

ON THE POSSIBLE MECHANISM OF CARCINOGENIC ACTION OF VINYL CHLORIDE

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INTRODUCTION

Although we have to date not studied the carcinogenicity or metabolism of vinyl chloride, some suggested modes and sites of action are presented in this paper. This presentation is based on our past experience with structure-activity relationships of direct-acting alkylating carcinogens and also on various important considerations on mechanisms of action of chemical carcinogens in general.

DIRECT-ACTING AND INDIRECT-ACTING ALKYLATING CARCINOGENS

Studies on the mechanism of the carcinogenic action of vinyl chloride involve the consideration of whether the chloroolefin is the active carcinogenic agent or whether activated carcinogenic metabolites are involved. Therefore, it is instructive to consider the same question in earlier studies with a wide variety of chemical carcinogens. No attempt is made here to give a review of the literature on this subject, but only an indication of what appears to us to be the current thinking in this area.

There is a considerable body of evidence which supports the view that many well-known carcinogenic agents such as nitrosamines, aromatic amines, azo dyes and aromatic hydrocarbons have to be metabolized *in situ* to their activated carcinogenic intermediates.<sup>1-3</sup> Although not firmly established for all of these carcinogens, much of the evidence suggests that the intermediate agents are alkylating in nature, i.e., electrophilic agents. Such electrophiles undergo a variety of reactions with nucleophiles *in vivo* and it is reasonable to assume that one or more of these nucleophilic reactions is or are responsible for the carcinogenicity of the parent compounds. These carcinogens can, therefore, be referred to as indirect-acting alkylating carcinogens. Many of these compounds are also capable of physical binding with tissue constituents. This noncovalent binding of aromatic hydrocarbons to nucleic acids, for example, has been extensively studied in many laboratories.<sup>4</sup> Most researchers in chemical carcinogenesis now believe that physical binding is not related to the critical interactions involved in carcinogenesis by aromatic carcinogens. However, these studies and conclusions derived from them do not exclude the possibility that physical binding with tissue constituents plays an important role for some chemical carcinogens.

The direct-acting alkylating carcinogens include such classes as the epoxides,  $\beta$ -lactones, and halo ethers. These agents do not have to be metabolized to exert their carcinogenic effect and for this reason they are usually carcinogenic only at the site of administration in laboratory experiments. There are some exceptions, e.g., propane sultone, which is chemically less reactive than other direct-

Van Duuren: Carcinogenic Action of Vinyl Chloride

acting alkylating carcinogens in this group,<sup>5</sup> hence giving rise to tumors at the site of administration and at distant sites.<sup>6</sup>

During the past decade we have determined the carcinogenicity of a series of approximately 100 direct-acting alkylating agents including the following chemical structural types: epoxides, lactones, their sulfur and nitrogen isomers, and halo ether compounds. The carcinogenicity assays were conducted by use of mice and rats by various routes of administration.<sup>7-11</sup> In parallel with the bioassays we examined the chemical reactivity of these compounds with a variety of nucleophiles by *in vitro* experiments. Nucleophilic reactions included rate of hydrolysis studies, rates of reaction with  $-S_2O_3^{2-}$  and  $-N_3^-$  ions,<sup>12,13</sup> reactions with and/or isolation of products of some of these agents with purines,<sup>14,15</sup> DNA,<sup>16,17</sup> and whole mouse skin chromatin.<sup>18</sup> In addition to these *in vitro* and *in vivo* studies we have studied the stereochemistry, molecular flexibility, and functionality (i.e., monofunctional, bifunctional or polyfunctional) of these molecules since these factors have an important bearing on cross-linking abilities in nucleic acids, reactions at phosphate sites in nucleic acids, cross-linking in the chromatin complex<sup>19</sup> and reactions with such body chemicals as cysteine and glutathione.<sup>20</sup> These studies have made a contribution to a basic understanding of the mode of action of direct-acting alkylating carcinogens. Furthermore, these studies have made it possible to predict carcinogenicity within this group of compounds, i.e., direct-acting alkylating carcinogens. Examples of these are:

- i. chloromethyl methyl ether (CME)<sup>14,15</sup>
- ii. bis(chloromethyl) ether (BCME)<sup>14,15</sup>
- iii. propane sultone<sup>5</sup>
- iv. N,N-dimethylcarbamyl chloride<sup>21</sup>

These compounds are cited because they were, and in some cases still are, being used extensively in industry and in the cases of compounds i and ii our findings have had far-reaching implications. As a result of our work on the carcinogenicity of CME and BCME, these two compounds were included in the recent list of human carcinogens by the U.S. Department of Labor.<sup>17,18</sup> Several recent epidemiologic studies in this country and abroad have implicated these two compounds as being responsible for lung cancer in workers exposed to either or both of these materials.<sup>19-22</sup>

ACTIVATED CARCINOGENIC INTERMEDIATES OF VINYL CHLORIDE

Vinyl chloride is a small molecule which is relatively unreactive compared to its allyl analogs and to such potent and chemically reactive direct-acting alkylating carcinogens as the  $\alpha$ -halo ethers mentioned above. It may bind to microsomal membranes or other constituents such as serum albumin prior to metabolism. It is nonetheless likely that it is metabolized to activated carcinogenic intermediates in the liver and other sites. We have considered the possibility that hydroperoxide, peroxide, or free radical intermediates may be involved. While such intermediates should be kept in mind, we feel that epoxide intermediates are likely candidates as activated carcinogenic intermediates, particularly in the liver. Many studies have shown that aromatic hydrocarbons are metabolized to epoxides *in vitro* in rat liver.<sup>23</sup> The liver is known to be particularly rich in such enzymes. These enzymes occur in other organs and tissues but at lower levels and the proposed epoxide intermediate can therefore also account for primary tumors induced at

other sites in the rat inhalation experiments reported by Viola *et al.*<sup>21</sup> and Maltoni.<sup>22</sup> Furthermore, our own studies on structure-activity relationships of epoxides, lactones, and halo ethers referred to above point very strongly to an epoxide, monochloroethylene oxide, as the activated carcinogenic intermediate of vinyl chloride. This is illustrated in FIGURE 1. This compound is not only an epoxide as are the carcinogens epichlorohydrin and glycidaldehyde; it is also an  $\alpha$ -chloro ether, like chloromethyl methyl ether and bis(chloromethyl) ether, i.e., the carbon atom bearing the cyclic ether (epoxide) oxygen also carries a chlorine atom. Such  $\alpha$ -halo ethers are known to be highly reactive alkylating agents.<sup>23</sup> BCME is a particularly potent direct-acting alkylating carcinogen resulting in tumors at the site of application, e.g., mouse skin, subcutaneous tissue in mice and rats, and rat lung.

The metabolic studies on trichloroethylene described below had resulted in the suggestion of an epoxide intermediate in the metabolism of trichloroethylene.<sup>24</sup>

Earlier attempts to synthesize an epoxide of vinyl chloride by peracid oxidation have failed for obvious reasons. The compound, monochloroethylene oxide, has been reported<sup>25</sup> but it has only been partially characterized. Its synthesis and spontaneous rearrangement to monochloroacetaldehyde are shown in FIGURE 2.

Monochloroacetaldehyde is a highly toxic agent<sup>26</sup> which is intensely irritating to eyes, skin, mucous membranes, and respiratory tract. Evidence has been presented which indicates that chloroacetaldehyde is an intermediate in the metabolism of 2-chloroethanol.<sup>27</sup> *In vivo* and *in vitro* experiments in rat liver have shown that chloroacetaldehyde reacts with glutathione to give S-carboxymethyl

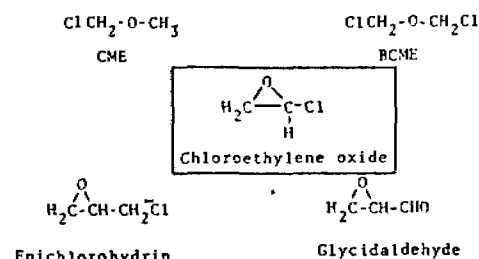


FIGURE 1. Alkylating carcinogens related to chloroethylene oxide.

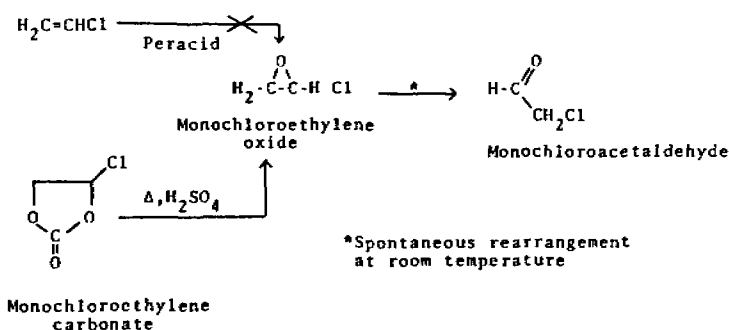


FIGURE 2. Synthesis and rearrangement of chloroethylene oxide.

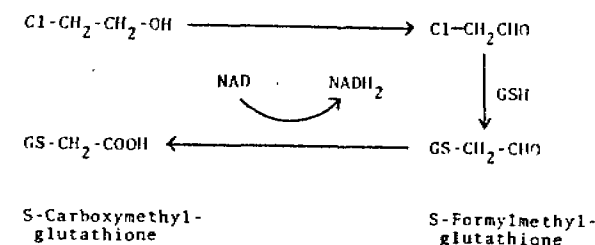


FIGURE 3. Metabolism of 2-chloroethanol in rat liver.

glutathione as a final metabolic product. These experiments are summarized in FIGURE 3.

The role of reactions of activated carcinogenic intermediates of compounds such as nitrosamines or of direct-acting alkylating carcinogens such as glycidaldehyde and other agents with DNA or its constituent bases has been extensively studied. Therefore, it is of interest to examine this type of reaction as shown in FIGURE 4. Glycidaldehyde (I) was shown in our earlier studies to react with guanine derivatives (II) to give the triheterocyclic ring compound III.<sup>19</sup> The spontaneous rearrangement product of monochloroethylene oxide is monochloroacetaldehyde as shown in FIGURE 2. Adenine and its derivatives (V, FIGURE 4) react with monochloroacetaldehyde (IV, FIGURE 4) to give a similar triheterocyclic product<sup>20,21</sup> (VI, FIGURE 4).

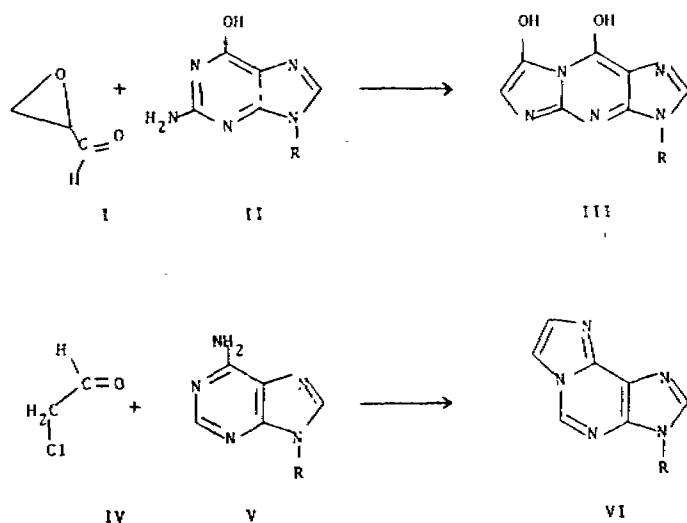
Some of the possible metabolic products of VC and the mechanisms of their formation are depicted in FIGURES 5 and 6. Analogous known *in vivo* and *in vitro* reaction schemes lend support to those given in FIGURES 5 and 6. It is clear from the schemes presented in FIGURES 5 and 6 that a variety of metabolic routes are possible and only laboratory experiments will enable us to determine the exact *in vivo* metabolic pathways. It is also expected that in the liver the  $\text{NAD}^+ \rightleftharpoons \text{NADH} + \text{H}^+$  and alcohol dehydrogenase pathways play an important role and that reactions with sulfhydryl compounds such as cysteine and glutathione are involved, as described below for trichloroethylene.

#### TRICHLOROETHYLENE (TCE)

This compound is a close structural analog of vinyl chloride and hence it is worth summarizing here our present knowledge concerning this compound. TCE is a liquid, b.p.  $87^\circ\text{C}$ ;<sup>28</sup> m.p.  $-88^\circ\text{C}$ .<sup>29</sup> It is prepared by the addition of chlorine to acetylene followed by treatment of the resultant tetrachloroethane with calcium hydroxide. TCE is extensively used in: a) the dry-cleaning industry; b) as a degreasing agent for metal surfaces, leather, and textiles, and c) as a solvent for the extraction of residual oils from vegetable oil cakes, etc. TCE has found wide use for these purposes because of its noninflammability.

Also as a result of its noninflammability, it is frequently used in open-vat operations in poorly ventilated work areas. TCE was used since 1941 as an anesthetic<sup>30</sup> but its use for this purpose has decreased sharply in recent years since it has been replaced by fluoro ethers and related anesthetics.

A number of reports have appeared on the acute toxic effects of TCE in animals and man.<sup>31-34</sup> Most of these studies were by inhalation exposure for short periods of time and few effects were observed. Mice given TCE by intraperitoneal injection showed mild hepatic dysfunction.<sup>34</sup> Mice exposed to TCE by inhalation



In structures II and III R = ribose or deoxyribose.

In structures V and VI R = -CH<sub>3</sub>, ribose or deoxyribose.

FIGURE 4. Reactions of glycidaldehyde and chloroacetaldehyde with purine derivatives.

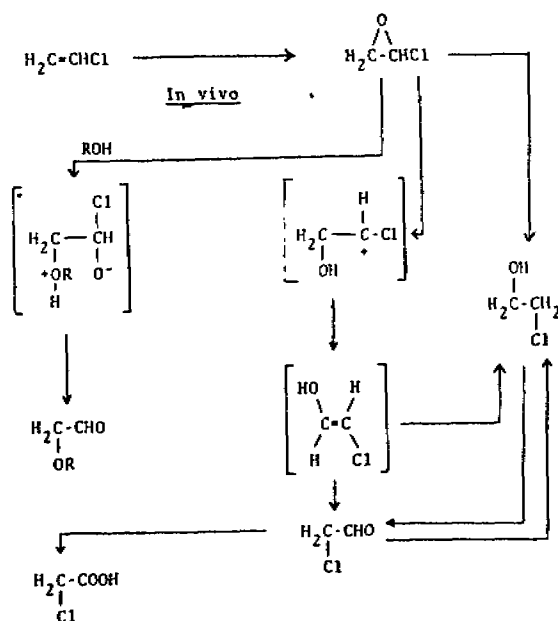


FIGURE 5. Possible reactions of vinyl chloride.

# Van Duuren: Carcinogenic Action of VC

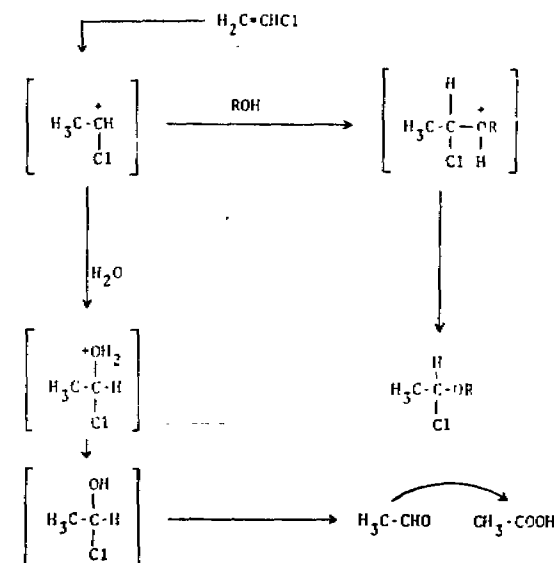


FIGURE 6. Possible reactions of vinyl chloride.

for up to 8 weeks showed fatty degeneration of the liver.<sup>28</sup> Only very limited studies appear to have been carried out on workers exposed to TCE.<sup>29</sup> To our knowledge no carcinogenic effects have been observed with TCE in experimental animals.

Several reports have appeared describing the metabolism of TCE in animals and man. Trichloroacetic acid and trichloroethanol or its glucuronide have been found in the urine of humans or dogs exposed to TCE by inhalation.<sup>30,31</sup> Powell,<sup>32</sup> as early as 1945, suggested a metabolic scheme shown in FIGURE 7 in which an epoxide was proposed as an intermediate. In a subsequent study<sup>33</sup> rats were fed <sup>14</sup>C-labeled TCE and the metabolites were isolated from the urine. Both trichloroacetic acid and trichloroethanol were found. The specific activities of the two metabolites isolated were approximately equal to that of the administered trichloroethylene. This result suggested that there is a pathway via trichloroacetaldehyde, as suggested by Powell<sup>32</sup> (FIGURE 7) and that no exchange of chloride with the body chloride pool occurred. Daniel<sup>34</sup> also found that trichloroethanol is not the precursor of trichloroacetic acid, i.e., these two metabolites are formed via independent pathways. Leibman<sup>35</sup> showed that trichloroethylene is converted to trichloroacetaldehyde (chloral) in liver microsomes of rats, rabbits

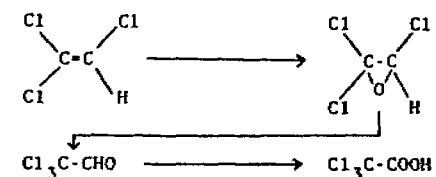


FIGURE 7. Proposed epoxide intermediate in the metabolism of trichloroethylene.

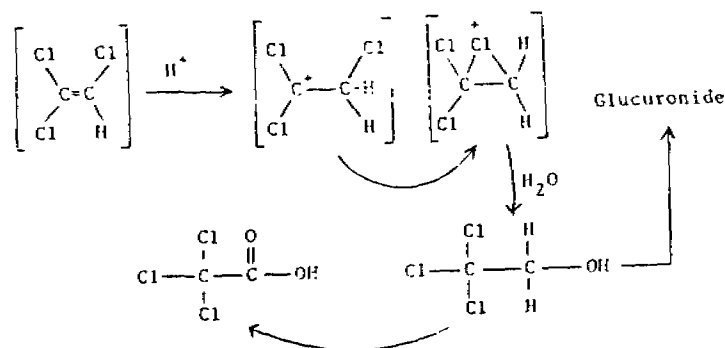


FIGURE 8. Other proposed metabolic intermediates of trichloroethylene.

and dogs in a reaction requiring  $\text{NADPH} + \text{H}^+$  and oxygen. Leibman<sup>10</sup> also examined the effects of activators and inhibitors of the enzyme system. Work by Byington and Leibman<sup>11</sup> presented similar evidence concerning the metabolism of TCE in rat liver. Other possible intermediates have been proposed,<sup>12</sup> e.g., a chloronium ion or carbonium ion intermediate as shown in FIGURE 8.

The effect of disulfiram [bis(diethylthiocarbamoyl)disulfide] on the metabolism of TCE has been examined in humans exposed to TCE by inhalation.<sup>13</sup> It was found that the elimination of trichloroethanol in the urine was decreased by ~50 percent and of trichloroacetic acid by ~80 percent when disulfiram was administered prior to TCE exposure. In the same experiment it was found that unchanged TCE excreted from the lungs increased sharply as compared to the controls. It was concluded that disulfiram inhibits the oxidation of TCE.

McKinney *et al.*<sup>14</sup> reported on the autooxidation of TCE and suggested that an epoxide intermediate may be formed. However, the isolation and identification of this compound has not yet been accomplished.

In summary, a number of metabolites of TCE have been isolated; however, the exact metabolic route, the nature of the intermediates involved, and the effects on liver function remain to be clarified.

#### INDUCTION OF ANGIOSARCOMAS OF THE LIVER IN HUMANS AND EXPERIMENTAL ANIMALS BY OTHER CHEMICAL AGENTS

Angiosarcoma of the liver, which has been much discussed at this conference in connection with vinyl chloride and polyvinyl chloride carcinogenesis, is a rare disease in humans. It is, therefore, worthwhile to mention some other chemicals which cause the same type of liver tumor in animals and man.

A number of reports have appeared indicating that vineyard workers in France and Germany exposed to arsenical insecticides had an unusually high incidence of these tumors.<sup>15-18</sup>

Regelson *et al.*<sup>19</sup> reported on one case of human hemangioendothelial sarcoma of the liver in a patient treated for psoriasis with sodium arsenite (Fowler's solution). This drug was still in use for this purpose at the time of the report of Regelson *et al.*<sup>19</sup>

Toth and co-workers<sup>20,21</sup> reported on the experimental induction of angiosarcoma of the liver in mice by treatment with dimethylnitrosamine<sup>20</sup> and 1,1-dimethylhydrazine.<sup>21</sup> Both compounds were administered orally in these ex-

periments. Dimethylnitrosamine administered to rats also resulted in angiosarcoma.<sup>22</sup> All three of these compounds are currently believed to be converted to alkylating agents *in vivo*. Rats fed a diet containing the plant *Senecio longilobus* (which contains pyrrolizidine alkaloids) resulted in the induction of a low incidence of these tumors.<sup>23</sup>

#### SUMMARY AND CONCLUSIONS

An attempt has been made in this report to bring together relevant information bearing on the possible mechanism of action of the carcinogen vinyl chloride. The relationship of indirect-acting alkylating carcinogens and their direct-acting counterparts is described. It is suggested that an  $\alpha$ -chloro ether or related -onium ion intermediate is involved as an activated carcinogenic intermediate *in vivo*. Based on earlier studies it is also suggested that noncovalent binding to serum albumin, microsomal membranes, or other target sites may precede activation to a carcinogenic intermediate. Serum albumin binding may be related to the site of origin of acroosteolysis, a disease associated with exposure to vinyl chloride. Trichloroethylene is a widely used industrial chemical. Based on its structural similarity to vinyl chloride and known information concerning its metabolism in animals and man, an  $\alpha$ -chloro ether or -onium ion is suggested as an important intermediate in its metabolism. It is likely to be carcinogenic, particularly to the liver but this remains to be established. Other chemical agents that have been shown to be carcinogenic to animals and man are briefly mentioned.

#### ACKNOWLEDGMENTS

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