BEFORE THE
UNITED STATES DEPARTMENT OF HEALTH AND HUMAN SERVICES
AGENCY FOR TOXIC SUBSTANCES
AND DISEASE REGISTRY
AND
THE U.S. ENVIRONMENTAL PROTECTION AGENCY

CHEMICAL MANUFACTURERS ASSOCIATION VINYLIDENE CHLORIDE PROGRAM PANEL COMMENTS ON DRAFT TOXICOLOGICAL PROFILE FOR 1,1-DICHLOROETHENE

Availability of Toxicological Profiles 53 Fed. Reg. 51192 (December 20, 1988)	Docket No. ATSDR - 7 FRL-88-29021

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The Chemical Manufacturers Association ("CMA") Vinylidene Chloride Program Panel ("VDC Panel" or "Panel") submits these comments and responds to the request for comments of the Agency for Toxic Substances and Disease Registry ("ATSDR") regarding the Draft Toxicological Profil for 1,1-Dichloroethene ("Draft") (53 Fed. Reg. 51192 (December 20, 1988)). The VDC Panel is an industry group organized as a special program of CMA, representing all United States manufacturers of 1,1-dichloroethene ("vinylidene chloride" or "VDC") and a substantial portion of the industry, both domestic and foreign, which converts VDC into other products.

Executive Summary

The Panel's comments are divided into two sections -- general comments on the approach taken in the Draft and specific comments on statements made regarding VDC in the Draft.

The Panel's principal concerns are as follows:

- Exposure to VDC is extremely rare and, when it exists, is at very low levels. The material is carefully controlled because of various non-cancer health effects, but to a level equivalent to the controls placed on vinyl chloride, a known carcinogen. The Draft should assess all toxicology endpoints with a view toward determining whether additional studies will truly lead to health effects information on which further regulatory controls might be based. If not, the need for additional information is highly questionable.
- The various data needs evaluations should be revised to reflect a careful scientific judgment as to whether additional studies are necessary to determine whether the substance poses a significant risk to health. Such a judgment is not presented in the Draft.

- o The large body of carcinogenicity and pharmacokinetic data should be fairly characterized as demonstrating that VDC is not likely to pose a significant carcinogenicity risk. In view of the large number of studies, additional research cannot be said to be necessary.
- o Suggestions of developmental effects are misleading unless they clearly state that all such effects in animal studies have been associated with maternal toxicity.

The comments also contain a number of additional comments on individual statements in the Draft.

A. General Comments

The Panel incorporates the general comments submitted by CMA on the second set of toxicological profiles. Comments of the Chemical Manufacturers Association on Generic Issues Raised by the Second 25 Toxicological Profiles, dated May 12, 1989.

The Panel is particularly concerned that the Draft fails to reflect an adequate evaluation of testing needs for VDC. Any evaluation of testing needs must begin with a consideration of the purpose of the tox profiles, as set

forth in 42 U.S.C. § 9604(i), and as repeated in the guidelines developed by ATSDR. The law provides for the preparation of tox profiles which contain the following:

- First, the profile shall include an examination, summary and interpretation of available toxicological information and epidemiologic evaluations "in order to ascertain the levels of significant human exposure for the substance and the associated acute, subacute, and chronic health effects."
- Second, the tox profile shall determine whether adequate information exists or is in the process of development "to determine levels of exposure which present a significant risk to human health of acute, subacute, and chronic health effects."
- Third, the tox profile shall, where appropriate, include "an identification of toxicological testing needed to identify the types or levels of exposure that may present significant risk of adverse health effects in humans." (42 U.S.C. § 9604(i)(3)).

ATSDR and EPA have promulgated Guidelines for Development of Toxicological Profiles. (52 Fed. Reg. 12870 (April 17, 1987)). The Guidelines provide that "the primary

focus of the toxicological profiles should be on the data most relevant for evaluating levels of significant human exposure and the acute, subacute, and chronic health effects of the subject hazardous substances (i.e., each profile will identify the quantity of a substance which represents a level of potential exposure that would constitute a public health concern based on available data)." (52 Fed. Reg. at 12871-72). The guidelines also state that "[t]he toxicological profiles also must focus on important data needs that preclude the determination of significant levels of human exposure or contribute substantially to the uncertainty of such levels." (52 Fed. Reg. at 12872).

The data needs evaluation is thus repeatedly tied into the need to identify <u>significant</u> exposure and <u>significant</u> risks. An evaluation that merely addresses what testing may be desirable or beneficial has little bearing on the issue before ATSDR. That issue, the Panel submits, is whether additional testing is needed (i.e., necessary) to determine significant exposure and significant health risks.

The Panel notes that Section 2.9 of the Draft, which discusses the requirements of Section 104(i)(5) of CERCLA (42 U.S.C. § 9604(i)(5)), describes ATSDR's role as simply assessing whether adequate information on the health

effects of the substance is available. The Draft fails to refer to Section 104(i)(3), which indicates the factors to be considered in evaluating what is "adequate" and what is "inadequate." As quoted above, the law defines the adequacy of data in terms of the need for additional data to assess levels of "significant exposure" which present a "significant risk to human health." The Panel believes that this failure to focus on the Congressionally mandated criteria whereby the adequacy of the database is to be determined has made it difficult for the Draft to give more than general conclusions regarding additional data that may be useful. Such conclusions fail to fulfill the function intended for the tox profiles. Each indication of a data need should be accompanied by a reasoned evaluation of precisely what data are truly needed to determine significant risk to human health.

Without a determination by ATSDR and EPA that additional data are necessary to determine whether levels of human exposure are significant and whether exposure presents a significant risk to human health, the data needs recommendations contained in the Draft will not fulfill the purpose set forth in CERCLA.

The failure to evaluate the significance of the so-called data gaps is particularly important for VDC, for

B. Specific Comments

CMA's specific comments are organized by health effect. The comments address carcinogenicity, the extent of exposure, developmental effects, reproductive effects, neurologic effects, and miscellaneous issues.

1. Carcinogenicity

a. The Draft does not adequately characterize the current data on carcinogenicity

The Draft discusses carcinogenicity data in several places and repeatedly fails to present a full and accurate picture of the existing database. The Panel submits that the existing carcinogenicity data are sufficient to determine whether VDC exposure presents significant risks.

It is extremely important that the general discussion of VDC carcinogenicity contained at various points in the Public Health Statement be complete and accurate. Statements which, by reason of omissions, may mislead the public should be amended to include at least a summary of the information being omitted so that the statements given are not misleading. For example, on page 3, the Draft contains the statement "[a]n increased risk for cancer has been demonstrated in animals exposed to DCE." Read by itself, this statement does not present a

which there is already a large volume of data and to which exposure is already extensively controlled. Part B of these comments discusses the available data. It is also important, and should be noted in the tox profile, that OSHA has recently amended the Z-Table regulation to lower the permissible exposure limit to VDC in the workplace to 1 ppm. (54 Fed. Reg. 2332, 2566-67 (Jan. 19, 1989)). This exposure level was not based upon carcinogenicity data, but rather upon other toxicological data. However, a safety factor was added to the evaluation on the basis of the one positive carcinogenicity bioassay reported in the Draft (see discussion below). As a result, VDC exposure in the workplace is restricted to the same level as exposure to vinyl chloride monomer, a known human carcinogen.

Because of the extensive limitations on VDC exposure levels, it is unlikely that existing exposure levels can be considered significant, regardless of the findings of additional research. It is extremely important that in evaluating the need for additional data, ATSDR consider the extent to which existing regulatory programs already restrict exposure to levels below what can be considered significant.

fair depiction of the large database which fails to demonstrate carcinogenicity in animals. The statement is misleading to the extent that it does not make clear to the reader that an increased risk for cancer has been demonstrated only in a single sex of a single strain of a single species of laboratory animal (the male Swiss mouse) or that that one study has been criticized.

The reference on page 19 to a cancer effect level ("CEL"), standing alone, fails to reveal the many inadequacies in the study on which it is based, and also fails to point out the many studies which did not produce evidence of carcinogenicity. The Panel submits that it is inappropriate to calculate a cancer effect level on the basis of the scant and flawed data that have been reported as positive.

The discussions in Sections 2.2.1.8 (cancer risk due to inhalation exposure), 2.2.2.8 (cancer risk due to oral exposure), 2.2.3.8 (cancer risk due to dermal exposure), 2.3 (relevance to public health) and 2.9 (adequacy of the database) contain a number of errors and should be revised.

Several of the introductory summary statements are shown later to be in error. For example, the statement at page 32 that "the results of studies by Maltoni et al.

(1985) in mice in rats and mice [sic] have provided some evidence of a carcinogenic effect associated with DCE exposure" is inaccurate. As the Draft later notes, the Maltoni study produced positive results only in mice. While Maltoni himself claimed to have found positive results in Sprague-Dawley rats, that conclusion has not been accepted by EPA and cannot be verified by Maltoni's published results. The Draft specifically points out the flaws which limit the usefulness of the Maltoni rat study (at p. 33). In view of those concerns, it is inappropriate to refer to the study as a positive study.

The reference at page 34 to the EPA q_1^* of 1.2 $(mg/kg/day)^{-1}$ for cancer risk, and the plotting of that value in Figure 2-1 is misleading. The q_1^* value is presented in such a way that it will be misunderstood

·ause of the need to convert inhalation dose to equivalent internal dose before inhalation risk upper bound values can be calculated.

Moreover, in view of the weaknesses of the Maltoni study and the contradictory data produced in other studies, it is inappropriate to suggest that a valid cancer risk level can be determined from those data. In fact, it should be pointed out that EPA has not regulated VDC as a probable carcinogen, but has instead regulated it in drinking water

on the basis of non-carcinogenicity data. Similarly, OSHA has not regulated VDC on the basis of carcinogenicity data, although the levels to which it is regulated are comparable to the levels for carcinogenicity data on the basis of multiple safety factors.

The Draft summarizes the available inhalation long-term chronic or oncogenicity bioassays on VDC and suggests that "[t]he negative or inconclusive findings of various inhalation studies may be partially explained by inadequate test conditions (EPA 1985a)." (Draft at p. 34). Similar objections are raised to the oral studies (p. 46) and are repeated in the over-all data needs assessment (p. 73).

The Panel believes that it is extremely ambiguous to state (p. 45) that "a trend toward increased incidence of malignant and nonmalignant tumors . . . has been reported in several studies." As pointed out in the Draft, the incidence of these tumors was not statistically significant. These are not positive studies, and therefore to suggest "a trend" without statistical significance is potentially misleading.

It is noteworthy that the limitations in the Maltoni study which are observed in Section 2.2.1.8 of the Draft are not repeated in the earlier Public Health

Statement. The Panel submits that the failure to point out these limitations, and the failure to point out the many studies which were negative, renders the statements made in the Public Health Statement misleading.

The summary of carcinogenicity data at page 56 accurately lists the large number of studies following inhalation, oral, dermal, and subcutaneous exposure routes and accurately notes that only one study (Maltoni et al. (1985)) provided evidence of a positive carcinogenic effect from DCE exposure. The Draft is inaccurate in indicating that the Maltoni study provided "clear evidence" (p. 56), in view of the many difficulties and problems with that study. Indeed, EPA's characterization of the data was as "limited evidence." (51 Fed. Reg. 28840, 28841 (August 12, 1986)). The Draft, we believe, inaccurately states that the results of all other carcinogenicity studies with laboratory animals have been "inconclusive." The Panel believes that, considering the large number of studies and the various routes of exposure, the carcinogenicity data for VDC is not inconclusive, but rather adequately demonstrates that VDC does not pose a carcinogenic risk to humans.

The Draft states that the initiation-promotion study by Van Duuren et al. was "positive" (p. 56). The study itself was not a positive study. In EPA's words:

> "The relevance of positive results in this tumor initiation study with regard to the assessment of human health effects is not clear, particularly since complete carcinogenic activity could not be demonstrated." (EPA Health Assessment Document for Vinylidene Chloride at 10-124 (Aug. 1985)).

Finally, the Draft summarizes the current state of the database with regard to chronic exposure and carcinogenicity as follows:

> "Data from animal studies on the chronic toxicity and carcinogenicity of DCE are sparse, and limited in their usefulness because of experimental design flaws. The data presented do not sufficiently characterize the carcinogenic or chronic toxic effects of DCE. However, the available information does suggest that DCE is carcinogenic in animals. Additional information on the chronic toxicity and carcinogenicity of DCE from well-conducted animal bioassays and human epidemiological studies using various routes of exposure would be useful in predicting the likelihood that such effects occur in humans." (Draft at p. 73).

The present database for VDC oncogenicity and chronic effects consists of 18 long-term studies. The Panel believes that the characterization of this database in the Draft as "sparse and limited in . . . usefulness" seriously misrepresents the weight of evidence. VDC was shown to produce tumors in only one sex (male) of one strain (Swiss) of one species (mouse). Contrary to the statement contained in the Draft, the data in these studies do sufficiently

characterize the carcinogenic or chronic toxic effects of VDC and indicate that VDC is not likely to pose a carcinogenic risk to humans.

The Panel submits that the objections posed by the Draft to the 17 negative long-term toxicity and/or oncogenicity studies are insu: ient to demonstrate that this enormous data base cannot ble the Administrator to make a reasonable determinatio. : whether of exposure to VDC presents a significant health risk. Moreover, the existing data regarding metabolic and pharmacokinetic species differences and regarding the mechanism of toxicity in the mouse provide a reasonable basis to predict that humans are unlikely to exhibit health effects comparable to the mouse.

b. The CMA-sponsored studies were well-designed and well-conducted

Two studies were performed for the CMA VDC Panel to assess the effects of VDC inhalation and ingestion in drinking water on Sprague-Dawley rats: McKenna et al. (1982) (published as Quast et al. (1986)); and Quast et al. (1983). The Draft fails to specify which studies suffer from which alleged defects. (See pp. 34, 46). It suggests that the maximum tolerated dose may not have been achieved and that otherwise "inadequate test conditions" might explain the results.

The Panel submits that these studies were well designed and conducted and, along with the NTP bioassay and the comparative pharmacokinetics, metabolism and mechanism of action studies, can provide a reasonable basis for a determination of significant risk.

The extensive data base on comparative pharmacokinetics and metabolism of VDC and the mechanism of action data indicate that the mouse is uniquely sensitive to the effects of VDC. The Panel believes that the long-term inhalation and drinking water studies using Sprague-Dawley rats (Quast et al. (1983), McKenna et al. (1982)) are appropriate for the purpose of assessing the risk for man.

The exposure period of the CMA inhalation study was 18 months, with a final sacrifice at 24 months. The normal lifespan of the Sprague-Dawley rat is considerably shorter than the Fischer 344 rat used by the NTP. In fact, the exposure period of the Sprague-Dawley rat in the inhalation study, 18 months, covered a portion of the animals' life-span comparable to the portion of the Fischer 344 rat life span covered by the 2-year exposures. The historical survival data on Sprague-Dawley rats at the laboratory in which the long-term inhalation and drinking water studies were conducted were, for males, 40-81% at 18 months and 6-24% at 24 months. The survival of male and

female Fischer 344 rats was 75% at 24 months. On the basis of the exposure time, the inhalation study on the Sprague-Dawley rat is believed to be appropriate for assessing the potential risk to man from exposure to VDC.

The dose levels for the long-term inhalation study using Sprague-Dawley rats, 25 and 75 ppm, were sufficiently lower than the concentrations, 125 and 200 ppm, which caused hepatocellular necrosis after 30 days exposure in the female Sprague-Dawley rat (Quast (1976)) so as not to compromise the interpretation of the study outcome. In both the inhalation and drinking water exposure regimens using the Sprague-Dawley rat, the criteria for maximum tolerated dose accepted in the EPA position paper on MTD were satisfied. The statement in the Draft that "[c]linical signs of toxicity were not generally observed" (p. 46) is not applicable to this study. The presence of vacuolization and fatty infiltration of hepatocytes are conditions that indicate that a MTD was achieved under the criteria set forth in the EPA position paper. See Harris, J.E. et al. (1986). Position Paper on Maximum Tolerated Dose in Oncogenicity Studies at p. 5.

This study represents the longest inhalation exposure duration (18 months) used in a laboratory investigation on toxicity and oncogenicity of VDC to date.

No oncogenic response was noted in this study, in spite of what amounted to a lifetime exposure for the Sprague-Dawley rat.

c. The NTP studies were conducted at concentrations selected according to EPA criteria

Contrary to the suggestion in the Draft, the design of the NTP studies has been accepted by EPA. The only objection raised by EPA in its Health Assessment Document was the suggestion that the bioassays may not have utilized a sufficiently high dose for either species. The doses were questioned because of the lack of an effect on weight gain or survival in the high dose population. EPA did note, however, that the cumulative dose of the NTP high dose mouse study was roughly equivalent to the high dose of the Maltoni inhalation study. EPA only suggested that the route of administration and the strain of mouse used might have made a difference in the results because of differences in distribution and metabolism.

The Panel submits that the NTP studies were conducted with an appropriately high dose, based upon the findings in the subchronic phase of the studies and in accordance with EPA's criteria. The dose levels were based upon levels which had produced body weight changes and histopathologic observations. The NTP studies, along with

the Quast et al. (1983) study, can be used for risk assessment purposes.

d. Other studies also provide valuable data

The Draft unfairly dismisses the contributions that the extensive data base can make to the weight of evidence present on the toxicity and oncogenicity of VDC, stating merely that the existing studies are inadequate. On the contrary, inhalation studies by Lee et al. (1978) and Hong et al. (1981) provide evidence of differences in species and strain sensitivity to the effects of VDC exposure. In these inhalation studies, exposures to 55 ppm VDC for 6 hours/day, 5 days/week for 12 months and 6 months, respectively, demonstrated no statistically significant differences in tumor occurrence between the exposed and control CD-1 mouse strains. The exposure duration in the Lee et al. (1978) study was equivalent to that in the Maltoni Swiss mouse study, while the dose was more than twice the dose that Maltoni et al. found as producing significant tissue injury and kidney adenocarcinomas in the Swiss mouse.

e. Metabolism and pharmacokinetic data provide a good framework to evaluate the mouse data for possible relevance to humans

The Panel submits that, given the extensive data on the pharmacokinetics and metabolism of VDC, the potential biological effects in man versus the rat and mouse are predictable on a comparative physiologic and metabolic basis. Accordingly, the Draft should conclude that a reasonable prediction of human health effects can be made on the basis of existing data. A large number of distribution, excretion, metabolism and oncogenicity studies have already been conducted, and the results of these studies have been summarized by EPA in the Health Assessment Document and the 1985 Drinking Water Criteria Document.

A consensus has developed among scientists who have studied the matter, including EPA scientists, regarding the mechanism by which the tumors were produced in the Maltoni Swiss mouse study. The oncogenic response, or lack thereof in species studied correlates with the extent to which the species metabolizes VDC to its toxic metabolites. An increased production of toxic metabolites in the mouse causes an increase in several toxicity endpoints. The oncogenic response in the Swiss mouse correlates with a high level of toxicity. The absence of an oncogenic response in other species correlates with lower levels of toxicity,

which in turn correlate with reduced production of toxic metabolites.

Animal bioassays with VDC have been uniformly negative, with one noteworthy exception. The suggestion in the Draft that the Maltoni rat inhalation study provided "some evidence" of carcinogenicity (p. 32) represents a serious mischaracterization of the results of that study. The Draft accurately points out the serious flaws in the Maltoni rat study (p. 33). In view of those flaws, the document should not characterize the study as producing "evidence of a carcinogenic effect associated with DCE exposure" (p. 32).

Tumors were produced in the kidneys of Swiss mice in the Maltoni inhalation study. However, as the Draft notes, these effects were accompanied by severe toxic effects. Indeed, they were seen at concentrations of VDC that were notably toxic and near the acutely lethal concentration. 1/ There exists considerable evidence of the greater sensitivity of this strain of mouse to VDC. This single positive oncogenicity study appears to be related to the significant tissue injury in male mice exposed to VDC.

1/ Maltoni et al. (1985) reported a high degree of toxicity and mortali[illegible] within one week at 200, 100 ppm and 50 ppm in the Swiss m[illegible]se. See EPA's Health Assessment Document for Vinylidene C[illegible]oride, Table 10-26, at page 10-91.

Attached as Appendix A is a data review entitled "Interpretive Review of the Animal Toxicological, Pharmacokinetic/Metabolism, Biomolecular and In-Vitro Mutagenicity Studies on Vinylidene Chloride and the Significance of the Findings for Man" by J.M. Norris and R.H. Reitz ("Interpretive Review"). The Interpretive Review contains a comprehensive overview of data relevant to an effort to extrapolate from existing animal data for human risk assessment purposes.

EPA scientists have also recognized how well-characterized the mechanism of VDC toxicity is. In its draft Drinking Water Criteria Document for VDC, EPA stated:

> "Very little is known about the mechanism of toxicity of cis- and trans-1,2-DCE. However, there has been considerable work directed toward defining the mechanism of toxicity of 1,1-DCE. . . . [M]ost of the acute and long-term toxic effects observed, e.g., hepatotoxicity, renal toxicity, mutagenicity and carcinogenicity, are due probably to the formation of toxic metabolites." 2/

As the Interpretive Review explains, data clearly demonstrate that VDC is metabolized much more rapidly by

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2/ E.P.A., Office of Drinking Water, Draft Criteria Document for the Dichloroethylenes (1,1-Dichloroethylene, cis-1,2-Dichloroethylene, and trans-1,2-Dichloroethylene) September 1985 ("EPA Criteria Document"), at VII-1.

mice than by rats.3/ It has been shown that there are much higher levels of covalently bound VDC metabolites in both the liver and kidney of mice than in the same organs of rats.4/ The formation of substantially large amounts of toxic metabolites in the mouse explains the greater toxicity of VDC to the mouse than to the rat.

The EPA Criteria Document noted:

> "It is generally believed that reaction of these metabolites, i.e,, 1,1-dichloroethylene oxide, chloroacetyl chloride and chloroacetic acid, with macromolecules leads to the observed toxic effects: hepatotoxicity, renal toxicity, mutagenicity, and carcinogenicity." (Id. at VII-3 (emphasis added)).

It also noted the many studies that have shown that mice are far more susceptible to kidney damage from VDC than are rats. (Id. at VII-5).

The extensive bioassay data confirm EPA's observation, quoted above, that the reaction of VDC metabolites is generally believed to be the cause of the observed toxic effects. Thus, no significant increase in tumors was shown in Maltoni's inhalation study with Sprague-Dawley rats, in his inhalation study with Chinese hamsters

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3/ Jones, B.K. and Hathaway, D.E. (1978). Differences in metabolism of vinylidene chloride between mice and rats, Br. J. Cancer, 37:411-417.

4/ EPA Criteria Document at VII-4.

or in his gavage study with Sprague-Dawley rats. It was only in his inhalation study with Swiss mice exposed to the nearly lethal dose of 25 ppm that an increase in kidney adenocarcinomas was observed. No other studies were positive, including an inhalation study with Wistar rats, Viola, P.L. and Caputo, A. (1977) an inhalation study with Sprague-Dawley rats, id., an inhalation study with CD-1 mice, Lee et al. (1978), an inhalation study with CD rats, id., another inhalation study with Sprague-Dawley rats, McKenna et al. (1982), an ingestion study with Sprague-Dawley rats, Quast et al. (1983), another inhalation study with CD mice, Hong et al. (1981), another inhalation study with CD rats, id., a skin application study with Swiss mice,5/ a subcutaneous injection study with Swiss mice, Van

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5/ Van Duuren et al. (1979). The Draft cites the Van Duuren skin application study suggesting that VDC acted as a tumor initiator. Results of a study for complete carcinogenic activity also conducted by Van Duuren were negative. The aforementioned tumor initiation study should not be cited as suggestive of VDC's carcinogenicity. EPA has concurred in this determination:

> "The relevance of positive results in this tumor initiation study with regard to the assessment of human health effects is not clear, particularly since complete carcinogenic activity could not be demonstrated. Maximally tolerated doses, estimated from preliminary short-term tests, were used in the tests for carcinogenicity performed by Van Duuren et al. (1979)." (EPA Health Assessment Document at 10-124).

Duuren et al. (1979), a third inhalation study with Sprague-Dawley rats, Maltoni (1977a), a gavage study with Sprague-Dawley rats, Maltoni et al. (1977b), a gavage study with Fischer 344 rats, NTP (1982), and a gavage study with B6C3F1 mice, id. See infra, pp. 29-31.

In view of the disparity between the mouse kidney adenocarcinomas and the results in seventeen other long-term studies, there is now a widely held view in the scientific community regarding the mechanism of tumor formation in the mouse study. In addition to the references summarized in the Interpretive Review, EPA has summarized that view as follows:

> "It has been suggested that development of kidney adenocarcinomas in [male] mice is due to the increased rate of biotransformation of 1,1-DCE in [male] mice, which produces a higher level of reactive intermediates that can subsequently react with macromolecules, e.g., DNA. A study has been made of the potential of 1,1-DCE to cause DNA alkylation, DNA repair and DNA replication in the liver and kidneys of mice and rats, and these results were compared with results obtained using the potent carcinogen dimethylnitrosamine. Tumorigenic doses of dimethylnitrosamine produced relatively little tissue damage but caused a high degree of DNA alkylation and DNA repair synthesis. In contrast, tumorigenic doses of 1,1-DCE resulted in massive tissue damage but induced minimal DNA alkylation or DNA repair synthesis. These data were interpreted as suggesting that tumors observed in mice exposed to 1,1-DCE

> arise primarily through effects of the chemical on nongenetic components of cells." (EPA Criteria Document at VII-8 (citations omitted)).6/

When this extensive data base is used to estimate potential risks to man, it can be seen that the Maltoni oncogenicity data have no known applicability to man. The rate of oxidative metabolism for halogenated hydrocarbons such as VDC appears to be related to body surface area, rather than body mass. Thus metabolic activation would occur more slowly in man than in small laboratory animals. This observation is consistent with findings of Reitz et al. (1980) and Jones and Hathaway (1978), who demonstrated that the rat metabolizes less VDC than the mouse; Andersen et al. (1980), who related the slower rate of metabolism of VDC in man versus the rat to the rate of pulmonary uptake; and Walker (1978), who reported that significant metabolic dissimilarities exist between man and the mouse relative to the monooxygenases which catalyze the metabolism of VDC to the reactive metabolites.

After summarizing the data showing interspecies differences in metabolic effects, the Agency noted in its Criteria Document:

6/ EPA cites Maltoni (1977); Hathaway (1977); Henschler and Bonse (1977); and Reitz (1980).

> "Since the rate at which an inhaled chemical is presented to the liver is related to pulmonary uptake, it would be expected that the smaller breathing volume (liters/kg/hr) in man relative to the rat would produce a corresponding slower rate of metabolism of 1,1-DCE in man ... and the formation of smaller amounts of toxic metabolite(s)." (EPA Criteria Document at VII-2 to -3).7/

Thus, the capability now exists to evaluate the relevance to man of the Maltoni study and the other bioassays. EPA's scientists have noted the significance of the metabolic dissimilarities in the mouse in comparison with other species. In view of the existing data, VDC toxicity and the potential risk to man are well-characterized.

Finally, the occurrence of renal tumors in Swiss mice was accompanied by significant cytotoxicity in the kidney. When cytotoxicity was absent or min al (at lower doses or in other species), oncogenicity was ot observed. Consequently, it appears likely that induction of cytotoxicity is associated with an increase in the incidence of tumors.

The extensive data available from pharmacokinetic and metabolism studies provide a framework by which to understand the species and strain differences observed in

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7/ EPA cited Andersen et al. (1980).

the large chronic data base. Similarly, these data may be used to better understand the relevance of the mouse tumor data to man.

2. Extent of Exposure

The data presented in the Public Health Statement (p. 1) with regard to air concentrations within manufacturing facilities are out-dated. Data submitted by the manufacturers to EPA on September 19, 1988 indicates that employees' potential 8-hour TWA exposure to VDC ranges from non-detectable to 31 ppm. The mean exposure is considerably less, and the 31 ppm represents an unusual condition in a single plant, and not normal operation. Data submitted by the manufacturers indicates that the average exposure in all exposure scenarios in manufacturers' plants is less than 1 ppm for one manufacturer and approximately 1.3 ppm for another manufacturer (excluding two off site samples). It is also necessary to clarify to readers of the Public Health Statement the likely significance of extremely low levels.

The statement at page 2 that VDC has been measured in food stuffs wrapped in plastic food packaging films at concentrations "that range from less than .005-0.01 ppm" should be clarified to indicate that VDC has not been found in all measurements of food stuffs wrapped in these

films. Thus, the concentrations range from 0 or non-detectable to 0.01 ppm. Unless such a change in made, the Draft suggests that VDC has been measured in every food sample.

The Draft recognizes that "enough information is available" on production use and release of VDC. However, it suggests that more information on how much VDC has been disposed at hazardous waste sites and how much has been abandoned "would be useful" (p. 82). While additional information would always be "useful," the key issue for the Draft is whether "adequate information . . . is available . . . to determine levels of exposure which present a significant risk . . . ." 42 U.S.C. § 9604(i)(3)(B). The Panel believes that such a determination can now be made without additional data regarding disposed VDC.

With regard to air emiss s, the Draft cites EPA's estimates of total annual air emissions of about 650 tons per year. The Draft accurately notes that more recent data indicate a reduction in the number of emission point sources and in the total amount of VDC released. SARA § 313 reported emissions for 1987 amounted to a total of 836,371 pounds. The Panel suggests that this more current information be used in the Draft.

The Draft should also be updated with the benefit of CMA's survey data generated and submitted to the EPA Office of Toxic Substances. Those data indicate that approximately 103.4 tons per year is released from manufacturing and using facilities.

3. Developmental Effects

The Public Health Statement unfairly states (at p. 3) that birth defects have been noted in the offspring of pregnant animals exposed to VDC. The notation that "[s]ickness of the mothers was also often observed" does not adequately explain to the lay reader the significance of the maternal toxicity findings. Similarly, the reference in Table 1-2 to 15 ppm as causing toxicity to mice fetuses and the discussion at pages 30, 52 is misleading.

One study relied upon by the Draft is Short et al. (1977a). The EPA Health Assessment Document stated, with regard to the Short study:

> "[A] conclusion of teratogenicity is weakened by additional effects on the pups as a result of maternal toxicity at nearly all concentrations of vinylidene chloride used." (Health Assessment Document at p. 10-61).

Another study cited is Murray et al. (1979). Although wavy ribs and delayed ossification in rat fetuses are mentioned in the Draft (p. 30), there is no mention of

the following statement in the EPA Health Assessment Document:

> "Wavy ribs and delayed ossification were interpreted as fetotoxic and embryotoxic manifestations of maternal toxicity at the higher exposure levels." (Health Assessment Document at p. 10-63).

Finally, the Draft refers to "increased resorption and skeletal alterations in rabbit fetuses" (p. 30). The EPA document states:

> "Again, the fetal anomalies observed in rabbits were attributed to maternal toxicity rather than to a teratogenic effect of vinylidene chloride." (Health Assessment Document at p. 10-65).

The Panel submits that a fair and accurate presentation of developmental toxicity data must include a clear discussion of the complications caused by the maternal toxicity. The EPA Health Assessment Document may provide a good model for such a discussion.

The Draft at page 74 correctly notes that adequate information exists, but states that the data support the conclusion that developmental effects "are most likely to occur in humans." While the effect of maternal toxicity is stated, the Draft implies that developmental effects will occur without maternal toxicity. Such a suggestion is totally without support in the database. A more accurate statement would indicate that developmental effects <u>may</u>

occur in humans at exposure levels that cause toxic effects in the mother.

4. Reproductive Effects

The Draft claims (at pp. 73-74) that a standard reproductive effects study of inhalation has not been conducted and "would be useful." The Panel believes that VDC has been adequately tested to determine whether it poses a significant risk of reproductive effects. The Short et al. (1977) studies described at page 31 provide an adequate basis to assess the reproductive effects of inhalation exposure.

5. Neurologic Effects

The Draft states that inhaled VDC "can induce neurotoxicity after short-term exposure" (p. 23) and that central nervous system toxicity has been observed (p. 52). The data described at page 29, however, indicate that the effects observed cannot be properly characterized as neurotoxic. The symptoms are better characterized as inebriation.

The data needs section states at page 74 that information is available, but that "more information would be useful in assessing the neurotoxic effects of prolonged exposure to [VDC]." The Panel believes that this is another example of a data needs evaluation that is not designed to

fulfill the purposes of the tox profile. Clearly, adequate information exists to determine whether VDC exposure causes a significant health risk. Prolonged exposure effects may be of interest in a hypothetical evaluation, but are of no relevance to a realistic risk assessment.

If the two cases of persistent cranial nerve disorders (p. 29) were more likely due to dichloroacetylene, it is inappropriate to mention them in this VDC Draft. The reference should be removed.

6. Miscellaneous Comments

The Draft should reflect the fact that VDC is also used as a chemical intermediate, not only as an ingredient in plastic products (p. 1).

In Section 1.2 (p. 1) it is stated that "[a]ir concentrations within manufacturing facilities have been measured from 6 ppm to 1900 ppm which exceeds the levels observed to affect health in animals." In Section 5.5 (p. 90), other exposure data are presented which indicate that typical VDC exposures are far lower (<5 ppm). The 1900 ppm level is obviously an extreme. In these cases, the median values must be presented to make a meaningful judgment. It also is not stated whether this exposure level was over an 8-hour work day or was a short term excursion. Lastly, OSHA has recently established a Permissible Exposure Limit of 1

ppm (8-hr. TWA) based on toxicity, not on carcinogenicity. As noted above, however, workplace exposure is already maintained at about this level.

In the last paragraph in this section, potential exposure to VDC from plastic packaging films is discussed. In addition to the data presented, it should also be mentioned that these packaging uses are regulated by the FDA and that small VDC residuals are considered by the FDA to present no hazard to the consumer.

In Section 1.3 (p. 2) it is stated that VDC "can probably also enter the body through the skin." No data to support this statement are presented in Section 2.2.3, and, therefore, this statement is inappropriate for Section 1 (Public Health Statement) and should be removed.

In Table 1-1 (p. 5) it should be noted that these levels are extrapolated from animal data. If they are, the use of the term "short-term exposure" for exposures less than 14 days becomes meaningless for health effects information, especially when the document cites exposure levels of 500 and 4000 ppm. Clearly, these exposure levels are well above the permissible exposure limit (1 ppm) and ACGIH TLV (5 ppm), and it is unrealistic to imply that these levels should or could be endured for up to 14 days.

In the second sentence of Section 2.3 (page 48), possible human exposure situations are listed. In discussing the potential for human exposure, it should be pointed out that the levels of exposure will vary greatly from situation to situation and even within each situation for different individuals.

In the next paragraph the effects of metabolism are briefly reviewed. However, one observation discussed in Section 2.6.3 (p. 65) is omitted here. That is the observation that though the pathways are similar in the rat and mouse, the rate of metabolism was greater in the mouse (resulting in a greater concentration of toxic metabolite).

In the second paragraph on page 82 it is stated that VDC has been found at 16% of all hazardous waste sites. It would be helpful to know at what level VDC was found.

In the last paragraph in Section 1.6, Minimal Risk Levels (MRL) are discussed. The reader is referred to Section 2 for information on how MRL's are derived. Since Section 1 is designed to stand alone, it should be made clear in this section that these values are derived by applying safety factors to LOAEL's. It should also be pointed out that actual risk levels are unknown and, in fact, may be zero.

In the third paragraph on page 27, it is concluded based on animal studies that "humans are at risk for DCE-induced liver toxicity following inhalation exposure to this chemical." As with all chemicals, it is "excessive" exposure that may result in liver effects. The word "excessive" should be inserted in this sentence, or the exposure levels resulting in human liver effects should be indicated if known.

In the third paragraph on page 43 the study by Nitschke et al. is cited. The sentence reads "Nitschke et al. (1983) observed fatty changes in the liver of rats exposed to DCE in the drinking water at levels equivalent to 7 mg DCE/kg body weight/day in vitro, during lactation, and through weaning into adulthood." What is the meaning of "in vitro" in this statement? It would appear to be an error since the study was conducted in vivo.

On page 81, 1989 production is estimated at 165-175 million pounds. The Panel estimates this number to be 230 million pounds.

On page 86, it is stated that "EPA (1983a) indicates that potentially significant concentrations could still be reached on an urban and regional scale, in areas near air emission sources." First, "potentially significant" is not quantified or defined. In addition, in

your previous discussion on releases (Section 5.2.1), there was no data presented to substantiate this claim. Was the concentration of VDC measured or is this a generalization based on volatile organic compounds? If there are no data, then this conclusion is inappropriate and misleading.

On page 88 one study of drinking water supplies is quoted (EPA 1985a). It would be informative to include the results of another recent (1984) and comprehensive study. (J.J. Westrick et al., The Groundwater Supply Survey, J. Amer. Waterworks Assoc., May, 1984, p. 52-59). In this survey, a total of 945 samples were analyzed for 29 volatile organic compounds and five trihalmethanes in finished wastes from groundwater sources nationwide. VDC was not detected at the quantification limit of 0.2 ppb in 97.7 percent (923 of 945) of the samples. The maximum value of VDC detected in 2.3 percent (22 or 945) of positive samples was 6.3 ppb, with a median value that ranged from 0.28 to 1.2 ppb in four data subsets. It could also be pointed out that the 6.3 ppb value is below the 7 ppb maximum contaminant level set by the Safe Drinking Water Act.

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Appendix A

INTERPRETIVE REVIEW OF THE ANIMAL TOXICOLOGICAL, PHARMACOKINETIC/METABOLISM, BIOMOLECULAR AND IN-VITRO MUTAGENICITY STUDIES ON VINYLIDENE CHLORIDE AND THE SIGNIFICANCE OF THE FINDINGS FOR MAN

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ABSTRACT

An interpretive review of the animal toxicologic and related data on vinylidene chloride (VDC) is presented. The appropriateness of extrapolating findings from the various laboratory animal species to man and the overall implications of the animal data for man are discussed. Pharmacokinetic/metabolism and biomolecular events in rodents exposed to VDC clearly demonstrate the inherent species differences which contribute to differences in target organ toxicity and in a species/strain-specific and sex-related tumorigenic response. The target organ toxicity in the rodent species is related to the capacity to biotransform VDC to reactive species with the mouse having a far greater capacity than the rat. The tumorigenic response to VDC appears to be mediated via a non-genetic mechanism, namely, recurrent tissue damage. Man, by virtue of having a slower rate of oxidative metabolism than either rodent species, is likely to be less sensitive to the toxic effects of VDC than the rat and significantly less sensitive than the mouse. Exposure to VDC concentrations not causing cytotoxicity would be unlikely to result in adverse health effects in man.

1. INTRODUCTION

The results of several long-term inhalation and ingestion studies on VDC (Rampy et al., 1977; Huntiston et al., 1978; McKenna et al., 1980; Maltoni et al., 1977, 1980; Viola and Caputo, 1977) have shown that the mouse is more sensitive than the rat or the hamster to toxic effects of VDC; mice exposed to 25 ppm showed liver and marked kidney changes whereas 25 ppm exposure of hamsters was a no-toxic effect concentration and 75 ppm exposure of rats caused only minimal, reversible liver changes. Furthermore, only following lifetime exposure of mice to vapor concentrations that were markedly toxic and near the acutely lethal level (Maltoni et al., 1977, 1980) was a tumorigenic response observed. Various shorter term studies have demonstrated significant differences between the rat and mouse in the expression of frank toxicity. The objective of this interpretive review is to present biological evidence that is relevant to the assessment of safety of man exposed to VDC at permissible levels in the workplace, or to trace quantities that may exist in the ambient environment.

2. ANIMAL TOXICOLOGICAL AND RELATED DATA

A. The Mouse is More Sensitive to the Toxic Effects of Vinylidene Chloride Than the Rat or the Hamster.

A direct comparison study to delineate the differences between male CD rats and male CD-1 mice following exposure to various vapor

concentrations of VDC up to 60 ppm, 23 hours/day, was conducted by Short et al., 1977. After 2 days of exposure to 60 ppm VDC, mortality differences between the species were dramatic, 80% mortality in the mice and 0% in the rats. VDC was observed to produce a greater effect as measured by elevated serum enzymes, in the liver of the mice than in the rats. A dose-related increase in both serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic-pyruvic transaminase (SGPT) occurred in mice exposed to VDC for one day. The serum enzymes in the rats were also increased, however, the increase, which was evident after the 2nd day of exposure to 60 ppm, was not nearly as large as that observed in the mice (Table 1).

TABLE 1

Effect of Vinylidene Chloride Exposure on Serum Enzymes in Male Rats and Mice

Days Exposure	Serum Enzyme	VDC - (ppm) Rats[a] 0	Rats[a] 60	Mice[b] 0	Mice[b] 60
1	SGOT	66±10	74±6	56±8	1946±271
	SGPT	28± 2	44±7	32±6	3043±209
2	SGOT	63± 4	264±33	82±30	751±150
	SGPT	34± 4	198±29	38±4	1112±226
3	SGOT	81± 4	238±47	64±14	no sur-
	SGPT	34± 6	122±29	29±2	vivors

[a]Control and 60 ppm n = 5.

[b]Control n = 4 or 5; 60 ppm day 1 n = 4, day 2 n = 2.

Adapted from Short et al., 1977

The toxicity to the liver of the mice and rats as revealed by the elevation of serum enzymes, was confirmed by histopathologic observations. Examination of kidney tissue of the CD-1 mice showed the kidney also to be a target organ with all the mice exposed to 15, 30 or 60 ppm VDC having severe tubular necrosis after 1 or 2 days of exposure; no kidney toxicity was observed in the rats at 60 ppm.

Longer-term exposure of Sprague-Dawley, CD and Alderley Park rats to VDC via inhalation or ingestion likewise revealed the liver to be the target organ in this species. Specifically, the results of a 90-day toxicity inhalation study and a two-year toxicity and oncogenicity study of Sprague-Dawley rats to 25 or 75 ppm VDC, 6 hours/day, 5 days/week, showed a minimal exposure-related effect characterized as a midzonal hepatocellular fatty change. This change was observed after 30 and 90 days in the 90-day study and after 3, 6 and 12 months in the 2-year study (Balmer et al., 1976; McKenna et al., 1980). The non-progressive liver change in the rats was readily reversible on cessation of the exposure after 18 months in the 2-year study (McKenna et al., 1980). No toxicologically significant exposure-related changes were observed in serum enzymes values in either study.

Lee et al., 1977 reported that the liver of CD rats exposed to 55 ppm VDC, 6 hours/day, 5 days/week for periods up to 12 months, showed marked or severe focal, disseminated vacuolization indicative of fatty change. No persistent change was found in serum enzyme values. Gage, 1970 reported that Wistar-derived Alderley Park rats

exposed to 500 ppm VDC, 6 hours/day, 5 days/week for 4 weeks showed liver cell degenerative changes whereas at 200 ppm no liver toxicity was observed. Serum enzymes determinations were not made in this study.

A 90-day toxicity study incorporating VDC in the drinking water of Sprague-Dawley rats at concentrations of 60, 100 or 200 ppm (equivalent average doses 6, 10 or 19 mg VDC/kg body weight/day for male rats and 8, 13 or 26 mg/kg/day for female rats) showed minimal hepatocellular fatty changes in rats ingesting 200 ppm VDC. The changes were not progressive in nature as demonstrated by the results of the 2-year toxicity and oncogenicity drinking water study conducted at the same concentrations (Humiston et al., 1978). The group of male rats ingesting 200 ppm VDC over a 2-year period showed, in addition to minimal hepatocellular changes, an increased incidence of periportal hepatocellular hypertrophy. The female rats at all dose levels showed minimal hepatocellular fatty change and periportal hepatocellular hypertrophy.

Species differences were likewise observed relative to the maximum dose tolerated by rats and mice in inhalation studies conducted. Specifically, histopathologic examination of animals on these studies established that 25 ppm in the mouse and 200 ppm in the Sprague-Dawley rat exceeded the maximum tolerated dose. Long-term studies on rats, Sprague-Dawley and Wistar, revealing that the highest level of inhaled VDC tolerated by rats was less than 200 ppm

were reported by Maltoni et al., 1977, 1980; Viola and Caputo, 1977. After only two sequential daily exposures of 4 hour duration to 200 ppm, VDC produced strong toxic effects, namely, fatty degeneration in the liver and early necrotic changes of renal tubuli in the Sprague-Dawley rat (Maltoni et al., 1977, 1980). The highest "bearable" level for prolonged exposure was 150 ppm. Likewise, Viola and Caputo, 1977, reported that the high exposure level in their original study on Wistar rats was lowered from 200 ppm to 100 ppm in the 6th month to avoid toxic reactions from occurring during the remainder of the 12-month study. A second study by these investigators was conducted on the Sprague-Dawley rat at 100 ppm and 75 ppm VDC; the pathologic findings in this study have not been reported.

A study on Swiss mice revealed high mortality and severe toxic effects as a result of exposure, 4 hours/day, to VDC vapor concentrations of 200 or 100 ppm for 2 days, and of 50 ppm for 4 days, and the mice exposed to 25 ppm reportedly showed severe toxic effects in kidneys and liver (Maltoni et al., 1977, 1980; Maltoni, 1977a). Based on the severity of the histopathological changes, 25 ppm was a concentration that exceeded the currently acceptable maximum tolerated dose for mice on a long-term study.

B. The Response Observed in Swiss Mice is Species/Strain-, Sex- and Dose-Related.

The response in the kidney of Swiss mice is apparently unique to that strain and is associated with the degree of toxicity expressed in the target organ. Strain specific toxicity data reported from two comparison studies revealed exposure-related signs of toxicity including mortality, lowered mean body weights, increased liver and kidney weights and increased serum glutamic pyruvic transaminase with significant differences existing between the sexes (Maltoni, 1977a; Henck et al., 1980). Maltoni, 1977a reported a sex-related difference in mortality in the various strains exposed to 200 ppm VDC, 4 hours/day for 2 consecutive days. Mortality among the male mice, and particularly the Balb/c and Swiss male mice was greater than that of the females:

TABLE 2

Mortality in Various Strains of Mice Exposed to 200 ppm Vinylidene Chloride

Mouse Strain	Mortality Rate	
	Male	Female
Balb/c	24/30	0/30
Swiss	51/60	0/60
C_3H	17/30	11/30
$C_{57}Bl_6$	7/30	0/30

The major histopathologic changes in these strains of mice reportedly occurred in the kidneys and liver with the Swiss male mice showing a marked effect.

A comparison study has been conducted on four strains of mice, Ha(ICR), $B_6C_3F_1$, CD-1 and CF-W, a strain of Swiss-Webster derived mice believed to be genetically comparable to Maltoni's Swiss mouse (Henck et al., 1980). The mice were exposed to 55, 100 or 200 ppm VDC for 6 hours/day, 5 consecutive days/week for a total of 10 exposures. No-exposure related mortality was observed among the mice exposed to 55 or 100 ppm. At 200 ppm male Ha(ICR), CD-1 and CF-W mice had higher mortality rates than the females of the respective strains, but no sex-related differences in mortality was observed in the $B_6C_3F_1$ mice (Table 3).

TABLE 3

Mortality in Various Strains of Mice Exposed to 200 ppm Vinylidene Chloride

Mouse Strain	Mortality Rate	
	Male	Female
Ha(ICR)	6/10	4/10
$B_6C_3F_1$	10/10	10/10
CD-1	10/10	0/10
CF-W	10/10	1/10

Gross and histopathologic examination of these strains of mice showed that the male mice at all exposure levels had a marked degree of nephrotoxicity with renal failure which accounted for the mortality. Renal toxicity was insignificant in all female mice when

compared to the male mice of the same strain. The cause of death in the Ha(ICR) and $B_6C_3F_1$ female mice was reportedly associated with acute hepatotoxicity.

The greater sensitivity of the Swiss mouse, and particularly the male mouse, to the toxic effects of VDC has been correlated with a tumorigenic response after repeated exposure to a concentration of 25 ppm. This concentration, which was near the lethal concentration (50 ppm) for the Swiss mice, produced marked changes in the kidneys of the mice. Exposure to 10 ppm, a concentration producing significantly less toxicity, did not result in a tumorigenic response.

No tumorigenic response was observed in a third species, the Chinese hamster, following inhalation exposure of 25 ppm VDC, 4 hours/day, 4 days/week for 1 year and held for 1 year post-exposure period before termination (Maltoni et al., 1977, 1980). No tumorigenic response was observed in Sprague-Dawley rats following exposure to 25 or 75 ppm VDC, 6 hours/day, 5 days/week for 18 months and held for observation for an additional 6 months (McKenna et al., 1982). Likewise no statistically significant increase in tumorigenic response was observed in CD rats exposed to 55 ppm, 6 hours/day, 5 days/week for 12 months or after 10 months of exposure followed by up to 12 month observation (Lee et al., 1978; Hong et al., 1981). No tumorigenic response was observed following oral administration via gavage of VDC in bioassays with the rat or the mouse (NCI, 1980;

Maltoni et al., 1977, 1980). NCI, 1982 reported that no tumorigenic response had been observed in a 2-year study in Fischer 344 rats, at dose levels, 1 or 5 mg/kg/day, nor in $B_6C_3F_1$ mice given 2 or 10 mg/kg daily. Maltoni et al., 1977, 1980 reported no tumorigenic response in Sprague-Dawley rats administered via gavage 20, 10 or 5 mg/kg/day and 0.5 mg/kg/day for 4 or 5 days/week for 1 year and held for 1 year post-exposure period before termination. Humiston et al., 1978 reported no tumorigenic response in Sprague-Dawley rats maintained on drinking water containing up to 200 ppm VDC and providing doses as high as 26 mg/kg/day.

C. The Toxicity and Tumorigenicity in Mice Correlates With the Greater Capacity of the Mouse to Metabolize Vinylidene Chloride.

In an attempt to understand the differences between the mouse and the rat, studies were designed to characterize the pharmacokinetics and metabolism in various strains of both rodent species. A comparison of the overall fate of inhaled or ingested VDC in rats and mice showed that the major differences were quantitative rather than qualitative. The most conspicuous difference was in the greater amount of the parent compound excreted via the pulmonary system by the rat as compared to the mouse following an oral dose of 50 mg/kg (Jones and Hathway, 1978a). Twenty-eight percent (28%) of the administered dose of VDC was excreted unchanged via the lungs of

Wistar derived Alderley Park rats whereas in the Alderley Park mice only 6% of the administered dose was thus excreted. McKenna et al., 1978 reported that Sprague-Dawley rats administered the same dose, 50 mg/kg, excreted 19% of the administered dose as unchanged VDC via the pulmonary system.

A major quantitative difference in the metabolism of VDC between the rat and mouse was in the considerably greater amount of N-acetyl-S-cysteinyl acetyl derivative formed by the mouse (Jones and Hathway, 1978a).

The quantitative differences in the metabolism of VDC between the species are attributable to a combination of physiological and biochemical factors. The initial step in the metabolic pathway for VDC (Figure 1) in mammals appears to be the epoxidation of VDC catalyzed by microsomal monooxygenases with further biotransformation, via two pathways, one to chloroacetic acid via rearrangement of the metabolite (I) 1,1-dichloroethylene oxide to (II) chloroacetyl chloride and (III) monochloroacetic acid, and the other ultimately to the glutathione conjugate, N-acetyl-S-cysteinyl acetyl derivative, (IV) N-acetyl-S-(carboxymethyl) cysteine, catalyzed by glutathione-S-transferases which are thought to have a physiological role in initiating the detoxification of potential alkylating agents (Boyland and Chasseaud, 1969). Specific epoxide-hydrating pathways appear to be of minimal significance in the metabolism of VDC to reactive species (Andersen et al., 1980). Since the mouse possesses high monooxygenase activity relative to the rat (Oesch et al., 1977;

Oesch, 1973), a greater proportion of VDC administered to the mouse is metabolized to the reactive metabolite, 1,1-dichloroethylene oxide. The relative proportions of the N-acetyl-S-cysteinyl acetyl derivative arising through the reaction of 1,1-dichloroethylene oxide with glutathione, in the mice and rats parallel with activities of liver glutathione-S-epoxide transferase in these species (Hayakawa et al., 1974). Since the mouse, however, excretes greater than expected amounts of the N-acetyl-S-cysteinyl acetyl derivative, it would appear that the greater production is due to the higher cytochrome P-450 activity which is found in the organs of the mouse (Litterst et al., 1975). This observation (Jones and Hathway, 1976a) is consistent with results of a pharmacokinetic comparison study reported by McKenna et al., 1977, 1979, in that not only was metabolism of VDC greater in the Ha(ICR) mouse than in Sprague-Dawley rat exposed to 10 ppm VDC for 6 hours, but the production of the reactive metabolites in the target tissues for VDC-induced toxicity was markedly greater in the mouse. The fact that the enhanced production of reactive VDC metabolite in the mouse tissue could not be solely attributed to the increased metabolism of VDC over that of the rat was indicated on normalization of the binding data for differences in metabolism. Thus the ratios of covalently bound ^{14}C-activity/gram of protein in the liver and particularly in the kidney of the mouse to the total VDC metabolized by the mouse were increased relative to ratios determined for the rat. The data obtained from this comparison study are summarized below:

TABLE 4

Vinylidene Chloride Metabolism and Covalently Bound ^{14}C-Activity in Male Sprague-Dawley Rats and Ha(ICR) Mice (10 ppm, 6 Hour Exposure)

Animal Species (Strain)	A Metabolized VDC (mg/Eq/kg)	Tissue	B Covalent Binding (ug Eq ^{14}C-VDC/g Protein)	Ratio B/A
Rat (Sprague-Dawley)	2.84±0.26	Liver	5.28±0.14	1.86
		Kidney	13.14±1.25	4.63
Mouse (Ha(ICR))	5.27±0.74	Liver	22.29±3.77	4.23
		Kidney	79.55±19.11	15.09

All values are x ± S.E.; n = 4.

Adapted from McKenna et al., 1979

McKenna et al., 1977, 1979 furthermore reported that the time course for the disappearance of covalently bound ^{14}C-activity from the liver and kidney of Sprague-Dawley rats and the liver of CD-1 mice exposed to 5 ppm or 100 ppm was consistent with those observed for normal protein turnover in rodent tissues, $t_{1/2}$ 51-65 hours (Omura et al., 1967). However, the time course for covalently bound ^{14}C-activity in mouse kidney was exposure-concentration dependent. At a concentration of 100 ppm the ^{14}C-activity was persistent ($t_{1/2}$ >500 hours), whereas at 5 ppm the time course was consistent with normal turnover time. The observed enhanced production in the mouse of covalently bound ^{14}C-activity in the liver (22.29 vs. 5.28 ug Eq ^{14}C-VDC/g protein) and particularly in the kidney (79.55 vs. 13.14 ug Eq ^{14}C-VDC/g protein) and the dose-dependent time course for disappearance of the ^{14}C-activity from the kidney correlate with the

reported greater susceptibility of the mouse relative to the rat to the toxic effects of VDC in these target organs and the tumorigenic response in the kidneys of Swiss mice following exposure to concentrations producing marked toxicity.

The transformation of the 1,1-dichloroethylene oxide to chloroacetic acid, via the second pathway, is a potentially saturable process (Hathway, 1977). Therefore since the availability of both 1,1-dichloroethylene oxide and its rearrangement product, chloroacetyl chloride is greater in the mouse than in the rat due to increased cytochrome P-450 activity in the mouse, the potential of these reactive species to bind with macromolecules is likewise greater. Thus saturation of the chloroacetic acid process is likely to be more significant in the mouse than in the rat (Jones and Hathway, 1978a).

D. The Mechanism of Action for the Vinylidene Chloride-Induced Tumors in the Mouse appears to be Non-Genetic.

The potential of VDC to cause DNA alkylation, DNA repair, and/or DNA replication with associated tissue damage in the rodent target organs has been researched in male Sprague-Dawley rats and male CD-1 mice exposed to 10 ppm and 50 ppm for 6 hours (Reitz et al., 1980).

Overall alkylation of DNA by VDC was minimal in both kidney and liver of the rat and mice following exposure to 50 ppm or 10 ppm and DNA repair, measured directly in the mice, showed a very small but

significant increase only in the kidneys of mice exposed to 50 ppm VDC (Table 5).

Failure to demonstrate significant genetic effects at the tumorigenic doses of VDC in mice suggests that the tumors in the kidneys of mice were unlikely to be mediated by direct genetic interaction.

There are a variety of indirect processes which can influence the tumor incidence during an animal bioassay. One such process is the production of recurrent cytotoxicity with concurrent cell death and subsequent cellular regeneration (Reitz et al., 1980a; Watanabe et al., 1980). This process is frequently encountered in animal biossays were maximally "tolerated" doses of chemical are administered in order to increase the chances of detecting a carcinogenic response.

During chronic administration of cytotoxic doses, DNA replication is stimulated in the affected tissues. Since there is a tiny, but still finite chance for error in each replication system, the net effect may be to increase the spontaneous mutation rate and ultimately affect the tumorigenic process.

Another consequence of increasing cell division is that the relative rates of DNA repair and DNA replication are altered. This may be very important because there is evidence that the existing DNA has been copied during replication. Consequently, repair becomes less

effective in protecting cells from the consequences of genetic damage after cellular replication. It appears that this type of cytotoxicity occurs in mice exposed to vinylidene chloride at near acutely-lethal vapor concentrations. eg., 25 ppm.

TABLE 5

DNA Alkylation and DNA Repair in Tissue of Mice and Rats Exposed to Vinylidene Chloric.., Ratio of Treated Animals to Controls

Species/Exposure/ Tissue	Alkylations/ Nucleotide ($\times 10^6$)	DNA Repair Ratio[a] (Observed/Expected[b] ± 95% Confidence Limits)
Mouse - 50 ppm		
Kidney	30	1.38±0.090*
Liver	6.1	1.16±0.363
- 10 ppm		
Kidney	11	1.16±0.255
Liver	0.94	0.764±0.222
Rat - 10 ppm		
Kidney	2.0	N.A.
Liver	0.87	N.A.

[a]Repair ratio of 1.0 indicates little or no repair is present; any increase above 1.0 is taken as indication that prior DNA damage has occurred.

[b]Observed hydroxy urea-resistant TdR incorporation to expected using control data.

*Ratio significantly greater than 1.0 ($p<0.05$, t-test).
N.A. - not available.

Adapted from Reitz et al., 1980b.

Reitz et al., 1980b reported dose-related tissue damage and increased DNA replication (Table 6) in the kidneys of mice. Effects comparable to those observed in the mouse kidney were not observed in the liver of either species or in the kidneys of the rats.

TABLE 6

DNA Synthesis in Tissues of Vinylidene Chloride Exposed to Rats and/or Mice

	VDC Concentration - ppm		
	Mouse		Rat
DNA Synthesis[a]	10	50	10
Kidney	7.72*	24.7*	2.20*
Liver	1.20	2.45	0.88

[a]DNA synthesis was estimated by determining specific radioactivity of DNA following (^{3}H) thymidine ((^{3}H)TdR) injection. (^{3}H)TdR was injected 48 hours after the treatment suspected of causing cytotoxicity. A positive response is indicated by an increased rate of incorporation of (^{3}H)TdR into DNA relative to a control group, resulting in a ratio greater than 1.0.

*Radio significantly greater than 1.0 ($p<0.05$, t-test).

Adapted from Reitz et al., 1980b.

The tissue damage in the kidneys of mice exposed to 50 ppm VDC for 6 hours determined directly after cessation of exposure at various times up to 192 hours thereafter showed toxic nephrosis immediately after exposure, progressive nephrosis at 8 and 24 hours, and regeneration after 96 hours. At the 10 ppm concentration slight dilation and swelling were observed immediately after exposure with nephrosis evident after 96 hours post-exposure.

3. IN-VITRO MUTAGENICITY TESTS

In-vitro comparison mutagenicity tests conducted on VDC using tissue extracts from rats and mice have given results consistent with species differences observed in in-vivo studies. Jones and Hathway, 1978b reported that Salmonella typhimurium strain TA1535 and TA100 exposed to VDC gave a weakly mutagenic response in the presence of S-9 mixture from normal mouse liver or kidney and a strongly mutagenic response in presence of S-9 mixture from Aroclor 1254-induced mice. In rats liver preparation from Aroclor-induced animals, gave a positive, but weak, bacterial mutagenic response, while preparations from normal animals were inactive. VDC was also shown to be weakly positive in Salmonella typhimurium strain TA 1535 and TA100 mediated by liver S-9 mixture from a human subject who had been on long-term phenobarbitone medication, but no bacterial mutagenic response was observed in the same test systems mediation by liver S-9 mixture from a normal human subject or from normal marmosets.

4. SIGNIFICANCE FOR MAN

The pharmacokinetic/metabolism and biomolecular data on VDC exposed rats and mice clearly indicate that inherent species differences contributed the observed differences in target organ toxicity, and in the tumorigenic response in the kidney of the mice following exposure to concentrations in excess of a maximum tolerated dose

(associated with significant organ toxity) for a long-term study. The capacity to metabolize VDC to the reactive metabolites correlated with the sensitivity to the toxic effects of VDC observed in these species. The mouse which has a greater capacity to metabolize VDC than does the rat, is more sensitive to VDC-caused tissue injury and tumorigenicity.

For many xenobiotics, including the halogenated hydrocarbons, the rate of oxidative metabolism appears to be roughly related to the body surface area (Weiss et al., 1977; Schmidt-Nielsen, 1970; Pinkel, 1958). Thus in man, the total amount of reactive species formed by the metabolism of VDC would be less than that formed by the laboratory rodents. This observation is consistent with the findings of Reitz et al., 1980 and Jones and Hathway, 1978a, who demonstrated that the rat metabolizes less VDC than the mouse and Walker, 1978 who reported significant metabolic dissimilarities exist between man and the mouse relative to the monooxygenases which catalyze the metabolism of VDC to the reactive species.

The correlation between metabolism and toxicity was confirmed at the macromolecular level. At concentrations causing degenerative/regenerative changes in the kidneys of mice, there was significant binding of the reactive species to cellular macromolecules, and a significant increase in DNA replication, with little effect upon DNA repair. Thus the probable mechanism of the tumor formation was shown to be primarily non-genetic.

The results of the mechanism studies indicate that the exposure of man to concentrations which do not produce cytotoxic effects would be unlikely to produce a tumorigenic response. Furthermore, the metabolic differences seen in the rodents and man make it unlikely that organ injury would result from exposure of man to the concentration reported in the ambient environment or in the workplace. The lack of adverse health effects reported by Ott et al., 1976 in a health survey study of 138 employees exposed to up to 70 ppm VDC is supportive of the finding that man is less susceptible to VDC than the rat and far less than the mouse.

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