CMA

CHEMICAL MANUFACTURERS ASSOCIATION

Vinyl Chloride Panel

Health Committee
FAX
May 8, 1996

TO:	Jim Barter	PPG Industries	412/434-2137
	Ed Beeler	GEON	216/930-3034
	John Gamble	Westlake	713/963-1540
	Mark Gruenwald	Borden	614/225-7638
	James Knaak	Occidental	716/286-3141
	Caffey Norman	Patton Boggs	202/457-6315
	Dave Penney	Vista	713/588-3478
	Dave Pun	Formosa	201/716-7283
	Jonathan Ramlow	Dow	517/636-1875
	Jay Strange	Georgia Gulf	504/687-0294

FROM: Has Shah CMA

RE: Conference Call

MESSAGE

Please review the enclosed information in preparation for the Vinyl Chloride Health Committee conference call, scheduled for May 9, 1996, at 10:00 a.m. EDT. Please call me at 703/741-5637 if you have any questions. I look forward to talking with you then.

SENDER: Elena Howell

PHONE: 703/741-5638

PAGES: 11 (including coversheet)

1300 Wilson Boulevard, Arlington, VA 22209 • Telephone 703-741-5000 • Fax 703-741-6000

URGENT FAX

RUSH TO: Dr. Robert Venezia, Chem Manu Assoc

FAX: 703 741-6091

FROM: RHR Toxicology Consulting

PAGES (INCLUDING THIS COVER): 3

May 7, 1996

Dear Dr. Venezia,

Here is a copy of the EMAIL which Ie submitted to IRIS in connection with the project which Mike Gargas and I have proposed to carry out for CMA's VC panel.

I apologize for submitting material prematurely — I did NOT want to miss the submission deadline and took the liberty of preparing a list for IRIS. I understand from Mike that CMA had planned to ask for an extension of the submission deadline. Sorry about that!

None of the materials have been submitted to IRIS; just the list as requested in the Federal Register Notice. Hope this has not caused you any inconvenience, and we look forward to your response to our proposal.

Dick Reitz

IRIS SUBMISSION --- to IRIS.comments@epamail.epa.gov, on May 2, 1996
RESPONSE TO REQUEST FOR SUPPLEMENTAL INFORMATION (VINYL CHLORIDE)
Federal Register Notice: Vol. 61, No. 64, April 2, 1996 (pages 14570-14571)

Gentlemen,

We believe you may find the following documents/items useful during your re-evaluation of the carcinogenic potency of vinyl chloride (CAS 75-01-4).

(1). Reitz, R.H., Gargas, M.L., Andersen, M.E., Provan, W.M., and Green, T.L. (1996). Predicting cancer risk from Vinyl Chloride exposure with a Physiologically Based Pharmacokinetic model. Toxicol. Appl. Pharmacol. 137, 253-267.

(2). VCDOSE2.CSL --- The extensively commented source code for a Physiologically Based Pharmacokinetic model capable of describing the uptake, distribution, and conv rsion of vinyl chloride to its reactive and genotoxic metabolites. This source code may be compiled to an executable model with the software packages ACSL-PC and/or SimuSolv.

(3) VCDOSE2.CMD --- The associated parameter definitions and procedures used simulat different species and exposure conditions with the PBPK model described above.

(4) Baretta, E.D., Stewart, R.D., and Mutchler, J.E. (1969) Monitoring exposures to vinyl chloride vapor: Breath analysis and continuous air sampling. Amer. Indust. Hygi n Assoc. Journal, 30, 537- 544.

(5) Buchter, A., Bolt, H.M., Filser, J., Goergens, H.W., Laib, R.J., and Bolt, W. (1978). Pharmakokinetik and karzinogenese von vinylchlorid abreitmedizinische risikobeurteilung.Verh. Dtsch. Ges. Arbeitsmed., 18, 111-124 <ENGLISH TRANSLATION>.

(6) Creech, J.L., & M.N. Johnson (1974), Angiocarcoma of the liver in the manufactur of PVC.J. Occup. Med., 16, 150-151.

(7) Drew, R.T., G.A. Boorman, J.K. Haseman, E.E. McConnell, W.M. Busey, & J.A. Moore (1983) The effect of age and exposure duration on cancer induction by a known carcinogen in rats, mice, and hamsters. Toxicol. Appl. Pharmacol., 68, 120-130.

(8) ECETOC (1988), Technical Report No. 31 "The mutagenicity and carcinogenicity of vinyl chloride: A historical review and assessment." ISSN 0773-8072-31, Brussels, Belgium.

(9) Gargas, M.L., Andersen, M.E. and Clewell, H.J. III. (1986). A physiologically-based simulation approach for determining metabolic constants from gas uptake data. Toxicol. Appl. Pharmacol. 86, 341-352.

(10) Gargas, M.L., Burgess, R.J., Voisard, D.E., Cason, G.H., and Andersen, M.E. (1989) Partition coefficients of low molecular weight volatile chemicals in various liquids and tissues. Toxicol. Appl. Pharamcol., 98, 87-99.

(11) Gehring, P.J., P.G. Watanabe, & C.N. Park (1978) Resolution of dose-response toxicity data for chemicals requiring metabolic activation. Toxicol. Appl. Pharmacool., 44, 581-591.

(12) Guengerich, F.P. & P.G. Watanabe (1979) Metabolism of (14C) and (36Cl)-labeled vinyl chloride in vivo and in vitro. Biochem. Pharmacol, 28, 589-596.

(13) Guengerich, F.P., Kim, D.H., and Iwasaki, M. (1991) Role of human cytochrome P-450 IIE1 in the oxidation of many low molecular weight cancer suspects. Chem. Res. Toxicol., 4, 168-179.

(14) Kappus, H., H.M. Bolt, A. Buchter, & W. Bolt (1976) Liver microsomal uptake of 14C-VC [illegible]

(15) R.L., and Woods, J.S. (1978) Carcinogenicity of vinyl chloride and vinylidene chloride. J. Toxicol. Envir. Hlth., 4, 15-26.

(16) Maltoni, C. (1974). "Vinyl Chloride Carcinogenicity: An Experimental Model for Carcinogenesis Studies.", monograph from the Institute of Oncology and Tumour Center, Bologna, Italy 40138.

(16) Maltoni, C., C. Lefemine, P. Chieco, & D. Carrettu (1974), Vinyl chloride carcinogenesis: Current results and perspectives. Med. Lav., 65, 421.

(17) Raucy, J.L., J.C. Kraner, & J.M. Lasker (1993) Bioactivation of halogenated hydrocarbons by Cytochrome P4502E1. Critical Reviews in Toxicology, 23, 1-20.

(18) Reitz, R.H., Mendrala, A.L. and Guengerich, F.P. (1989). In Vitro Metabolism of Methylene Chloride in Human and Animal Tissues: Use in Physiologically-Based Pharmacokinetic Models. Toxicol. Appl. Pharmacol. 97, 230-246.

(19) Simonato, L., L'Abbe, K.A., Andersen, A., Belli, S., Comba, P., Engholm, G., Ferro, G., Hagmar, L., Langard, S., Lundberg, I., Pérastu, R., Thomas, P., Winkelmann, R., and Saracci, R. (1991) A collaborative study of cancer incidence and mortality among vinyl chloride workers. Scand. J. Work Environ. Health, 17, 159-169.

(20) Viola, P.L. (1970), Pathology of vinyl chloride. Med. Lav., 61, 174.

(21) Viola, P.L., A. Bigotti, and A. Caputo (1971) Oncogenic response of rat skin, lungs, and bones to vinyl chloride. Cancer Res., 31, 516-522.

(22) Watanabe, P.G., G.R. McGowan, E.O. Madrid, & P.J. Gehring (1976) Fate of 14C-vinyl chloride following inhalation exposure in rats. Toxicol. Appl. Pharmacool., 37, 49-59.

LABORATORY OF MOLECULAR CARCINOGENESIS AND MUTAGENESIS
THE UNIVERSITY OF NORTH CAROLINA AT CHAPEL HILL
CAMPUS BOX 7400, ROSENAU HALL
SOUTH COLUMBIA STREET
CHAPEL HILL, NC 27599
PHONE 919-966-6142
FAX 919-966-6123

FACSIMILE TRANSMISSION

To:	Bob Venezia
Address:	CMA
FAX number:	703-741-6091
From:	James A. Swenberg, D.V.M., Ph.D.
Date:	May 7, 1996

Bob:

Here is the research proposal/protocol. I made a few changes from our discussions in New Jersey. I sent a copy to Jim Knaak for his comments. He said that the issue of dynamic versus static exposures will be determined in the future. Dr. Rudy Jager has some dynamic exposure systems that will use even less than the 27 gm. As I do not have access to the needed information, I cannot go any farther today. I hope that this is what you need. If you have any questions, please give me a call tomarrow.

PROTOCOL FOR MOLECULAR DOSIMETRY STUDIES ON VINYL CHLORIDE

James A. Swenberg, D.V.M., Ph.D.
University of North Carolina
May 7, 1996

Listed below is a series of studies on the formation and repair of DNA adducts induced by vinyl chloride (VC) that will provide a vastly improved understanding of the mechanisms of vinyl chloride carcinogenesis. These studies will provide information relevant to cancer risk assessments of vinyl chloride. The studies would be conducted in a manner that will complement the two generation reproductive and developmental toxicity studies that will be conducted at Huntingdon Life Sciences Laboratory.

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 2E1. The adduct and carcinogenesis data are all from "high" exposures of ~500 ppm. No information exists on lower exposures. In addition, we have clearly demonstrated that DNA adducts identical to those formed by VC are formed endogenously in unexposed rats, mice and humans. We have developed ultrasensitive and highly specific assays for the DNA adducts of VC. These include immunoaffinity/^{32}P-postlableing methods for 1,N^6-ethenodeoxyadenosine (EdA) and 3,N^4-ethenodeoxycytidine (EdC), and GC/MS methods for N^2,3-ethenoguanine (EG). By adding animals to the reproductive and developmental toxicity studies, we will be able to assess the effect of exposure concentration on 1) the molecular dose of etheno adducts, the primary promutagenic DNA adducts of VC; 2) determine if there are differences in the dose-response between adult and weanling rats; 3) examine the major DNA repair pathways for VC DNA adducts; and 4) characterize the utility of using DNA adducts excreted in the urine as a biomarker of exposure.

Molecular Dosimetry Studies in Nonparenchymal Cells (NPC) versus Hepatocytes

Understanding why VC targets the endothelial cell for its carcinogenic effect is critical for proper risk assessment. We have preliminary data on EG in vinyl fluoride-exposed rats (2500 ppm, 6 hr/day, 5 days/wk, 4 wks) which shows that the number of adducts is ~2.5-fold higher in NPC than hepatocytes, even though metabolism of VF to its electrophile is thought to only take place in hepatocytes. Since the NPC are exposed by diffusion of VC or VF electrophiles from neighboring hepatocytes and therefore have lower exposures, this suggested that NPC were deficient in DNA repair activity for EG. To test this hypothesis, we have developed a RT-PCR method for quantitating methylpurine-DNA glycosylase (MPG) in tissues and cells. Preliminary data have shown that NPC have <20% of the MPG activity present in hepatocytes. In addition, we have shown that NPC have 5-fold greater numbers of abasic sites in their DNA. Abasic sites are formed when MPG excises a damaged base, such as EG, EA and EC. These data suggest that NPC are deficient in a second step in the DNA repair of VC adducts. We think that the most likely candidate is a deficiency in AP endonuclease. The difference between hepatocyte and NPC abasic sites was seen in rats exposed to VF, but not in controls. The combination of two defects in DNA repair in the target cells for VC carcinogenesis provides a strong scientific basis for the induction of hemangiosarcomas by this important chemical. In view of the fact that the increase in abasic sites was only seen in highly exposed rats shows the need to understand the dose response relationship for this endpoint. By conducting 4 week exposures to 0, 10, 100, and 1000 ppm VC and measuring etheno adducts, MPG expression and abasic sites, we will be able to determine if nonlinearities exist in the molecular dose of VC, in MPG expression, and in the number of abasic sites present in the DNA. Our hypothesis is that EG will show a supralinear dose response; that MPG will be similar in exposed and unexposed hepatocytes, but lower in both exposed and unexposed NPC; but that abasic sites will exhibit a sublinear dose response characteristic of saturation of DNA repair in NPC, but not hepatocytes.

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

Our data showing endogenous formation of etheno DNA adducts that are identical to those induced by VC have important implications. We had previously published a paper that concluded that the DNA adducts of VC were highly persistent. We now believe that this was not correct, in that what we were calling persistent DNA adducts were actually the endogenously formed adducts at steady-state. By utilizing [$^{13}C_2$]-VC, the formation and repair of VC-induced DNA adducts can be studied relative to those adducts formed endogenously. I believe that these studies will critically impact on any risk assessment dealing with low exposures, such as might be associated with accidental releases that reach the fenceline.

We will examine the amount of [$^{13}C_2$]-VC induced EG in rats exposed to 10, 100, or 1000 ppm for 5 days (6 hrs/day). These same animals will be housed in metabolism cages for 3 days prior to exposure and during each day's post exposure holding period (days 1-4) so that urine can be collected. The urine will be analyzed for endogenous and induced DNA adducts that have been repaired by the MPG pathway using GC/MS. We will also develop immunoaffinity-LC/MS methods for all three etheno bases. In addition, it may be possible to identify and quantitate [$^{13}C_2$]-VC metabolites in the urine using NMR. A second set of [$^{13}C_2$]-VC exposures is contemplated that would consist of single exposures. These will be designed after we get data from the above study.

Significance

We have developed highly sensitive and specific assays for the promutagenic DNA adducts of vinyl chloride over the past 10 years. By exposing additional animals in the Huntingdon study, we can use these methods to answer several questions related to improved risk assessment of VC. First, the molecular dosimetry data will be the only information of its type that addresses dose-response. All other data have come from high exposures of 500-600 ppm. Second, we will address the issue of differences between weanling and adult animals. While we already know that preweanling rats are more sensitive than adults to the formation of DNA adducts and carcinogenesis at high concentrations, it is not known if this is true for low exposures. Since the only VC exposures that children encounter would be extremely low, the 10 ppm data are of particular importance. Just because preweanling rats exposed to concentrations that saturate metabolism develop more adducts does not necessarily mean that young animals exposed to low concentrations will also develop more DNA adducts than adults. Our recent development of an assay for MPG and the finding that NPC are deficient in this DNA repair pathway provides the first mechanistic data that explains why VC causes hemangiosarcomas. The additional finding of greatly increased numbers of abasic sites in the NPC DNA further highlights the importance of cell-specific differences in DNA repair. The experiments outlined above will provide compelling data on the importance of DNA repair in VC carcinogenesis. Finally, the use of $[^{13}C_2]$-VC will allow us to differentiate between induced and endogenous DNA adduct formation. If large numbers of induced EG are formed, they may lead to increased numbers of endogenous adducts due to competition for DNA repair. On the other hand, we will be able to determine the extent of increased DNA damage relative to endogenous adducts for all three exposure groups. For example, we might find that 10 ppm VC for 5 days only doubles the amount of EG present endogenously. Such a finding should have great impact on low dose risk assessments. We would then look at various single exposures to simulate fenceline exposure scenarios to determine if there was any detectable increase in risk. Similar studies could be used to mimic occupational or environmental exposures to VC.

Protocols

Study I. Adult Rats Exposed to Vinyl Chloride for 1 Week

Route: Whole body exposure conducted during the two generation animal portion of the main study

Exposure groups: 0, 10, 100, or 1000 ppm

No. of rats/group: 32

Total: 128

Exposure duration: 1 week (6 hr/day, 5 days)

Species/strain: CD Rat

Age: Approximately 12 weeks of age

Sex: Males

Sacrifice: 8 rats/ group at end of last exposure (non-perfused), and 8 rats/ group at end of last exposure and at 3 and 7 days post-exposure (perfused)

Urine collections: during Days 1-4 of exposure (collected during nonexposure times)

Study II. Weanling Rats Exposed to Vinyl Chloride for 1 Week

Route: Whole body exposure conducted during the two generation animal portion of the main study

Exposure groups: 0, 10, 100, or 1000 ppm

No. of rats/group: 32

Total: 128

Exposure duration: 1 week (6 hr/day, 5 days/week)

Species/strain: CD Rat

Age: Approximately 21 days of age

Sex: Males

Sacrifice: 8 rats/ group at end of last exposure (non-perfused), and 8 rats/ group at end of last exposure and at 3 and 7 days post-exposure (perfused)

Urine collections: during Days 1-4 of exposure (collected during nonexposure times)

Study III. Adult Rats Exposed to Vinyl Chloride for 4 Weeks

Route: Whole body exposure conducted during the two generation animal portion of the main study

Exposure groups: 0, 10, 100, or 1000 ppm

No. of rats/group: 32

Total: 128

Exposure duration: 4 weeks (6 hr/day, 5 days/week)

Species/strain: CD Rat

Age: Approximately 12 weeks of age

Sex: Males

Sacrifice: 8 rats/ group at end of last exposure (non-perfused), and 8 rats/ group at end of last exposure and at 3 and 7 days post-exposure (perfused)

Urine collections: during last week of exposure (collected during nonexposure times)

Study IV. Adult Rats Exposed to [$^{13}C_2$]-Vinyl Chloride for 1 Week

Route: Nose only exposure

Exposure groups: 10, 100, or 1000 ppm

No. of rats/group: 8

Total: 24

Exposure duration: 5 days (6 hr/day)

Species/strain: CD Rat

Age: Approximately 12 weeks of age

Sex: Males

Sacrifice: 8 rats/ group at end of last exposure (perfused)

Urine collections: 3 days prior to exposure and Days 1-4 during exposure (collected during nonexposure times)

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