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# Prenatal Susceptibility to Carcinogenesis by Xenobiotic Substances Including Vinyl Chloride

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**The carcinogenicity of vinyl chloride for experimental animals when administered transplacentally is reviewed in comparison with known transplacental carcinogens, including those that, like vinyl chloride, are dependent on enzyme-mediated metabolic conversion to a reactive intermediate in maternal or fetal tissues. Vinyl chloride is converted by mixed-function oxidases to the reactive metabolite chlorooxirane, the carcinogenicity of which is also reviewed. Vinyl chloride is unequivocally a transplacental carcinogen for the rat. No evidence exists, however, to support the hypothesis that exposure of male rats to vinyl chloride or any other carcinogen confers an increased risk of tumor development on their progeny. Many structural analogs of vinyl chloride, i.e., substituted ethylenes, are also carcinogenic for adult animals, and can with confidence likewise be predicted to be effective transplacental carcinogens.**

## Introduction

The carcinogenicity of vinyl chloride and its predilection for the hepatic blood vessels of both experimental animals and man is now well recognized, and the substance is rightly regarded as a serious hazard in the workplace. Moreover, the demonstration by Maltoni (*1*) that vinyl chloride is not only carcinogenic for adult rodents but is a transplacental carcinogen for the rat as well, has raised concern over a possible risk of carcinogenesis in children born to mothers who had been employed in vinyl chloride manufacturing during their pregnancy. In the United States this has also led to questions of whether it is reasonable to single out women in the workplace as individuals especially at risk and whether to do so unfairly infringes upon their rights to equality in employment. It has been asked whether men may not equally be at risk in terms of the potential of workplace exposure to vinyl chloride and related compounds to cause genetic damage in workers so exposed, and in this indirect way to contribute to an increased risk of cancer in their offspring. These are important questions, not all of which can be fully answered at the present time. Our laboratory has not been engaged in research on vinyl chloride and therefore can contribute no new data to what has been presented during the course of this conference. However, we have been engaged for many years in studying the phenomena of prenatal carcinogenesis by a variety of chemical carcinogens and in species as diverse as the mouse and subhuman primates, and it is the purpose of this presentation to provide a context of current knowledge about the phenomena of prenatal carcinogenesis within which the risk of prenatal exposure to this agent and related compounds can be evaluated.

## Mechanisms of Chemical Carcinogenesis

When one tests a substance or a mixture of substances for carcinogenic activity in rodents, a positive result commonly takes one or more of the following three forms: (1) a higher incidence of tumors of one or more organ systems is observed

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among treated animals in comparison with controls; if several different doses have been administered, test animals receiving higher dosage levels (except at very high, toxic levels) have a higher incidence of tumors than test animals receiving lower dosage levels; (2) a higher multiplicity of tumors occurs in one or more organ systems in treated versus control animals; if more than one dosage level is given to different groups of test animals, multiplicity, like tumor incidence, also varies in rough proportion to dose, and animals that receive the highest nontoxic dosage regimen develop the highest multiplicity of tumors; (3) the latency for tumor development is shortened in test animals that develop tumors in comparison with control animals that develop the same tumors; this effect also may be proportional to dose, so that animals that receive the highest dosage of test compound develop tumors after the shortest period of time from the beginning of treatment. The results of a bioassay by itself can only assert that a substance indeed is carcinogenic, in the purely phenomenological sense of the term, by one or more of the above criteria. A bioassay says nothing about the mechanism by which carcinogenesis has been effected. This is a significant point, as evidence is steadily accumulating that not all of the extremely diverse agents capable of inducing tumors in animals or man act by the same mechanism.

There exist a wide variety of chemicals that by these criteria have been shown to have the capacity to induce tumors in animals. These agents are extremely diverse in their chemical features, and include certain heavy metal cations; mineral fibers such as asbestos; and organic polymers, both soluble (iron–dextran; DEAE–dextran) and insoluble, such as plastic films. By far the greatest number of known substances carcinogenic for man or animals, however, are small organic molecules. For the overwhelming majority of these carcinogens there is a common mechanism of action. Agents belonging to this class either react chemically, or are transformed in the course of metabolism to products that react chemically with intracellular nucleophiles, including nucleic acids, to form covalent bonds. Such reactions are irreversible, and proceed through the intermediate formation of an electropositive, or electrophilic, intermediate (*2*). Agents of this sort react with DNA to induce mutations and can be detected by their capacity to induce nonscheduled DNA repair synthesis in nondividing cells; the term "genotoxic" is becoming accepted as a descriptive term for agents of this class, to which vinyl chloride belongs.

Most genotoxic carcinogens are not themselves chemically reactive and require metabolic transformation to a chemically reactive metabolite or ultimate carcinogen. Although there exist a variety of mechanisms involving different enzyme systems by which such changes can be effected, the most common route, which is applicable to vinyl chloride, involves the mixed function oxidases (*3*). This is a class of cytochrome-containing enzymes, dependent on molecular oxygen and reduced nicotinamide-adenine dinucleotide phosphate (NADPH), which have very broad substrate specificities and are capable of catalyzing a variety of reactions, including the epoxidation of carbon-carbon double bonds, the oxidation of aromatic compounds through the formation of arene oxides, and the oxidative dealkylation of compounds such as nitrosamines by hydroxylation of carbon atoms alpha to a nitrogen or oxygen atom. The capacity of these enzyme systems to metabolize different classes of foreign substances, including chemical carcinogens, varies from individual to individual and from tissue to tissue, and is in part responsible for variation among individuals and from one organ system to another in susceptibility to different types of chemical carcinogens.

## Transplacental Carcinogenesis in Experimental Animals

To demonstrate transplacental carcinogenesis, experiments must be designed to eliminate the possibility of exposure to a chemical carcinogen by any route other than across the placenta. For rats and mice, in which species most such experiments have been carried out, this is usually accomplished by allowing the carcinogen-treated, timed pregnant female to deliver her young on top of a wire mesh screen so that the pups, as they are delivered, fall through the mesh into a cage below which is inhabited by a lactating female. With luck, the latter will gather up the newborns and care for them as her own, raising them to maturity without their having come postnatally into contact with their carcinogen-contaminated natural mother. For mice, such screens are generally made of 1/2 in. hardware cloth; for rats, poultry fencing generally proves adequate.

General features of experimental transplacental carcinogenesis have been reviewed (*4-6*). The periods of susceptibility to different types of transplacental toxic effects in rodents by chemical carcinogens are strictly related to stages of prenatal development. Exposure during the interval between conception and implantation of the blastocyst will either be without effect or will be embryocidal. Exposure of rats and mice to the same agent

between approximately days 7 and 10, or between implantation of the blastocyst and development of the true placenta, when embryogenesis is occurring with great rapidity, may be lethal to the conceptus; at nonlethal doses there may be severe developmental abnormalities. At lower dosage levels, in the range of exposures that are carcinogenic during later periods of development, tumors are generally not induced in offspring of animals exposed to carcinogens during this period even though their life expectancy is not significantly shorter than that of untreated offspring. After day 12, however, and usually with increasing efficiency thereafter until termination of pregnancy, exposure of a gravid female to a carcinogen will cause tumors to develop in her offspring. Tumors develop in different organ systems in different species in response to a given agent, but are generally at least in part morphologically and anatomically similar to those inducible by postnatal exposure to the same agent. A given agent may not affect a given tissue or organ similarly in all species, and the tumor spectrum seen as a consequence of prenatal exposure may vary markedly from one species to another.

The most potent transplacental carcinogens are direct-acting alkylating agents, which, like methylnitrosourea (Fig. 1), decompose to reactive intermediates without enzymatic catalysis. The next higher homolog of this compound, ethylnitrosourea (ENU), is extremely active transplacentally and has been extensively studied in both rodent and nonrodent species. When this compound is administered as a single injection to a pregnant rat on one of the first 11 days of gestation, high doses result in devastating teratogenic effects or in death of the embryo, but not in tumorigenesis in surviving offspring. Beginning on day 12 and with increasing efficiency thereafter the offspring, which appear normal at birth if dosage is kept at a level below the acutely toxic range, subsequently develop tumors of the central and peripheral nervous system, with overt signs of disease developing 2 months to 2 years after birth (*7*). All three of the parameters previously mentioned as indices of carcinogenicity can be observed in experiments with ENU; the incidence of tumors and the multiplicity of tumors of the nervous system in treated offspring are both directly proportional to the dose administered to the mother, while latency is inversely proportional to dose. Despite the fact that ENU is a direct acting agent and is distributed nearly uniformly throughout the tissues of the rat fetus, tumors rarely appear in organ systems other than the nervous system. Occasionally, tumors of the kidney are seen, and an occasional offspring may develop leukemia. Epithelial tumors of the liver and the lung, for example, are virtually never encountered in rats transplacentally exposed to ENU.

When one compares the dose-response relationships for offspring of rats treated on day 15 of gestation with the response of adult rats to the same agent, it is found that the offspring are approximately 50-fold more susceptible than adults to the carcinogenic effects of this agent: the dose necessary to induce one or more neurogenic tumors in 50% of exposed adult rats is on the order of 150 mg/kg, but the corresponding dose for transplacentally exposed offspring is approximately 3 mg/kg (calculated on the basis of the mother's total body weight).

If exactly the same sort of experiment is conducted in the mouse, however, the results are qualitatively much different. Tumors of the nervous system are relatively rare in mice following transplacental exposure to ENU. As in rats, however, transplacentally treated offspring exposed on or after day 12 of gestation develop tumors. In contrast to rats, mice exposed transplacentally to ENU develop epithelial tumors of the lung and hepatocellular tumors of the liver in high incidence and multiplicity (*8*). In both species, the fetus is quantitatively more susceptible than the adult: susceptibility is greatest during the second half of the period of gestation, and increases as gestation proceeds towards parturition. Adult animals are less sensitive, by one to two decimal orders of magnitude, to the carcinogenic effects of ENU. This much higher prenatal susceptibility is one of the principal reasons for concern that transplacental exposure to carcinogens in the mother's workplace or environment may be of significant risk for the human fetus.

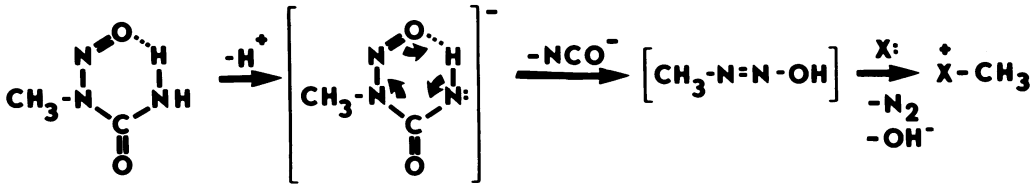


FIGURE 1. Decomposition of methylnitrosourea to yield a reactive alkylating diazonium hydroxide product. The initial step is pH-dependent, but does not require enzyme-mediated catalysis.

Recent studies carried out with ENU in our laboratories have shown, however, that two significant differences from the patterns of response seen in rodents occur when transplacental carcinogenesis experiments are carried out in a nonhuman primate. Different tissues are affected by the carcinogen, and the period of greatest prenatal susceptibility is early rather than late in gestation. Experiments on transplacental carcinogenesis in the Old World monkey, *Erythrocebus patas*, whose gestation period averages 170 days, have clearly shown that ENU is a transplacental carcinogen for this primate (*9*), but that it causes tumors principally of the vascular connective tissues and to a lesser extent the liver, kidney and brain, a pattern of response different from either the rat or the mouse. Tumor incidence is higher and latency is shorter in offspring than in pregnant or nonpregnant treated adults; in that respect the pattern resembles that seen in both rodent species, but neither the rat or the mouse yields a spectrum of tumors predictive of the results of exposing monkeys to the same agent. In our experiments pregnant patas monkeys were exposed to ENU during either the first half or the second half of gestation, or throughout pregnancy. It has become strikingly apparent that animals exposed for the first time at day 30 are at much higher risk for tumor development than animals given comparable exposure but beginning even 30 days later in gestation. This marks a significant departure from the patterns seen in rodents and suggests that the period of maximum intrinsic susceptibility to chemical carcinogens in other primates, including man, may well be during the first trimester of pregnancy and in that respect may resemble the period of greatest susceptibility to teratogens. Obviously, this includes the early fraction of gestation during which, at the time of possibly greatest vulnerability of her conceptus, a woman may not know for certain that she is pregnant and may therefore not be warned to take special precautions to prevent exposure to noxious agents.

In other important respects, such as the greater susceptibility of the fetus, experience in the patas monkey is comparable to that in the rat and mouse and suggests that the phenomenon of high fetal susceptibility to carcinogens is a general one. The fact that the organ systems principally affected by ENU in this species are different from those of the rodent species further emphasizes that one should be extremely cautious in extrapolating from any other species to man in predicting the site of action of a chemical carcinogen, even a direct acting one. The one common pattern valid across species lines in the matter of prenatal organ specificity is that the nervous system and the kidneys appear to be susceptible to at least some extent to this agent in all species tested, an observation which brings to mind the fact that tumors of the kidney and nervous system predominate among solid tumors of childhood.

## Transplacental Carcinogenesis by Metabolism-Dependent Carcinogens

The vast majority of genotoxic chemical carcinogens are not direct-acting, but, as indicated previously, require metabolic conversion to a chemically reactive ultimate carcinogen in order to effect carcinogenesis. It has been shown that in rodents, the mixed function oxidase enzymes principally involved in activation of chemical carcinogens are present at low or virtually undetectable levels in fetal tissues until immediately prior to parturition, and even then are present at levels that are minuscule in comparison with those in adult tissues (*10*). The picture is complicated by the fact that these enzymes are inducible, and their levels in tissues such as the liver may be significantly altered by exposure to chemical agents that are substrates for these enzymes, including carcinogens such as methylcholanthrene (*3*). The role of enzyme induction in modifying fetal susceptibility to transplacental carcinogens has not yet been well studied and remains conjectural. However, the low levels of enzymes present in noninduced fetal rodent tissues result in extremely inefficient conversion of most substances to reactive ultimate carcinogenic metabolites. Thus, when the chemically reactive metabolite is very unstable, i.e., has an extremely short half-life under physiologic conditions, it cannot be effectively generated in maternal tissues and transported via maternal and fetal bloodstreams to fetal tissues. Very short-lived reactive metabolites must be generated in situ in any fetal tissue in which carcinogenesis is to occur. Agents whose carcinogenicity is mediated by such metabolites are extremely poor transplacental carcinogens. An example is dimethylnitrosamine (DMN; Fig. 2). DNM is metabolized by mixed function oxidases by the *N*-dealkylation mechanism, generating an intermediate methyl(hydroxymethyl)-nitrosamine which is far too unstable to demonstrate even spectroscopically. It has never been synthesized. This is an excellent example of an agent which presumably must be formed by fetal enzymes. When the parent compound, DMN, was tested for transplacental carcinogenic activity it was found as expected to be much less efficient in offspring than in their mothers in the induction of tumors of the kidney (*11*).

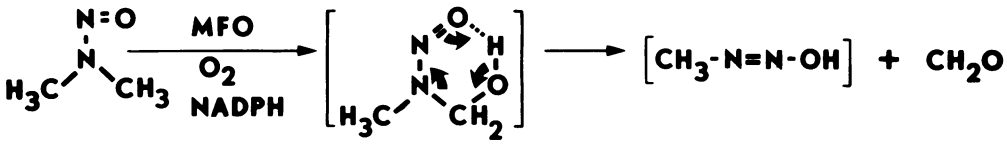


FIGURE 2. Metabolic activation of dimethylnitrosamine by a mixed-function oxidase (MFO) to a reactive intermediate formally equivalent to that generated nonenzymatically from methylnitrosourea as diagrammed in Figure 1. The enzyme catalyzed *N*-demethylation reaction requires molecular oxygen and NADPH, and yields a product too unstable and short-lived to isolate or synthesize.

From the example of dimethylnitrosamine, one might be tempted to infer that the transplacental route of exposure is insignificant for metabolism-dependent carcinogens and, since this encompasses the vast majority of genotoxic carcinogens, is not a significant route of human exposure. Such a prediction is probably wrong. Proximate and ultimate carcinogenic metabolites of many metabolism dependent carcinogens are much more stable than methyl(hydroxymethyl)nitrosamine. An excellent example is afforded by the polynuclear aromatic hydrocarbons, especially 7,12-dimethylbenz[a]anthracene (DMBA, Fig. 3) which in common with many of the other carcinogenic substances belonging to this chemical class can undergo metabolism to a variety of chemically reactive, arene oxide metabolites which are all ultimate carcinogens of varying potency. The most carcinogenic metabolites of this type of compound are the bay region diolepoxides (*12*), formed by three sequential metabolic steps as indicated in Figure 3. The arene oxides, dihydrodiol, and diolepoxide (Fig. 3) have all been synthesized and are sufficiently stable for not only synthesis and characterization, but for direct testing of carcinogenic potency in experimental animals (*13*, *14*). Accordingly, it is quite possible that metabolites of this sort may be formed in maternal tissues and may succeed in traversing the maternal bloodstream and placenta to reach the fetus. When the parent hydrocarbon DMBA was tested for transplacental carcinogenicity in rats by using a foster nursing procedure (*15*), it was found to be extremely potent. The experiment was terminated at 52 weeks with only 20% of the offspring still surviving; large numbers of tumors were seen in the central and peripheral nervous systems, the kidneys, and blood vessels, as well as in other sites. Thus, not only is this particular metabolism dependent agent a potent transplacental carcinogen, but it actually affects a broader spectrum of organ systems in the fetus of the rat than the extremely potent direct acting carcinogen ENU.

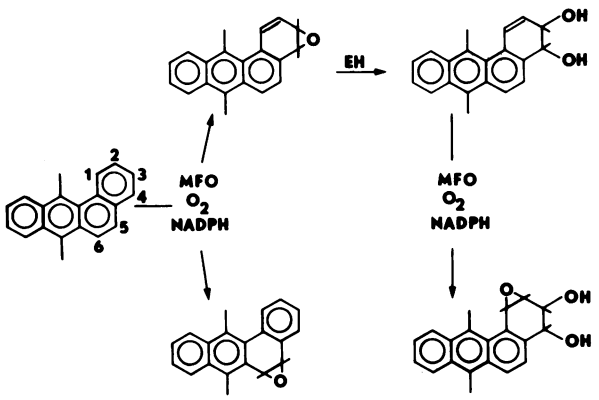


FIGURE 3. Some mixed-function oxidase reactions of the polynuclear aromatic hydrocarbon 7,12-dimethylbenz[a]anthracene (DMBA), yielding various mutagenic arene oxide products. These may subsequently be hydrolyzed by epoxide hydratase (EH) to dihydrodiols, which in turn may serve as substrates for MFO reactions that yield diolepoxides. Although carcinogenic and very reactive, diolepoxides can be synthesized.

## Metabolism and Carcinogenicity of Vinyl Chloride

The carcinogenicity of vinyl chloride for rats, mice, and hamsters over an extremely wide range of doses as well as its transplacental carcinogenic effects in rats, are presented by Maltoni (*16*); an earlier version of his data for rats (*1*) is summarized in Table 1. It can be seen that on inhalation of vinyl chloride, tumors are induced in dose-dependent fashion in adult rats in the liver, nasal cavity, kidneys, Zymbal's gland, and most importantly and consistently, in the blood vessels, especially those of the liver. Transplacental exposure for a period of one week, between the 12th and 18th days of pregnancy, generated tumors in three of these tissues, the kidney, blood vessels, and Zymbal's gland, in the offspring (Table 2). Although no controls were included specifically in the transplacental study, the very large series of historical control animals carefully examined in that laboratory are convincing proof that the elevated incidence of tumors of these three tissues is real, and that vinyl chloride is effectively a transplacental carcinogen in the rat. It is noteworthy that the sites and kinds of tumors in-

duced by vinyl chloride are different in part from those that resulted from transplacental exposure to any of the agents discussed previously, none of which affected the Zymbal's glands. The tissues and organs affected in offspring exposed transplacentally to vinyl chloride included some but not all those in which tumors developed in adults subjected to much more prolonged exposures (cf. Tables 1 and 2), and in the transplacental study, significant numbers of tumors were induced in the offspring but not in the mothers.

Vinyl chloride is a metabolism-dependent carcinogen, dependent for its carcinogenicity on mixed function oxidases (*17*) which convert vinyl chloride to its epoxide derivative chloroethylene oxide (chlorooxirane, Fig. 4). The carcinogencity of chloroethylene oxide and of its rearrangement product, chloroacetaldehyde, were recently investigated by researchers at the International Agency for Research on Cancer in Lyon, France, who tested both compounds by subcutaneous injection and by skin painting, the latter followed by phorbol ester promotion, in mice. Chloroethylene oxide proved to be an effective carcinogen, inducing both papillomas and carcinomas in the skin and giving rise to sarcomas on injection (Table 3). Its rearrangement product, chloroacetaldehyde, was toxic, but not demonstrably carcinogenic (*18*). It was noted by the IARC investigators is that the half-life of chloroethylene oxide for hydrolysis at 37° C is on the order of 0.9 min. This is sufficient time for chloroethylene oxide formed in maternal tissues to reach the fetus by way of the placenta, and provides strong suggestive evidence that maternal metabolism contributes to transplacental carcinogenicity of vinyl chloride. To date, however, chloroethylene oxide has not itself been tested for transplacental carcinogencity.

## Analogs of Vinyl Chloride

It is important to note that vinyl chloride is by no means unique with respect to chemical structure, and that a wide variety of compounds are in current use in large volumes in industrial processes which differ from vinyl chloride only by further substitution of the vinyl chloride molecule. A partial list of such substances is given in Table 4. Furthermore

**Table 1. Tumors in Sprague-Dawley rats after inhalation exposure to vinyl chloride, 4 hr daily and 5 days weekly for 52 weeks. Partial results after 135 weeks.[a]**

| | | Rats with tumors | | | | | |
|---|---|---|---|---|---|---|---|
| | | | | Angiosarcoma | | | |
| Vinyl chloride, ppm | Rats at risk (both sexes) | Zymbal's gland carcinoma | Nephroblastoma | Liver | Other | Liver cell tumors | Nasal cavity[b] |
| 30,000 | 60 | 35 | 0 | 18 | 1 | 1 | 1 |
| 10,000 | 69 | 16 | 5 | 9 | 3 | 1 | 7 |
| 6,000 | 72 | 7 | 4 | 13 | 3 | 1 | 3 |
| 2,500 | 74 | 2 | 6 | 13 | 3 | 2 | 5 |
| 500 | 67 | 4 | 4 | 7 | 2 | 3 | 0 |
| 250 | 67 | 0 | 6 | 4 | 2 | 0 | 0 |
| 50 | 64 | 0 | 1 | 1 | 1 | 0 | 0 |
| None | 68 | 0 | 0 | 0 | 0 | 0 | 0 |

[a]Data from Maltoni (*1*).
[b]Originally reported (*1*) as neuroblastoma of the brain.

**Table 2. Tumors in female Sprague-Dawley rats exposed to vinyl chloride by inhalation 4 hr daily from day 12 through day 18 of gestation, and in their offspring.[a]**

| | | | | Rats with tumors after 115 weeks | | | |
|---|---|---|---|---|---|---|---|
| | | Rats exposed | | | | Angiosarcoma | |
| Generation | Vinyl chloride concentration (ppm) | Total | Survivors | Zymbal's gland carcinoma | Nephroblastoma | Liver | Other |
| Parents | 10,000 | 30 | 30 | 1 | 0 | 0 | 1 |
| | 6,000 | 30 | 30 | 0 | 0 | 0 | 0 |
| Offspring | 10,000 | 54 | 12 | 3 | 1 | 0 | 2 |
| | 6,000 | 32 | 8 | 1 | 0 | 0 | 2 |

[a]Data from Maltoni (*1*).

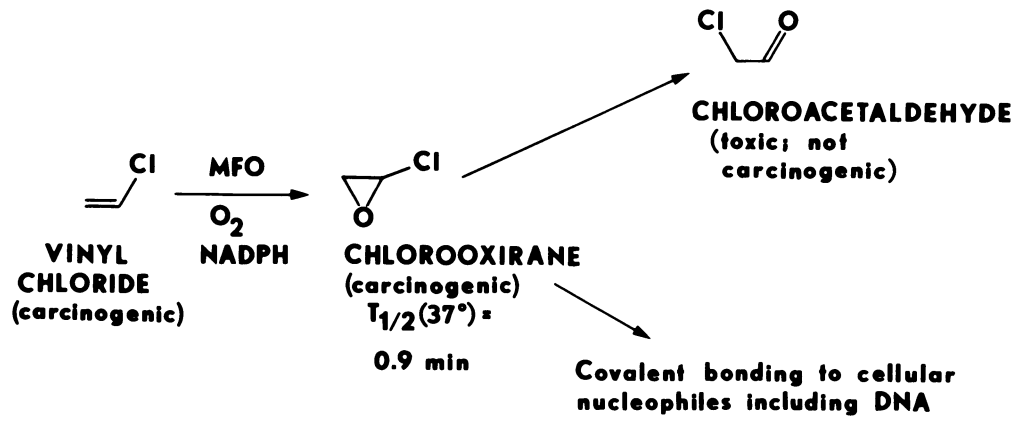


FIGURE 4. Metabolism of vinyl chloride by mixed function oxidases to chlorooxirane, a carcinogenic substance that is stable enough to synthesize.

**Table 3. Carcinogenicity of chloroethylene oxide in adult mice.[a]**

| Effective number | Route | Tumor-bearing animals, % | Duration of experiment, days |
|---|---|---|---|
| 28 M | 32 SC injections | 15 (54) | 549 |
| 24 F | | 12 (50) | 549 |
| Control | | | |
| 30 M | | 0 (0) | 549 |
| 28 M | 1 mg initiation (skin); TPA promotion | 26 (93), papillomas<br>5 (18), carcinomas | 590 |
| Control | | | |
| 28 M | | 4 (15), papillomas<br>0, carcinomas | 590 |

[a]Data from Zajdela et al. (*18*).

**Table 4. Carcinogenicity in adult rodents of structural analogs of vinyl chloride reviewed in the IARC Monograph Series.**

| Compound | Structure | Reference | Site of lesions | |
|---|---|---|---|---|
| | | | Rats | Mice |
| Vinyl chloride | $CH_2=CHCl$ | (*22a*) | Angiosarcoma<br>Zymbal's gland<br>CNS<br>Kidney | Angiosarcoma<br>Lung<br>Mammary |
| Vinylidene chloride | $CH_2=CCl_2$ | (*22b*) | Similar to vinyl chloride, but incomplete in 1979 | |
| Trichlorethylene | $CHCl=CCl_2$ | (*23a*) | (Inadequate) | Lung, liver |
| Tetrachlorethylene | $CCl_2=CCl_2$ | (*23b*) | Negative | Liver |
| Chloroprene | $CH_2=CH\text{-}CCl=CH_2$ | (*22c*) | Inadequate | - |

there is an even larger list of substances that resemble vinyl chloride in that they are substituted derivatives of ethylene, but differ in that they lack a chlorine atom. A very brief list of important derivatives of this sort is given in Table 5. A much longer list is given in the review by Posner and Falk (*19*). It is reasonable to postulate that metabolism via reactive epoxides is one route of metabolism to be expected for all these compounds, and mutagenic metabolites of some of them have been demonstrated (*20*, *21*). If mutagenic epoxides are formed in significant quantities in maternal tissues and are chemically reactive, yet stable enough to reach the fetus, it is probable that they too would have some measure of transplacental carcinogenic activity. Some of the substances listed in Tables 4 and 5 have been reviewed for risk of carcinogenicity to man in the IARC monograph series (*22*, *23*). Other compounds

Table 5. Compounds structurally similar to vinyl chloride that may have similar carcinogenic activity.

| Compound | Structure | Reference | Site of lesion | |
|---|---|---|---|---|
| | | | Rats | Mice |
| Vinyl bromide | $CH_2=CHBr$ | Not yet reviewed | | |
| Styrene | $Ch_2=CHC_6H_5$ | (*22d*) | – | Lung (?) |
| Acrylonitrile | $CH_2=CHCN$ | (*22e*) | Brain<br>Forestomach<br>Zymbal's gland | |
| Acrylamide | $CH_2=CHCONH_2$ | Not yet reviewed | | |
| Ethyl acrylate | $CH_2=CHCOOC_2H_5$ | Not yet reviewed | | |

listed here have been tested in the United States by the National Cancer Institute-National Toxicology Program. As a significant number have been found to be carcinogenic in at least one rodent species, it is reasonable to regard the entire class with suspicion as possible potential carcinogens and transplacental carcinogens pending the acquisition of further data.

## Effects of Transplacental Carcinogens on Subsequent Generations: Lack of Male Parental Risk for Prenatal Carcinogenesis

In the context of our present knowledge, prenatal carcinogenesis is virtually synonymous with transplacental carcinogenesis. That is, there is very little evidence to indicate that exposure of either male or female parents to a chemical carcinogen prior to conception confers an increased risk of carcinogenesis on their offspring. It should be pointed out that carcinogenic risk is not the only risk, and that exposure of males to toxic agents may cause sterility or reduce fertility. In rodents it has also been shown that dominant lethal mutations may be induced in this manner, the consequence of which is death of the conceptus (*24*). These hazards however are different from carcinogenic risk for surviving offspring, which has not been shown to be a consequence of exposure of adult males or of adult females prior to conception.

The experimental data which are relevant to this question generally concern what has been termed the "second generation effect" in prenatal carcinogenesis, which has been reviewed by Tomatis (*25*). The general design of experiments of this sort is as follows: The female of a parental generation, P, is given a chemical carcinogen during pregnancy. Male and female offspring of the treated female, constituting the $F_1$ generation, are then at risk for transplacental carcinogenesis. On attaining sexual maturity, and before tumors resulting from transplacental exposure appear, $F_1$ males and females are mated to produce an $F_2$ generation. Both parents of the $F_2$ generation had been exposed to carcinogen during prenatal life, but at no time were the $F_2$ animals exposed. In addition, males of the prenatally exposed $F_1$ generation were bred with untreated females to produce offspring designated $F_2M$, whose male parents only had been subjected to chemical carcinogens. Likewise, the $F_1$ females were bred with untreated control males to produce a $F_2$ F generation, of which only female parents had experienced exposure to chemical carcinogens. Similar experiments have been continued to the third and higher generations in attempts to demonstrate a higher incidence of tumors in comparison with untreated controls. Such experiments have been carried out with polynuclear aromatic hydrocarbons, direct acting nitrosourea carcinogens, and with ethyl carbamate, in three laboratories. They have consistently demonstrated that a small, often statistically insignificant but nevertheless reproducible and persistent excess risk of carcinogenesis is present in $F_2$ and $F_2F$ generations. $F_2M$ offspring in general have shown no excess risk. The numbers of tumors observed in the second generations in experiments of this sort have invariably been extremely small, leaving much to be desired in terms of statistical significance. However, these studies constitute the only experimental evidence for a chemically induced enhanced susceptibility to cancer mediated by damage to the germ cells. Present evidence indicates that if this effect is real, it is most significant for the female germ cells, which are undergoing rapid mitotic division during the final week of intrauterine development in the female rat or mouse fetus.

On the basis of present information, therefore, it is clear that prenatal carcinogenesis by chemicals is principally a direct effect of chemicals or their fetal or maternal metabolites upon fetal tissues, rather than upon the germ cells of the parents of either

sex; if an effect mediated via damage to parental germ cells exists, that damage with its attendant increased risk of carcinogenesis in the offspring appears greatest when the female parent is exposed to a carcinogen; and that the fetus is at risk on account of maternal exposures to potential carcinogens, not only because it is inevitably exposed to any agent that may find its way into the maternal bloodstream, but also because carcinogenic or toxic metabolites which may be even more dangerous than the environmental precursor may be generated by maternal tissues and transferred via the placenta to the fetus.

The incidence of tumors that will develop in a given organ system following transplacental exposure to a carcinogen is not necessarily the maximum incidence possible. Cocarcinogenic phenomena, in which exposure to a second, noncarcinogenic agent accelerates the development and increases the yield of tumors resulting from a previous carcinogenic exposure, are well demonstrated in transplacental carcinogenesis (*26*), especially in the mouse where they have been documented for the skin and for the liver. Potential tumor cells may remain quiescent for prolonged periods after exposure to the inducing agent, and in evaluating the significance of transplacental exposure one must bear in mind that subsequent, postnatal exposure to noncarcinogenic promoting agents may act in a strongly synergistic fashion with prenatal exposure to a carcinogen and may greatly increase the risk of carcinogenesis.

Finally, all agents known to be prenatal—that is, transplacental—carcinogens are also carcinogenic to some extent during postnatal life in one or more species. To some extent, this is a consequence of the manner in which the science of transplacental carcinogenesis has developed; known carcinogens have been selected for testing for transplacental effects. Nonetheless, the generalization holds that there is no known purely transplacental carcinogen.

## REFERENCES


1. Maltoni, C. Predictive value of carcinogenesis bioassays. Ann. N.Y. Acad. Sci. 271: 431-443 (1976).
2. Miller, E. C., and Miller, J. A. The metabolism of chemical carcinogens to reactive electrophiles and their possible mechanisms of action in carcinogenesis. In: Chemical Carcinogens, (ACS Monograph 173), C. E. Searle, ed., American Chemical Society, Washington, D.C., 1976, pp. 737-762.
3. Lu, A. Y. H., and Levin, W. The resolution of the liver microsomal hydroxylation system. Biochem. Biophys. Acta 344: 205-240 (1974).
4. Tomatis, L., and Mohr, U., Eds. Transplacental Carcinogenesis (IARC Scientific Publications No. 4). International Agency for Research on Cancer, Lyon, 1973.
5. Rice, J. M., Ed. Perinatal Carcinogenesis (NCI Monograph 51). U.S. Government Printing Office, Washington, D.C., 1979.
6. Rice, J. M. Carcinogenesis: a late effect of irreversible toxic damage during development. Environ. Health Perspect. 18: 133-139 (1976).
7. Ivankovic, S. and Druckrey, H. Transplacentare Erzeugung maligner Tumoren des Nervensystems. I. Äthylnitrosoharnstoff (ANH) an BD-IX Ratten. Z. Krebsforsch. 71: 320-360 (1968).
8. Vesselinovitch, S. D., Koka, M., Rao, K. V. N., Mihailovich, N., and Rice, J. M. Prenatal carcinogenesis by ethylnitrosourea in mice. Cancer Res. 37: 1822-1828 (1977).
9. Rice, J. M., Palmer, A. E., London, W. T., Sly, D. L. and Williams, G. M. Transplacental effects of ethylnitrosourea in the patas monkey. In: Tumours of Early Life in Man and Animals, L. Severi, Ed., Perugia Quadrennial International Conferences on Cancer, Perugia, Italy, 1978, pp. 893-906.
10. Lucier, G. W., Lui, E. M. K., and Lamartiniere, G. A. Metabolic activation/deactivation reactions during prenatal development. Environ. Health Perspect. 29: 7-16 (1979).
11. Alexandrov, V. A. Blastomogenic effect of dimethylnitrosamine on pregnant rats and their offspring. Nature 218: 280-281 (1968).
12. Jerina, D. M., Thakker, D. R., Yagi, H., Levin, W., Wood, A. W., and Conney, A. H. Carcinogenicity of benzo[a]pyrene derivatives: the bay region theory. Pure Appl. Chem. 50: 1033-1044 (1978).
13. Sukumaran, K. B., and Harvey, R. G. Synthesis of *trans*-3,4-dihyroxy-3,4-dihydro-7,12-dimethylbenz[a]anthracene, a highly carcinogenic metabolite of 7,12-dimethylbenz[a]anthracene. J. Am. Chem. Soc. 101: 1353-1354 (1979).
14. Slaga, T. J., Huberman, E., Digiovan, J., Gleason, G., and Harvey, R. G. Importance of the bay region diol-epoxide in 7,12-dimethylbenz[a]anthracene skin tumor initiation and mutagenesis. Cancer Lett. 6: 213-220 (1979).
15. Rice, J. M., Joshi, S. R., Shenefelt, R. E., and Wenk, M. Transplacental carcinogenic activity of 7,12-dimethylbenz-[a]anthracene. In: Carcinogenesis, Vol. 3: Polynuclear Aromatic Hydrocarbons. P. W. Jones and R. I. Freudenthal, eds. Raven Press, New York, 1978, pp. 413-422.
16. Maltoni, C., Lefemine, G., Ciliberti, A., Cotti, G., and Carretti, D. Carcinogenicity bioassays of vinyl chloride monomer: a model of risk assessment on an experimental basis. Environ. Health Perspect. 41: 3-30 (1981).
17. Salmon, A. G. Cytochrome P-450 and the metabolism of vinyl chloride. Cancer Lett. 2: 109-114 (1976).
18. Zajdela, F., Croisy, A., Barbin, A., Malaveille, C., Tomatis, L., and Bartsch, H. Carcinogenicity of chloroethylene oxide, an ultimate reactive metabolite of vinyl chloride, and bis(chloromethyl)ether after subcutaneous administration and in initiation-promotion experiments in mice. Cancer Res. 40: 352-356 (1980).
19. Posner, H. S., and Falk, H. L. Haloethylene-related compounds of industrial, environmental, and medical significance. Environ. Health Perspect. 21: 293-326 (1977).
20. Bartsch, H. Malaveille, C., Barbin, A., and Planche, G. Mutagenic and alkylating metabolites of haloethylenes, chlorobutadienes and dichlorobutenes produced by rodent or human liver tissue. Arch. Toxicol. 41: 249-277 (1979).
21. Simmon, V. F., and Baden, J. M. Mutagenic activity of vinyl compounds and derived epoxides. Mutat. Res. 78: 227-231 (1980).
22. IARC. IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. Vol. 19, Some Monomers, Plastics and Synthetic Elastowers, and Acrolein, International Agency for Research on Cancer, Lyon, 1979, (a) pp. 377-437; (b) pp. 452-453; (c) pp. 131-156;

(d) pp. 231-274; (e) pp. 73-133.
23. IARC. IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. Vol. 20, Some Halogenated Hydrocarbons, International Agency for Research on Cancer, Lyon, 1979, (a) pp. 545-572; (b) pp. 491-514.
24. Health and Welfare Canada. The Testing of Chemicals for Carcinogenicity, Mutagenicity, Carcinogenicity. Health and Welfare Canada, Ottawa, 1975.
25. Tomatis, L. Prenatal exposure to chemical carcinogens and its effect on subsequent generations. Natl. Cancer Inst. Monogr. 51: 159-184 (1979).
26. Goerttler, K., and Lohrke, H. Diaplacental carcinogenesis: initiation with the carcinogens dimethylbenzanthracene (DMBA) and urethane during fetal life and postnatal promotion with the phorbol ester TPA in a modified 2-stage Berenblum/Mottram experiment. Virchows Arch. Pathol. A 372: 29-38 (1976).