THE UNIVERSITY OF NORTH CAROLINA
AT
CHAPEL HILL

The School of Public Health
Department of
Environmental Sciences and Engineering

The University of North Carolina at Chapel Hill
CB# 7400, Rosenau Hall
Chapel Hill, N.C. 27599-7400

June 6, 1995

Have Bill review this logistically feasible?

Dr. Hasmukh C. Shah
Manager, Vinyl Chloride Panel
Chemical Manufacturers Association
2501 M Street, NW
Washington, D.C. 20037

Dear Dr. Shah:

Dr. Jim Knaak asked me to write to you with a proposal for additional studies on vinyl chloride research that I would be interested in conducting in collaboration with the CMA. I FAXed Jim a copy of this and he will bring it to your meeting this week. I am sending you a copy by FAX and by Federal Express, so that you have a hard copy for the meeting. If you have any questions, please give me a call. Thank you for your continued interest in our research.

Sincerely,

James A. Swenberg, D.V.M., Ph.D.
Director, Curriculum in Toxicology
Professor, Environmental Sciences and
Engineering, and Pathology

R&S151299

PROPOSED STUDIES ON VINYL CHLORIDE

James A. Swenberg, D.V.M., Ph.D.

University of North Carolina

June 5, 1995

Listed below is a series of studies on the formation and repair of DNA adducts induced by vinyl chloride (VC) that will provide molecular dosimetry information relevant to risk assessment. These studies would be conducted in a manner that will complement the two generation reproductive and developmental toxicity studies currently being planned.

Dose-response Studies

Presently, we have data showing that preweanling rats are more susceptible than adults to VC carcinogenesis, that preweanlings develop 3-fold greater numbers of VC DNA adducts, and that preweanlings have 3-fold higher expression of CYP 2E. The adduct and carcinogenesis data are all from "high" exposures of ~500 ppm. No information exists on lower exposures. By adding additional animals to the reproductive and developmental toxicity studies, we could assess the effect of exposure concentration on the molecular dose of ethenoguanine, the primary promutagenic DNA adduct of VC in adult and preweanling rats. Experimental design: 5 adult + 5 preweanling rats per dose (4 doses, including controls). The projected cost is $10,000.

Concentration x Time Studies

No information on C x T and VC molecular dosimetry are available. Such information would assist in extrapolating risks for brief exposure from carcinogenesis data. While I do not know what exposure concentrations will be used in the reproductive and developmental toxicity studies, several C x T groups could be constructed to examine this issue. For example, 1000 ppm x 1 hour, versus 500 ppm x 2 hours, versus 250 ppm x 4 hours, versus 100 ppm x 10 hours. Again, 5 adults and 5 preweanling rats per group would provide a reasonable number of samples. It would also be possible to compare naive versus pre-exposed rats. Project cost would be $10,000-20,000.

Molecular Dosimetry Studies in Nonparenchymal Cells (NPC) versus Hepatocytes

Understanding why VC targets the endothelial cell for its carcinogenic effect is critical to proper risk assessment. We have preliminary data on ethenoguanine adducts in vinyl fluoride-exposed rats (2500 ppm, 6 hr/day, 5 days/wk, 4 wks) that shows that the number of adducts is ~10-fold higher in NPC than hepatocytes. It is of great interest to determine if important differences exist between adults and preweanling rats, and between high and low exposures. I would propose using a one week exposure to the four exposure groups investigated above under dose-response in adult and preweanling rats. DNA adducts would be measured at the end of the exposure and 3, 7 and 14 days post exposure (4 doses, 2 ages, 4 times, 3 tissue/cells = 96 samples). In addition, the expression of the major DNA repair pathway, N-methylpurine-DNA glycosylase (MPG) would be examined in NPC and hepatocytes from these animals. The cost of this study would be $40,000.

Studies Using [$^{13}C_2$]-VC

When I met with the CMA Vinyl Chloride Panel, I requested that you consider having [$^{13}C_2$]-VC synthesized so that studies on the formation and repair of VC-induced DNA adducts could be studied relative to those adducts formed endogenously. I believe that these studies will critically impact on any risk assessment on accidental exposures. There are many studies that could be done.

First, the number of adducts formed by [$^{13}C_2$]-VC needs to be determined at several exposure concentrations to identify the molecular dose of induced versus endogenous adducts. I propose that closed chamber exposures to 500, 250, 100, 50, 25, 10, 5, 2.5, 1 and 0 ppm (5 rats, 6 hrs) be used. The cost of this portion is $35,000 and CMA supplies the [$^{13}C_2$]-VC.

Next, I would examine the rate of repair of [$^{13}C_2$]-VC induced DNA adducts in a high, mid and low exposure group (3 concentrations x 5 rats x 4 times). These same animals would be housed in metabolism cages during the post exposure holding periods so that urine could be collected. The urine would be analyzed for endogenous and induced DNA adducts that had been repaired by the MPG pathway. In addition, it may be possible to identify and quantitate [$^{13}C_2$]-VC metabolites in the urine using NMR. Drs. Fennell and Sumner at CIIT would be possible collaborators on this. The cost of this would be $40,000.

HPRT Mutations in VC-exposed Mice versus VC Workers

As part of our Superfund grant we are determining the HPRT mutation frequency and mutational spectra in workers in the vinyl chloride plants of France. The cohort contains over 400 workers, some of who were exposed to high concentrations of VC prior to 1974. Of these workers, some have markedly elevated numbers of mutations, while others have no increase. We are determining the molecular nature of these mutations and examining possible factors involved in differences in inter-individual variability including MPG expression and GST and CYP 2E1 genotyping. We would like to be able to compare the human data with an *in vivo* animal model. To this extent, we would like to have B6C3F1 mice exposed to the highest concentration of VC (500 - 1000 ppm) that you are using in the reproductive and developmental toxicity studies for ~8 weeks. We could go with as little as 4 weeks, but feel that the longer time would increase the number of mutants available for molecular analysis. The test animal would be 3 weeks old at the beginning of exposure. A total of 48 exposed and 24 control mice would be used. The mice would be shipped to UNC in groups of 12 exposed and 6 controls at 2, 4, 6 and 8 weeks post exposure, where we would isolate the HPRT mutants and conduct the molecular analyses of the type of mutations that were present. We have comparable data on human cells in culture exposed to VC, chloroethylene oxide (CEO) and chloroacetaldehyde. The cost of the HPRT analyses for this experiment would be $27,000.

Studies on VC-induced DNA Adducts in Human Endothelial Cells

Human endothelial cells are now commercially available. These cells could be used to compare the formation and repair of VC DNA adducts in human versus rat endothelial cells. We would expose endothelial cells to CEO or VC and determine the number of ethenoguanine, ethenocytidine and ethenoadenine DNA adducts at various times after exposure. In addition, the expression of MPG would be quantified. One reason that this experiment is important is that human angiosarcomas arising in VC workers have shown p53 mutations at AT base pairs, whereas, ethenoadenine adducts appear to be rapidly repaired in rodent liver, but ethenoguanine adducts accumulate. Therefore, we would expect that most mutations would occur at GC base pairs. By

comparing both the molecular dose of DNA adducts and the expression of MPG in human versus rodent cells, we will better understand the cause of this difference and be able to select the most appropriate biomarker for humans. The cost of this research would be $13,000.

Budget

The total budget for these studies is $175,000 -185,000. In addition, the CMA would need to cover the costs of animals, exposures and the synthesis of [$^{13}C_2$]-VC. The studies would be conducted over a two year time frame and could begin as soon as the project is funded. The research can be provided as a gift, with no overhead, or as a grant, with 44.5% overhead. If you select the latter, I will need to submit a formal proposal once we have finalized the research program.