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CHLOROETHYLENES: A Mechanistic Approach to Human Risk Evaluation

Trevor Green

ICI Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, SK10 4TJ, United Kingdom

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INTRODUCTION

The four chlorinated ethylenes, vinyl chloride (chloroethylene), vinylidene chloride (1,1-dichloroethylene), 1,1,2-trichloroethylene and 1,1,2,2-tetrachloroethylene form a group of structurally related chemicals with properties invaluable to modern industrial society. Polymers and copolymers of vinyl chloride and vinylidene chloride have become the most important synthetic resins in use today, with worldwide production of PVC exceeding 18 million tons in 1988. The solvent properties of trichloroethylene and tetrachloroethylene have resulted in their widespread use in metal degreasing, dry cleaning, and a wide variety of industrial applications. These chemicals have now been in common use for more than 50 years. During this time workers have been exposed to a wide range of concentrations, in some cases for periods of 20 years or more, which has allowed a significant data base to be compiled about the short- and long-term effects of these chemicals on human health. In the last 20 years this information has been supplemented by numerous studies in laboratory animals.

The chloroethylenes are either gases or volatile liquids and the principal route of exposure is by inhalation. Exposure to high concentrations results in CNS depression and narcosis (1–4); trichloroethylene, for example, has been used as an anesthetic for many short-duration surgical procedures (5). Other effects have been reported, ranging from acro-osteolysis, part of a phenomenon known as vinyl chloride disease (6), to liver, kidney, and lung changes

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(7, 8). Attempts to reproduce acro-osteolysis in laboratory animals led to the discovery of the carcinogenicity of vinyl chloride. In a study exposing rats to vinyl chloride by inhalation, Viola (9) and Viola et al (10) reported an increased incidence of skin, lung, and bone tumors. Maltoni et al (11) confirmed these findings and also reported an increased incidence of angiosarcoma of the liver. In the same year, 1974, Creech & Johnson (12) reported three deaths from angiosarcoma of the liver among workers employed on a vinyl chloride plant in the USA. The rarity of this type of tumor and the results of Maltoni's animal studies immediately established a connection between exposure to vinyl chloride and human liver cancer. This led to one of the most extensive epidemiological and toxicological studies of any industrial chemical.

One consequence of the work on vinyl chloride was the subsequent testing of the other chloroethylenes for the ability to cause cancer. That the structurally similar chloroethylenes would have properties similar to vinyl chloride appeared to be confirmed when each was shown to cause cancer in laboratory animals. There were, however, significant differences. Whereas vinyl chloride was established as a human carcinogen and was found to be mutagenic and carcinogenic in all tests used to measure these responses, a marked lack of mutagenicity and species-, strain-, and sex-dependent responses in two-year cancer studies characterized the evaluation of the other chloroethylenes. Furthermore, there was no evidence from occupationally exposed populations of any increase in any type of human cancer. Consequently, the risks to man from exposure to these chloroethylenes were much less well defined than for vinyl chloride.

The variable results in the cancer bioassays and the lack of activity in many mutagenicity tests spawned a series of mechanistic studies on the differences between vinyl chloride and the other three chemicals in this group. According to these studies, at least two, if not all three, polychlorinated ethylenes act in an entirely different manner from vinyl chloride and involve mechanisms that were unknown in 1974 when the carcinogenicity of vinyl chloride was first discovered. Consequently, the risks to man are better understood and a scientific basis now exists for the control and use of these chemicals. This paper reviews the mechanisms of action of each of the chlorinated ethylenes and considers the relevance of the animal studies to people exposed to these chemicals. The cancer studies and mutagenicity assays have been reviewed by others and are only considered if relevant to the mechanisms involved in the development of cancer.

VINYL CHLORIDE

By 1986, twelve epidemiology studies had identified 120 cases of vinyl chloride-induced angiosarcoma of the liver in populations employed in

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the manufacture of vinyl chloride (13), compared to an incidence in the general population of 0.10 per million. Increases in other tumors including liver, brain, lung, thyroid, lymphatic tissue, and skin were noted but a relationship could not be established between them and vinyl chloride exposure.

Hemangiosarcoma of the liver was also induced in rats, mice, and hamsters exposed to vinyl chloride (11). As in man, such tumors are extremely rare in these species. Other tumors associated with vinyl chloride exposure included zymbal gland tumors in rats and hamsters, nephroblastomas in rats, pulmonary and mammary gland tumors in mice, and forestomach papillomas and melanomas in hamsters. The same range of tumors was seen in rats following either oral administration or inhalation exposure. Tumors were observed over a wide range of doses ($\times 10^3$) from as low as 10ppm and, in some studies, after short exposures (14). The wide range of effects in several species was considered characteristic of a genotoxic carcinogen. This view was supported by the short-term mutagenicity tests. Vinyl chloride was mutagenic and clastogenic in vivo, and in vitro when in the presence of an appropriate metabolic activating system (8). Clastogenicity has also been reported in workers exposed to high concentrations of vinyl chloride (15).

There seems little doubt that vinyl chloride is mutagenic and carcinogenic as a result of its metabolism by microsomal mixed function oxidases (cytochromes P-450) to chloro-oxirane (chloroethylene oxide) (16, 17). This highly electrophilic epoxide is a potent mutagen when tested directly or when generated from vinyl chloride in the presence of an appropriate metabolizing system (18, 19). DNA-alkylation products that lead to mispairing, depurination, and fragmentation have been isolated from animals exposed to vinyl chloride and from a variety of in vitro systems (20, 21). These products are consistent with chloro-oxirane being the ultimate carcinogenic form of vinyl chloride. The other known mutagenic metabolite of vinyl chloride is chloroacetaldehyde, the rearrangement product of chloro-oxirane (19). Although less mutagenic than the epoxide, chloroacetaldehyde is known to react with DNA to give the same alkylation products and could therefore have a role in the carcinogenicity of vinyl chloride (20).

The major detoxification pathway for these two mutagenic metabolites is by conjugation with glutathione leading to the excretion of S-(2-hydroxyethyl) cysteine and thiodiglycolic acid in urine (Figure 1) (22, 23). The epoxide also appears to be a substrate for epoxide hydratase and gives an unstable diol that is further metabolized to become incorporated into the citric acid cycle and yield carbon dioxide in vivo. That vinyl chloride metabolism is a saturable process (24) is reflected by the incidences of angiosarcoma and the reduction of the slope of the dose-response curve to zero at higher dose levels (14). There are no known major species differences in the metabolism of vinyl chloride.

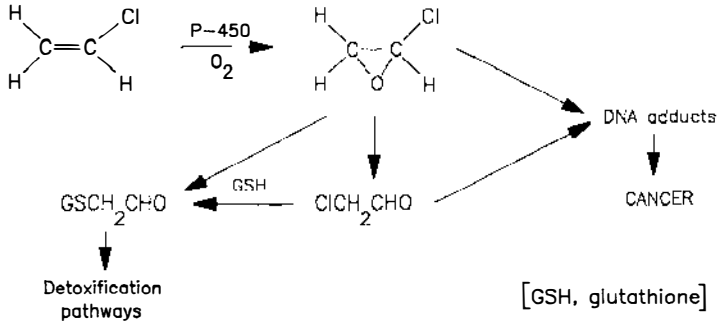

Figure 1 The metabolic activation of vinyl chloride.

The vast amount of information available about vinyl chloride, which is very briefly summarized here, corroborates the mutagenicity and carcinogenicity of this chemical. This conclusion is consistent with its metabolism to a mutagenic epoxide and the development of cancer by a mechanism involving alkylation of DNA. Vinyl chloride is therefore a classical genotoxin causing cancer by somatic mutation.

THE MUTAGENICITY AND CARCINOGENICITY OF VINYLIDENE CHLORIDE, TRI- AND TETRA-CHLOROETHYLENE

Vinylidene chloride, trichloroethylene, and tetrachloroethylene are all metabolized by cytochrome P-450 to products consistent with the formation of an epoxide intermediate (25, 26) and are identical in this respect to vinyl chloride. Although the stability and reactivity of the epoxides have been intensively investigated (27–30), there is no direct evidence that they exist as free chemical species in biological systems. It has been suggested that these epoxides never leave the enzyme site as free species (31), unlike vinyl chloride epoxide, which has been detected spectroscopically and trapped with a variety of nucleophiles (32, 33). A further indirect measure of the formation and reactivity of these epoxides is the mutagenicity of the parent chemical in vivo, and in vitro in the presence of suitable metabolic activation. Again, these results provide little evidence that these epoxides exist in mammalian systems. Weak responses have been seen for vinylidene chloride in prokaryotic and some eukaryotic systems (34, 35). The responses are highly dependent upon species or the source of the metabolic activation used in the in vitro tests, with mouse tissues the most active (36, 37). A similar pattern is seen for trichloroethylene where the responses are perhaps even weaker, e.g. no evidence of point mutations in bacteria (38, 39), some in yeast (40, 41), but chromosomal effects in the mouse micronucleus assay (42). Tetrachloroethylene appears not to be mutagenic or clastogenic either in vivo or in vitro

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in tests where pure material was used (39, 43). DNA-binding studies have been reported for each of the chloroethylenes, although there is little evidence of binding except with vinyl chloride where specific adducts have been identified (44–47). There is therefore a clear gradation from a potent mutagen and clastogen, vinyl chloride, through to tetrachloroethylene, which appears to have no genotoxic properties in short-term tests. This conclusion is based on an overview of a complex area that in detail is beyond the scope of this review. Many of the mutagenicity tests reported in the literature are confounded by the use of impure material and the presence of mutagenic stabilizers that have been used in some commercial grades of these chemicals. Nonetheless, the conclusion is valid when properly conducted tests of pure material are considered.

The pattern of inconsistent responses seen in the mutagenicity tests is repeated in the two-year cancer studies. The carcinogenicity of vinylidene chloride, the closest analog of vinyl chloride and therefore the one that might be expected to most resemble vinyl chloride in its properties, has never been fully resolved. Of eighteen chronic studies only one reported a carcinogenic effect (48). Male Swiss mice exposed to 25ppm by inhalation had a low incidence of renal adenocarcinoma. The effect was not seen at 10ppm, nor in female mice, in other species at higher dose levels, or in mice of other strains. In light of these negative results, this apparently real but unique response fails to resolve the issue of whether or not vinylidene chloride is a carcinogenic hazard to man. A further bioassay is unlikely to resolve this issue.

Trichloroethylene and tetrachloroethylene have also shown marked species-, strain- and sex-specific responses in two-year studies (43, 49–54). Both chemicals are clearly carcinogenic in the liver of B6C3F1 mice in several studies by either inhalation or ingestion (32, 49–51). However, trichloroethylene did not induce tumors in Swiss mice (52) nor did either chemical cause liver tumors in rats (43, 49–51). A low incidence of kidney tumors was reported in male rats exposed to tetrachloroethylene in two studies (43, 53), and lung tumors have been observed in CD-1 mice exposed to trichloroethylene in another study (54).

Thus, in contrast to vinyl chloride where there are consistent responses in the cancer bioassays and a clear genotoxic mechanism, the other chloroethylenes fail to give a clear indication of their genotoxic potential in short-term tests and the cancer studies fail to give a clear indication of the hazard to man. Many of these issues have largely been resolved by mechanistic studies that explained the species and sex differences, and linked the relevance of the animal studies to exposed human populations.

Vinylidene Chloride-Induced Mouse Kidney Tumors

Perhaps the least satisfactory explanation of a species-specific response among the chloroethylenes is found for vinylidene chloride. This may reflect

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the degree of uncertainty over its carcinogenicity. Kidney tumors were found in only one study that used Swiss mice and then in only two out of eighteen surviving males (48). Severe nephrotoxicity was a feature of the study at the dose level where tumors were seen but not at lower dose levels where no tumors occurred. Of the species and strains used in the eighteen cancer studies, the male Swiss mouse was more susceptible than rats, hamsters, or other strains of mice to the nephrotoxic effects of vinylidene chloride. Thus, it is tempting to make a connection between the kidney damage and the low incidence of tumors seen in these mice.

Other possible mechanisms have been considered. Vinylidene chloride is metabolized by a saturable pathway to a putative epoxide intermediate that rearranges to chloroacetyl chloride (25, 55). The major detoxification pathways for these intermediates are hydrolysis and conjugation with glutathione, resulting in a variety of metabolites and the depletion of hepatic glutathione levels. Studies have investigated the metabolism and pharmacokinetics of vinylidene chloride in different species and organs, seeking an explanation for the response seen exclusively in the mouse kidney. Alkylation of macromolecules is highest in the mouse kidney (56–58) and is dependent on cellular glutathione levels (59). Oesch et al (36) compared the effects of vinylidene chloride on hepatic and renal enzymes and reported a reduction in epoxide hydratase and glutathione-S-transferase activity in male mouse kidney that was not seen in female mice or in rats of either sex. Although a reduction in these detoxification enzymes is consistent with the outcome of the cancer studies, one enzyme, epoxide hydratase, apparently plays a very minor role in the metabolism of vinylidene chloride.

In conclusion, higher metabolic rates, higher levels of covalent binding, and possible reductions in the levels of detoxifying enzymes are all consistent with the male Swiss mouse's susceptibility to kidney tumors. Implicit is the belief that vinylidene chloride is carcinogenic as a result of its metabolism to a reactive electrophile such as the epoxide. However, the crucial factor in determining the species and strain differences may be the susceptibility of the Swiss mouse to the cytotoxic effects of vinylidene chloride. There is some evidence that tumors are not seen in the absence of toxicity; this suggests that the weak alkylating effects of vinylidene chloride metabolites may not be sufficient to cause cancer. Kidney damage and the resulting cell division may facilitate the expression of the weak genotoxic potential of the metabolites or, alternatively, kidney damage may alone lead to cancer by a nongenotoxic mechanism. Whichever mechanism applies, the effects seem to be unique to the male Swiss mouse and to have little relevance to other species, including man.

Tri- and Tetrachloroethylene Induced Mouse Liver Tumors

Both trichloroethylene and tetrachlorethylene cause a very similar increase in the incidence of hepatocellular carcinoma in B6C3F1 mice following either

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oral administration or inhalation exposure for two years (43, 49–51). Short-term exposure to these chemicals causes liver growth and marked peroxisome proliferation in mice but not rats (60–62). The link between peroxisome proliferation and cancer in rodents strongly suggests that this response is the basis of the species difference in carcinogenicity (63–65). The identification and subsequent cancer bioassay of the metabolite responsible for the peroxisome proliferation confirmed this hypothesis. Both chemicals are metabolized to trichloroacetic acid (Figure 2), which causes peroxisome proliferation in rats and mice in vivo and in rat and mouse hepatocytes in vitro (60). A two-year study in which trichloroacetic acid was administered to B6C3F1 mice in drinking water found a 32% incidence of hepatocellular carcinoma within 61 weeks of the start of the study (66). Tumors were not found in the livers of control animals at that time. There can be little doubt therefore that trichloroacetic acid is the metabolite of trichloroethylene and tetrachloroethylene responsible for the liver tumors seen in mice. These studies also demonstrate that the epoxide intermediates proposed in the metabolism of both chemicals have no role in the initiation or development of these tumors.

Trichloroacetic acid is a common metabolite of trichloroethylene and tetrachloroethylene in most animal species including rats, mice, and man (26, 67–70). When trichloroacetic acid was administered to rats and mice hepatic peroxisome proliferation was seen in both species (60). However, this response and liver cancer were seen only in mice and not rats when the parent chemicals were administered in the two-year cancer studies. The reason for this apparent anomaly is pharmacokinetic; trichloroacetic acid-induced peroxisome proliferation is a threshold phenomenon (60). In the mouse, the metabolism of trichloroethylene and tetrachloroethylene is linear over a wide range of dose levels and the threshold is easily exceeded at the high dose

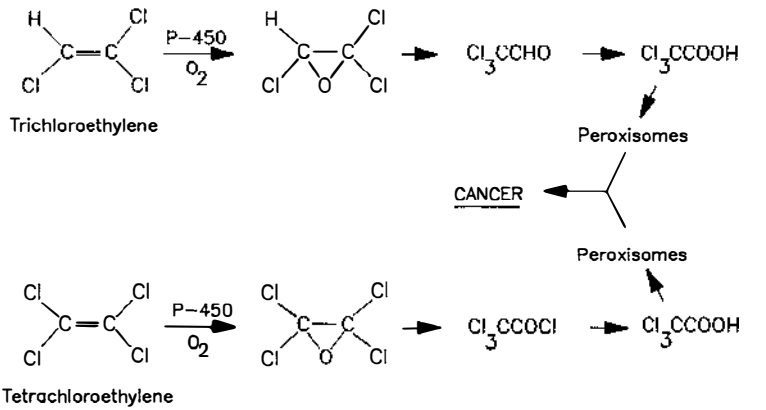

Figure 2 The metabolism of tri- and tetrachloroethylene in relation to the development of liver tumours in $B_6C_3F_1$ mice.

levels used in the cancer studies. In the rat, metabolism becomes saturated at relatively low dose levels and the threshold concentration of trichloroacetic acid required to induce peroxisome proliferation is not exceeded at any dose of the parent chemical (26, 60, 71). The effects of this species difference in metabolism is clearly seen in the blood levels of trichloroacetic acid in the two species. For example, the peak in blood levels was seven times higher in the mouse than the rat when both species were exposed to 400ppm tetrachloroethylene for six hours (72).

These experiments produced an entirely consistent explanation for the mechanism of action of these two chemicals and for the basis of the rodent species differences. The mechanism involves peroxisome proliferation mediated by a common metabolite, trichloroacetic acid. The finding that trichloroacetic acid is a complete carcinogen appears to rule out any role for electrophilic epoxide metabolites in the hepatocarcinogenicity of these chemicals, which is consistent with their lack of mutagenicity. Also consistent with a nongenotoxic mechanism is the nature of the threshold response for peroxisome proliferation, which is also the basis of the species difference between rats and mice and is derived from simple pharmacokinetic differences between the two species. Thus, mice are susceptible to liver cancer as a direct result of the high metabolic rates frequently found in this species and rats are protected by lower rates and saturation of the metabolic pathway.

The above experiments and bioassays all used the B6C3F1 strain of mouse, and for the trichloroethylene studies, oral gavage as the route of administration. The Swiss mouse exposed by inhalation is apparently not susceptible to trichloroethylene-induced liver cancer in the same way (52), although the reasons for this strain difference are unknown. Swiss mice metabolize trichloroethylene at a similar rate and to a similar extent and are also susceptible to peroxisome proliferation (60, 71). Thus, other than differences in the dose and route of administration in the two cancer bioassays, and the known sensitivity of the B6C3F1 mouse to liver carcinogens, there is no clear explanation for this strain difference in carcinogenicity.

The relevance of the mechanism identified in B6C3F1 mice for humans exposed to tri- and tetrachloroethylene has been assessed in a number of studies comparing metabolic rates in mice, rats, and man, and the response of human liver tissue to the key metabolite, trichloroacetic acid (60). The metabolism of both trichloroethylene and tetrachloroethylene is limited in man by saturation of the metabolic pathway at relatively low exposure levels (67, 73, 74). In this respect, man resembles the rat and may not be able to produce sufficient trichloroacetic acid to stimulate peroxisome proliferation. There is, however, an even more significant difference between mice, rats, and man in their response to trichloroacetic acid. The peroxisome proliferation seen in vivo in rats and mice treated with trichloroacetic acid has been reproduced in vitro using mouse and rat hepatocytes (60). Under the same

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conditions, trichloroacetic acid failed to induce peroxisome proliferation in human hepatocytes. Consequently, the mechanism believed to operate in the mouse appears to be unique to that species. Only the mouse has the combination of high metabolic rates and the ability to respond to trichloroacetic acid as a peroxisome proliferator that results in cancer. The rat is limited by metabolic saturation; man is limited by both metabolic saturation and a lack of trichloroacetic acid-stimulated peroxisome proliferation. These conclusions are perhaps not too surprising. The mouse as the smallest of the species of interest would be expected to have the highest metabolic rates. It is also well known that peroxisome proliferation differs according to species and is much more common in rodents than in any other species.

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Tetrachloroethylene Induced Rat Kidney Tumors

A low incidence of renal tubular cell adenomas and adenocarcinomas was found in male F344 rats exposed to tetrachloroethylene by inhalation for a lifetime (43). The incidence was not statistically significant, but because adenomas are rare in control F344 rats and malignant kidney tumors are not normally observed at all, several studies have sought a mechanistic explanation for their origin.

Previous lifetime studies of tetrachloroethylene in rats have all been affected by poor survival within the dosed groups (51, 76). In each case there was evidence that survival was reduced as a result of chemically induced nephropathy distinguishable from the age-related nephropathy normally seen in this strain of rat. Of the species tested, the male rat was the most sensitive to these effects. Mortality was slight in the NTP inhalation study (43), but again there was evidence of kidney damage characterized by tubular enlargement and hyperplasia. There are therefore striking similarities between tetrachloroethylene and vinylidene chloride in that the species most sensitive to the nephrotoxic effects of the chemical has a low incidence of kidney tumors after two years of exposure. It is possible that a similar mechanism involving sustained cytotoxicity and cell division may apply.

Other well-defined mechanisms have been proposed to account for these tumors. High oral doses of tetrachloroethylene (1–1.5g/kg/day) cause an accumulation of the protein alpha-2u-globulin (hyaline droplets) in renal proximal tubular cells of male rats (75, 77–79). Tubular casts were formed and focal areas of proximal tubular regeneration were apparent in these studies. The effects were specific to the male rat and were not seen in females or in mice of either sex. Hyaline droplet formation or protein droplet nephropathy has been linked with a significant number of male rat-specific kidney carcinogens and is believed to be part of a cycle of necrosis and cellular regeneration that results in cancer (77, 80–83). Such a mechanism would explain the specificity of the tumors and would not be dissimilar in effect to the cytotoxicity and hyperplasia reported from the two-year studies. Howev-

er, although these responses were observed after high oral doses of tetrachloroethylene, they were not seen after inhalation exposure for 28 days (75) at the dose levels (up to 400ppm) used in the NTP inhalation study (43). Apparently, hyaline droplet formation, like peroxisome proliferation and other nongenotoxic responses, is a threshold phenomenon and the highest dose used in the NTP study is below the threshold. Further short-term studies using higher doses (1000ppm) by inhalation did induce this response in male rats (75). Thus, although a well-established link exists between hyaline droplet formation and renal cancer in male rats, the lack of effect at the top dose level used in the two-year inhalation study (43) questions the significance of this phenomena in the development of tumors in that study. A contribution from hyaline droplet formation to the development of these tumors cannot entirely be ruled out based on the results of a 28-day study. The potential for causing this effect has clearly been established and hyaline droplet formation may still occur during a lifetime study, even at the lower dose levels used in the NTP study (43).

Yet another mechanism has been proposed to explain the low incidence of male rat kidney tumors seen after exposure to tetrachloroethylene. Unlike the previous mechanisms, this one involves the formation of a mutagenic metabolite in the kidney. Tetrachloroethylene is metabolized by a second minor pathway involving hepatic glutathione conjugation (75, 84) (Figure 3). The conjugate is metabolized by the mercapturic acid pathway and excreted in urine as the N-acetyl cysteine derivative. The precursor of this metabolite, the cysteine conjugate of tetrachloroethylene, is a substrate for the renal enzyme β-lyase and is mutagenic in the Ames bacterial mutation assay when activated by rat kidney fractions (75, 85, 86). The lack of response of tetrachloroethylene itself in this assay is probably due to a failure of the standard test to

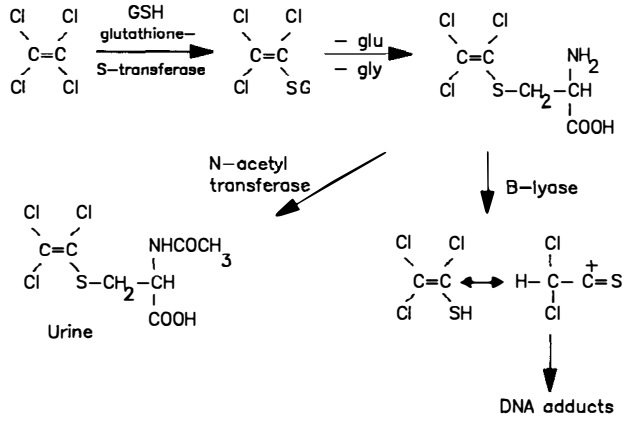

Figure 3 The metabolism of tetrachloroethylene by glutathione conjugation and activation by renal β-lyase in the rat.

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replicate the number of steps involved in the activation of the chemical by this pathway. These studies have been taken as evidence for the involvement of genotoxicity in the development of these tumors. Activity by this pathway is consistent with the male rat as the susceptible species, since glutathione conjugation and B-lyase activity is greater in male than female rats or mice of either sex (75). The pathway is minor, even in the male rat, and only increases significantly when the major cytochrome P-450 pathway has saturated (75).

A surfeit of explanations exists for what is actually a very low incidence of tumors in one sex of one species of laboratory animal. This may equally be true with most mechanisms of carcinogenesis in that many factors influence the initiation and development of tumors. In this case it is entirely possible, if not probable, that the overall mechanism involves a low level of genotoxicity derived from glutathione conjugation and activation by β-lyase, in conjunction with cell division stimulated by cytotoxicity or hyaline droplet formation. Each apparently separate mechanism may in combination provide the initiation and promotion steps believed to be essential for chemical carcinogenesis.

Most aspects of the mechanisms believed to be responsible for the kidney tumors seen in male rats are in fact unique to the male rat. Hyaline droplet formation and the protein alpha-2u-globulin are found only in the male rat and not in other laboratory animals or in man. Even in the male rat the effect is highly dose-dependent. Cytotoxicity and kidney damage are features of high continuous exposures and are unlikely to occur during occupational use of tetrachloroethylene. The other mechanism involving glutathione conjugation has been investigated in a series of studies comparing the key metabolic steps in rat, mouse, and human tissues (75). Human kidney possesses the enzyme β-lyase with activity towards the cysteine conjugate of tetrachloroethylene similar to that in the mouse and approximately 30-fold lower than that in male rat kidney. Experiments using human liver fractions failed to detect glutathione conjugation of tetrachloroethylene and concluded, based on the limit of detection of the assay, that the difference in rate between the rat and man was at least an order of magnitude.

In conclusion, three possible mechanisms have been reported to explain the male rat kidney tumors; each is very much dependent upon dose, two exhibit genuine thresholds, and the third is based on a pathway that is minor at low dose levels. It is unlikely that these mechanisms will occur in man; glutathione conjugation has not been detected in human liver, hyaline droplet formation is exclusive to the male rat and chronic toxicity is only likely to occur after exposure to high concentrations over a prolonged period.

OTHER TUMORS

In addition to the multiplicity of tumor types reported following vinyl chloride exposure, other tumors have also been reported for trichloroethylene and

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tetrachloroethylene. An increased incidence of lung adenomas and adenocarcinomas has been reported in mice exposed to trichloroethylene by inhalation (54) and an increase in mononuclear cell leukemia was seen in F344 rats exposed to tetrachloroethylene (43). A recent NTP study (87) exposing four strains of rat to trichloroethylene also reported a low incidence of kidney tumors.

No satisfactory explanations for the mechanisms involved in the development of these tumors are presently available, although two of the results may be features of the strain of animal used and the conduct of the study rather than a true carcinogenic response to the chemical. The incidence of mononuclear cell leukemia in control F344 rats in the study (43) was 56% (males) and although this was statistically increased by exposure to tetrachloroethylene, its relevance for man is questionable. This type of tumor was not seen in other strains of rat, is known to be of high and variable incidence in the F344 rat, and is not found in man.

The low incidence of kidney tumors reported in rats exposed to trichloroethylene appears to be largely a result of chronic toxicity rather than from a more specific mechanism. Although reported, this study (87) was considered inadequate for assessing carcinogenicity because of poor survival within the dosed groups. Nephrotoxicity was again a feature of the study and the lack of dose-, sex-, or strain-dependence on the distribution of the kidney tumors suggests that toxicity may well be the cause of the low incidence observed. Other mechanisms, detected with tetrachloroethylene, apparently are either absent or present at much lower levels.

CONCLUSIONS

The discovery of the carcinogenicity of vinyl chloride led to the assumption that the other chloroethylenes would be similarly carcinogenic. This belief was well founded in that each is carcinogenic in at least one animal species. At this point the similarities between vinyl chloride and the other chloroethylenes end. The expectation that they act in the same way as vinyl chloride through mutagenic epoxide metabolites has not been fullfilled. New mechanisms have been discovered involving peroxisomes, hyaline droplets, and new metabolic pathways that were unknown in the 1970s when vinyl chloride was first investigated. Many of the new mechanisms are believed to be nongenotoxic; they exhibit thresholds and in many cases show marked species-specificity. Chronic toxicity features in all studies where kidney tumors have been observed. That a variety of mechanisms can operate within a close structural group of chemicals is perhaps the most significant conclusion from these studies. Within this finding the species-, sex- and target-organ specificity of these chemicals has been explained and the need clearly illus-

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trated for this level of understanding to adequately evaluate the risks to man. The known mechanisms for the chloroethylenes are summarized in Table 1.

The question remains of the use of this type of work in human-risk assessment. The chloroethylenes illustrate clearly the pitfalls of assuming that structurally similar chemicals act by the same mechanism and can therefore be grouped together for risk assessment. Studies comparing mechanisms and species suggest that the risks to man from exposure to vinylidene chloride, trichloroethylene and tetrachloroethylene are considerably less than those associated with vinyl chloride. In some cases there are qualitative, and in others marked quantitative, differences between laboratory animals and man. Quantitative risk assessment is inappropriate where qualitative differences occur between species in response to peroxisome proliferators such as the metabolite trichloroacetic acid, or differences in tetrachloroethylene-induced hyaline-droplet formation. Where quantitative differences in metabolism and pharmacokinetics do exist, such procedures may be appropriate if they take into account those differences in the calculation of the risk to man. Even if the more generic weight-of-evidence approach is taken, the species differences in the cancer studies of vinylidene chloride, trichloroethylene and tetrachloroethylene, their lack of mutagenicity, and the mechanisms involved in the development of the tumors, all suggest the risks to man are minimal.

In-depth mechanistic investigations and species comparisons are still in their infancy and have yet to play a major role in human risk assessment. Clearly, however, such an approach is important and can assist in separating the high-risk chemicals such as vinyl chloride from species-specific carcinogens that may pose little or no threat to man at occupational or environmen-

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Table 1 Known mechanisms for the carcinogenicity of the chloroethylenes

Chemicals	Carcinogenic Metabolite	Mechanism	Tumors
Vinyl chloride	Epoxide/chloroacetaldehyde	DNA alkylation	Multiple All species
Vinylidene chloride	Epoxide/acid chloride?	DNA?	Male mouse kidney
	Unknown	Chronic toxicity	Male mouse kidney
Trichloroethylene	Trichloroacetic acid	Peroxisome proliferation	Mouse liver
Tetrachloroethylene	Trichloroacetic acid	Peroxisomes	Mouse liver
	Cysteine conjugate	DNA adducts	Rat kidney
	Unknown	Hyaline droplets	Rat kidney
	Unknown	Chronic toxicity	Rat kidney

tal exposure levels. As this area develops and our understanding of these mechanisms improves, the challenge for regulatory authorities will be to incorporate such data into the risk-assessment process, rather than rely solely on inconsistent animal bioassays and short-term tests.

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