, in particular the more sensitive groups such as the developing fetus and newborn, or that segment which experiences dietary deficiencies.

It has been reported that the enzymes responsible for ethanol metabolism may be involved in the metabolism of VCM (Gehring, 1974) since the effects of other chlorinated hydrocarbons, such as trichlorenthylene and chloral hydrate, which are biologically transformed by alcoholic dehydrogenase are potentiated by ethyl alcohol, it appears that comparable effects may occur with VCM. These investigations may help to determine a mechanism of action for this carcinogenic agent as well as for other related potentially carcinogenic chlorinated hydrocarbons.

There is no information available on the carcinogenic effects of VCM given together with common co-polymeric agents such as vinylidene chloride. Since both of these co-polymers have similar properties and structures, there is a need to investigate what effects this combination may have on the biological outcome.

Purpose

The purpose of this study is threefold:

a) To investigate the effects of VCM on the developing fetus through inhalation exposure in an effort to determine the risk of developing oncogenic lesions via the transplacental route and to determine the effects of inhalation exposure

to VCM on the neonate.

b) To determine whether certain dietary factors such as vitamin C deficiency or ethanol consumption may influence the biological response to VCM.

c) To develop cell culture techniques useful for the rapid screening of potential oncogenic agents, such as VCM and co-factors. These techniques will be used to provide the means for studying mechanisms of the action of similar agents and to serve as an indicator of dose-effect responses.

Scope of Work

A. Inhalation exposure of pregnant animals to levels of VCM to determine the potential transplacental oncogenic and other biological effects.

Sprague-Dawley rats will be obtained from Charles River Laboratories either timed-pregnant or they will be bred in our animal facilities. In the first experiment, twenty pregnant rats will be exposed to 20,000 ppm vinyl chloride, or as agreed upon with the contract officer, in an inhalation chamber from the 10th day of festation to the time of delivery. After birth, litters of five young will remain with each mother. Additional young will be sacrificed and cleared by the Schultz-Dawson method to visualize the skeleton for osseous changes. The mothers and their surviving young will be kept in box cages until weaning at four weeks of age. Then the mothers and their young will be fed Purina Lab Chow and maintained in rooms controlled for temperature, humidity and light cycle. The 100 surviving young will be examined for congenital malformations and then observed for signs of tumors and other diseases, particularly acro-osteolysis. X-ray examination will be done in selected cases at various times. Observations may have to be continued for one to two years.

After weaning of the first batch of young from exposed mothers, another batch of twenty pregnant females will be subjected to a similar vinyl chloride treatment. In this experiment, ten mothers with their litters of five

young each will be handled like the animals described above. Another group of ten mothers and their young will be kept in the vinyl chloride chamber until after weaning. In this way, 50 neonates will be exposed to test their susceptibility to vinyl chloride. This second batch of mothers and offspring will be treated like the first and observed for oncologic and other morbid effects.

Control mothers will be subjected to chamber treatment with only absolute filter air.

B. Interaction effects of VCM with other agents.

It is proposed that male rats will be subjected to various dietary manipulations and exposed to one or two levels of VCM (as agreed upon by the contract officer). The animals will be housed in a room with controlled lighting cycle, temperature, and humidity, and fed liquid diets ad libitum (16 hours/day). The liquid diets (General Biochemical, Chagrin Falls, Ohio) will be purchased in dry form and reconstituted as needed. The diets will consist of a control diet (without ethanol) and the same diet supplemented with 5% ethanol Appropriate controls will be used.

In addition to rats fed an ethanol supplemented diet and exposed to VCM, guinea pigs on vitamin C deficient diets and their controls will be used. The protocol involving guinea pigs will serve a dual purpose: a second species susceptibility to VCM and the co-factor response to vitamin C.

All exposures will be maintained for 12 months, 5 days/week, 4 hours/day to the VCM, or as agreed upon by the project officer. In addition, rats will be exposed to VCM and vinylidene chloride at levels agreed upon with the contract officer.

The exposure chambers will be located in a large room which has been previously used for human inhalation exposures. This room is very suitable for the proposed testing since it is sealed to insure against leakage. The main chamber has a double door entry. This chamber in a chamber arrangement will

subatmospheric pressures to insure against leakage. Air flow in the outer chamber is in a ceiling to floor direction with a complete air change every four minutes. Makeup air for the exposure chamber and negative pressure room will be prefiltered through absolute filters. Periodic sampling of both chambers using Sipin pumps and activated charcoal tubes will be made.

In addition, appropriate protection of personnel will be provided, including mandatory wearing of full face gas masks with outside air, supplied by the umbilical cord arrangement, emergency full face organic vapor canister type gas masks, and portable air supplies with rechargeable type cylinders. Periodic personnel monitoring will be made using Sipin pumps and charcoal tubes.

All VCM from the exposure chamber and the negative pressure room will be removed from the air using a fabricated activated charcoal filter. Spent activated charcoal will be removed from the filter by personnel, appropriately protected, and placed into heavy plastic bags. These bags will be incinerated.

A similar chamber for control exposure will be used.

Preliminary experiments will determine the amount of exhaled VCM from exposed animals and the time at which they can be moved to housing quarters after exposure. In the event the exhaled air concentrations are unacceptable, the animals will be housed in suitable quarters, which are isolated from all personnel.

Either the charcoal tube method or gas tight syringes will be used to collect the samples. An analytical gas chromatographic method to be used for determining VCM levels in the exposure chambers was developed by our in-house analytical department. However, a number of methods could be used for the analysis of VCM including a halide meter, long pathlength (Brooks-Moran) IR spectrophotometer or a gas chromatograph with a flame ionization detector. The GC is the method preferred. Facilities and personnel experience in analytical

procedures are available for this project.

The first year will be entirely devoted to setting up, calibration and exposing the pregnant animals from Part A. No additional animals (on various dietary regimens) will be exposed during the first year.

The second year will be devoted to inhalation exposure at one or two different levels of VCM, e.g., 50 and 500 ppm as agreed upon with the contract officer. Animals will be maintained on the proper deficiency or supplemented diets coupled with various exposure regimens as shown in the following table. Suitable control animals will also be used.

Diet		Number of Animals	VCM Exposure (ppm)
1) Vitamin C deficient	guinea pigs	40 X 2	50, 500
2) Vitamin C sufficient	guinea pigs	40 X 2	50, 500
3) Vitamin A deficient	guinea pigs	40	None
4) Vitamin C sufficient	guinea pigs	40	None
5) Control diet with 5% Ethanol	rats	40 X 2	50, 500
6) Control diet	rats	40 X 2	50, 500
7) Control diet with 5% Ethanol	rats	40	None
8) Control diet	rats	40	None
9) Control diet	rats	40	VCM + Vinylidene Cl.

During the third year animal exposures and observations will be continued, as based on previous data. The animals must be kept under observation for one to two years.

C. Tissue and blood samples from the exposed animals (part B) will be collected and transported to ETBL, MERC - Cincinnati for clinical chemistry and pathological determinations with special emphasis on electronmicroscopy.

The number of animals to be used will be determined as agreed upon by the project officer. The current figures represent ten animals from each group for the sampling. This portion of the work will begin the second year or where appropriate the first year as agreed upon with the project officer.

D. Tissue Culture Lab - Proposed for Second Year and Beyond

The malignant potential of chemical carcinogens has been demonstrated by in vitro cell culture methods. This technique should also be applicable to the vinyl chloride monomer. Three in vitro carcinogenic assay systems are in general use: 1. the hamster embryo cell system; 2. mouse cell lines; and 3. the 3T3 cell line.

This proposed work for a rapid screening system will be developed using the hamster embryo cell system without a feeder layer. Twelve to 14 day old fetuses are sacrificed and their cells grown in culture. The VCM will be added to the cultures in the gas phase in a mixture of 5% CO_2 in air. The design of the chamber and the concentrations of VCM will be determined in consultation with the contractor. It should be possible to obtain a dose response curve in this system with concentrations between 5 - 20,000 ppm VCM. The cells will be exposed for seven days, allowed to grow for an additional 8 - 10 days in control medium and then fixed and stained. Determination of cytotoxicity and examination for transformed cells is done in the same dish. Cytotoxicity is determined as a percentage of cells exposed to the agent against control.

The host mediated assay is of particular interest to this project because VCM is a gas and this is probably the route of entrance into the animal or man. This project is also interested in the effect of this agent on the embryo. In this assay the pregnant hamster or rat will be exposed to VCM in an inhalation chamber 48 hours before the fetuses are obtained from the mother and sacrificed. The fetal cells are then cultured and observed for transformation as above.

These techniques have proved quite satisfactory for the study of a

number of carcinogenic agents and may be the most satisfactory in this case, but because of the unusual nature of this carcinogen several other techniques will be used in consultation with the contractor. Endothelial cells are available for exposure to VCM as well as human fibroblastic cells. These will be used in tests similar to those outlined above. A somewhat different, but very interesting approach to this study, will be the observation of hyperplastic or premalignant changes of tissues in organ culture. These studies will be similar to those previously reported by Dirksen and Palekar on rat trachea. Rat blood vessels and small pieces of rat liver will be grown in organ culture in an exposure chamber with an atmospheric mixture of VCM, CO_2 and air or O_2. The tissue will be placed on rayon rafts and floated on medium in organ culture dishes. The tissues will be fixed at weekly intervals for five weeks and provided to the contractor for pathological examination. These changes may be similar to changes in the rats exposed to VCM in the inhalation chambers.

Periodic reports will be submitted to the project officer on the status of the experiments.

Facilities

The department possesses extensive animal quarters used for both short and long range toxicologic and metabolic studies on experimental animals. A special area was recently constructed permitting the maintenance of animals on rigidly controlled diets, including diets deficient in trace metals, etc.

The main area contains more than 10,000 square feet of animal rooms for acute and chronic experiments for most laboratory animals. Large air

the carcinogenic response of skin to various carcinogens (pure, complex mixt cocarcinogens, etc.), inhalation studies using coal tar are currently under

Five stainless steel, rectangular exposure chambers (Young and Bet Co., Cincinnati) are operational. Each has a cone shape top and bottom and capacity of 12 ft.^{3} (340L). In addition, a larger chamber (24 ft.^{3}) of th same design is available. These chambers are normally operated at airflows permit one exchange of air per minute. Each chamber can accomodate 60 rats rats capacity - large chamber) or 90 hamsters or 9 rabbits in individually h stainless steel holding cages.

After the primary air supply is drawn through an absolute filter t contaminent is added and introduced into the chamber, which permits very uni dispersion as determined by tests in the laboratory. The chambers are opera under subatmospheric pressures and the exhaust air is passed through either absolute filter, charcoal filter, or a water spray to remove the contaminant exhausting to the atmosphere.

In addition, four other types of chambers are used. One hexagonal stainless steel chamber with a capacity of 700 liters is operated in a manner similar to that described above. Four 32 liter capacity semihemispherical gl chambers are also employed. This particular type is useful for rangefinding tigation as well as for chronic exposures. Each chamber can accomodate up to 10 rats. Three chamber rooms, each 12.5 ft. long X 7.5 ft. wide X 9 ft. high with a volume of 850 ft.^{3} (24,700L), can singularly accomodate two large cage racks for 120 individually housed rats. Air is circulated to these chambers a rate of 150 ft.^{3} per minute to each chamber by means of two blowers (size C American Blower Co.) with absolute filters positioned on both the inlet and e ducts. These chambers are also kept under a slight subatmospheric pressure.

Contaminant generation equipment for inhalation chambers includes:

1) Dust generation - 7 Wright Dust Feeders (L. Adams Ltd., London, England) equipped with cyclones and 15 dust tubes. A supply of replacement parts is maintained.

2) Aerosol generating equipment including aspirating equipment such as that used for coal tar inhalation.

3) Fumes - various techniques as used for the generation of metal fumes.

4) Vapors - generated by modified Greenberg-Smith Impingers.

5) Gaseous exposure using various regulator valves and orifaces.

All chambers have indicating monometers (Magnehelic) to measure subatmospheric pressures. Air flow is monitored with either stainless steel orifaces or rotometers.

Other air flow calibration and monitoring equipment includes: 9 Rockwell dry test meters (175 $ft.^3$/hr. capacity); one calibrated meter 140 $ft.^3$ test meters; one wet test meter (3 liters/revolution) for calibration of low flow rate rotometers.

Routine sampling of the contaminant, etc. is performed hourly. Sampling equipment routinely used includes: 12 MSA midget bubblers, Greenberg-Smith Impingers with fritted disc; 7 Anderson cascade Impactors for particle size distribution; and various filter holder samplers for gravimetric and/or analytical procedures. In addition the industrial hygiene and analytical departments have a fairly complete and varied line of supplementary sampling equipment that is readily available. Thirteen gast pumps (S-10311, 4-0522, 1-0822) of various air flow capabilities and many other standby pumps are used routinely.

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