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Proposed upgrade of Trichloroethylene to known human carcinogen based on recent published data that indicate an excess of kidney cancers in workers exposed to trichloroethylene.

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Summary

The possible modes of action of trichloroethylene as a potential rat and human renal toxin and carcinogen are reviewed. Exposure levels, toxicity, genotoxicity and metabolic activation are seen as critical aspects of the mode of action. The following points are noted.

Human kidney cancer is reported to occur, in the German studies, following exposure to very high nephrotoxic doses of trichloroethylene. Data to substantiate the magnitude of exposure and evidence for renal toxicity in humans is lacking. Anecdotal reports of exposure do not correlate with cancer incidence.

Trichloroethylene is a weak renal carcinogen in the rat in a single valid study. It did not cause renal cancer in the mouse or hamster, or in the rat in two studies. Renal cancer has never been seen in the absence of toxicity.

The DCVC pathway is a very minor pathway, less than 0.01% of the dose and 7000 fold less than the cytochrome P-450 pathway in humans exposed to 160 ppm.

The amounts of DCVC formed from trichloroethylene are 3 orders of magnitude lower than the renal NOEL for DCVC in the rat.

More DCVC is formed from trichloroethylene in the mouse than the rat, and DCVC itself is more toxic in the mouse than the rat.

There is no evidence that DCVC is either mutagenic or carcinogenic in vivo.

Evidence of a hot spot mutation in the human VHL gene in a single study has not been reproduced in a similar study.

Alternative modes of action have been proposed to explain the kidney damage in the rat.

Overall, the trichloroethylene data is weak, key aspects are absent, many of the results are not reproducible between studies and a mode of action has not been established. The data are inadequate to classify trichloroethylene as a human carcinogen.

Introduction

The evidence being used to support the proposed upgrade of trichloroethylene comes from:-

- Epidemiology; principally studies in Germany where small populations were reportedly exposed to be uniquely high concentrations of trichloroethylene.

- Rat cancer bioassays where the same tumour type was seen.

- A common mode of action in rats and in humans.

In this submission, the question is asked, has a mode of action been established that is consistent with the development of kidney tumours in rats and humans?

A number of different aspects of trichloroethylene toxicology are relevant to an understanding of its mode of action as a renal carcinogen. Kidney tumours are reported to be increased in rats and humans only after exposure to very high nephrotoxic dose levels of trichloroethylene. The magnitude of exposure, its characterisation, and the accompanying toxicity, are therefore relevant. A metabolic pathway has been proposed which leads to metabolites which are toxic to the kidney and mutagenic in bacteria. Evidence of mutations has also been reported in human kidney tumours taken from individuals exposed to trichloroethylene. Thus, metabolism and potential genotoxicity are the other critical aspects of the mode of action

A careful analysis, given below, of each of these areas reveals a lack of data, and also inconsistent data, which leads to the conclusion that the evidence available to classify trichloroethylene as a human carcinogen is inadequate.

Human Exposure

The remarkably high incidence of kidney cancer in a small population of 169 individuals in a cardboard factory in Germany is attributed, by the authors, to the uniquely high concentrations of trichloroethylene which occurred in that factory (Henschler et al.1995). The same conclusion is reached in the study reported by Vamvakas et al. (1998).

The populations in these studies were small, <200 people, compared to the large epidemiology studies with total cohorts of about 30,000 individuals.

Neither atmospheric monitoring, or biological monitoring of exposure was undertaken. Assessment of exposure is based on recollection of physical symptoms which occurred at the time. This assessment was made 20-30 years after the factory closed and exposure ended.

The incidences of cancer did not correlate with exposure. Most of the cancers were seen in individuals employed as locksmiths and electricians and not in the highest exposed group in the Henschler study.

The claim that these exposures were uniquely high is untenable. Trichloroethylene has been in commercial production for almost 70 years and was used without controls or knowledge of adverse long term health effects for decades. Consequently, comparable exposures have occurred elsewhere. It is also particularly difficult to rationalise why people employed as locksmiths and electricians in Germany should be exposed to concentrations of trichloroethylene which did not occur elsewhere.

Conclusion: There are no measurements of exposure to trichloroethylene or other possible confounding factors. There is lack of a correlation between exposure and cancer incidence. Finally, the Henschler study is a cluster study that should not be considered as definitive but as 'hypothesis generating' and should not be used to make a causal inference.

The Significance of the Animal bioassays

There have been seven lifetime cancer bioassays in rats, seven in mice and one in hamsters. There has been no site concordance between the species and none of the tumours have occurred reproducibly in all studies, even within the same species.

Of the studies in rats, four were by gavage and three by inhalation. Because bioassays were conducted at maximum tolerated doses, dose levels between the studies were comparable, around 500-600 ppm by inhalation and up to 1000 mg/kg by gavage. Three studies, two by inhalation (Henschler et al 1980; Fukuda et al. 1980), and one by gavage (NCI, 1976) did not find any increase in kidney cancer. Two studies conducted by NTP (1988; 1990) using the gavage route, did find a sporadic increase in kidney tumours whose incidences were neither dose, sex or strain related. Both studies were judged (by NTP) to be inadequate for the purpose of determining carcinogenicity due to poor survival (NTP TR 243, 273) and deficiencies of the conduct of the studies (TR 273). In the inhalation study reported by Maltoni et al (1988), a small increase in kidney tumours was seen in male rats (4/130) at the top dose level of 600 ppm. Kidney toxicity was a common finding in all of the studies.

Kidney cancer has not been seen in mice in seven studies, nor in hamsters in a single study.

Conclusion: The kidney tumour incidence in the rat is low and frequently did not achieve statistical significance. Most studies exceeded the MTD, in one, the conduct of the study was inadequate. The finding of an increase in kidney cancer in the rat is not reproducible and these tumours have never been seen in mice or hamsters. Kidney tumours have only been seen at the highest dose levels and have never been seen in the absence of kidney toxicity. The evidence for renal cancer is, therefore, limited and confined to the rat.

Metabolic Activation

In addition to the major cytochrome P-450 pathway, trichloroethylene is also metabolised by conjugation with glutathione (Goeptar et al. 1995). The derived cysteine conjugate, S-(1,2-dichlorovinyl)-L-cysteine (DCVC; also present as the 2,2- isomer), is known to be

nephrotoxic following activation by the renal enzyme β-lyase. Although this pathway has been identified in rats and humans, a link between this pathway and the development of kidney cancer has not been established for the following reasons.

The DCVC pathway has been assessed in vivo by measurements of N-acetyl DCVC in urine. Between 50-73% of a dose of DCVC itself is excreted in this way in the rat (Goeptar et al. 1995). The DCVC pathway is a very minor pathway for trichloroethylene in both rats and humans, typically less than 0.01% of the dose (Birner et al. 1993; Goeptar et al. 1995; Green et al. 1997). In human volunteers, the amount metabolised by this pathway was 7000-fold lower than that by the cytochrome P450 pathway at exposures of 160 ppm (Bernauer et al. 1996).

There is no evidence to suggest that the DCVC pathway becomes a major pathway at the high dose levels associated with renal cancer. In the human volunteer study reported by Bernauer et al (1996), urinary N-acetyl DCVC levels decreased with increasing dose relative to metabolites from the cytochrome P-450 pathway (ratio P450:GST, 3292:1 at 40ppm and 7163:1 at 160ppm). Over the range of dose levels used (40-160 ppm) cytochrome P-450 metabolism was essentially linear (3.74 fold increase in metabolism for a 4-fold increase in dose) whereas GST metabolism only increased 1.7-fold.

The amount of DCVC formed by the metabolism of trichloroethylene in vivo is three orders of magnitude lower than the NOEL for kidney damage in rats dosed with DCVC itself (Green et al. 1997).

More DCVC is formed in the mouse than the rat following exposure to equivalent dose levels of trichloroethylene. Furthermore, DCVC is 5-10 fold more toxic in the mouse than the rat and caused an increase in renal cell division in the mouse, but not the rat (Birner et al. 1993; Eyre et al. 1995a,b; Green et al. 1997)

There is no evidence whatsoever to show that DCVC when formed as a minor metabolite of trichloroethylene is causally related to the development of either kidney toxicity or kidney cancer. DCVC has not been tested in a full cancer bioassay, in fact, the limited evidence available suggests that DCVC may cause liver cancer in rats rather than kidney cancer (Terracini and Parker, 1965). Equally, although a bacterial mutagen, its ability to cause genetic damage in vivo, including in the rat kidney, has not been established.

Recently alternative explanations for trichloroethylene induced kidney damage in rats have been proposed. It has been reported that trichloroethylene interferes with folate metabolism causing a chronic acidosis which results in kidney damage in rats (Green et al. 1998; Dow and Green, 2000). This mechanism, unlike DCVC activation, is consistent with kidney damage only occurring as a result of chronic high exposure to trichloroethylene.

Conclusion: Although it has been assumed that DCVC is responsible for the renal effects of trichloroethylene, evidence to support these claims is lacking. In fact, the evidence suggests that DCVC is not responsible. The DCVC hypothesis fails to explain the species differences in renal carcinogenicity between rats and mice. The amounts of DCVC formed from trichloroethylene are more than three orders of magnitude lower than a toxic dose of this material. There is no evidence to suggest that the DCVC pathway becomes a major pathway at high dose levels and, DCVC has not been shown to be either carcinogenic or mutagenic in vivo. Even Henschler and co-workers in a recent review of this area (Dekant and Henschler, 1999) conclude that "the present data do not permit a definite assessment of risk of renal tumour formation by trichloroethylene in humans on the basis of available mechanistic or epidemiological data".

Alternative explanations are starting to emerge and the mode of action of trichloroethylene as either a nephrotoxin or rat renal carcinogen, and its relevance to humans, have yet to be established.

Toxicity

In animals, renal cancer has only been seen in the presence of life shortening renal toxicity and it is assumed that toxicity is the fundamental cause of the renal tumours reported in the Henschler study. A number of studies have looked at the same German populations used in the cancer studies and claim to have detected kidney damage (Bruning and Bolt, 2000). These studies have several inherent weaknesses:

Kidney toxicity has been measured more than 20 years after exposure ended.

As with the German cancer studies, exposure levels are unknown.

The reported toxicities do not reflect the magnitude of exposure as assessed by job description.

The parameters used, urinary GST alpha and microglobulin excretion, vary widely in the normal population and are affected by age and sex, and a wide range of drugs, environmental and lifestyle factors (Yu et al. 1983; Waller et al. 1989; Gan et al. 1994). The populations exposed to trichloroethylene in these studies are small, < 50 exposed individuals, and the studies do not have the power to detect any change, particularly more than 20 years post exposure. The large number of potential confounding factors which may have effected the outcome of the study since exposure to trichloroethylene ended have not been considered.

Conclusion: There is no meaningful evidence of renal toxicity in the populations exposed to trichloroethylene in Germany 20-30 years ago.

Genotoxicity

Recent major reviews of the potential genotoxicity of trichloroethylene have been consistent in concluding that trichloroethylene is unlikely to cause cancer by chemically induced somatic mutations (Fahrig et al. 1995; Moore and Harrington-Brock, 2000).

Although DCVC is mutagenic in bacteria, it has not been shown to be genotoxic in the rat either in the kidney, or at any other site, in vivo. In addition, although chlorothioketene, the proposed reactive intermediate formed from DCVC is DNA reactive in inert solvents, DNA adducts could not be detected under physiological conditions (Volkel and Dekant, 1998). It seems unlikely therefore that DCVC will be genotoxic in the rat kidney in vivo.

Brauch et al (1999) described a "hot spot" mutation at nucleoide 454 of the VHL gene which was only present in renal tumours from individuals exposed to trichloroethylene during metal degreasing. This study suffers from the same fundamental weakness as the cancer and toxicity studies, namely the poor characterisation of the study population with respect to exposure. No direct measurements were available and categorisation of exposure relied on recall of physical symptoms. In some instances this information was obtained from the relatives of deceased members of the study population. It also seems unlikely that a small electrophile such as the chlorothioketene derived from DCVC should cause a specific single mutation of this type. A second similar study by Schraml et al (1999), also carried out in Germany, similarly analysed tumour tissue from trichloroethylene exposed patients for mutations in the VHL gene. This study found no unique phenotype, genotype, or mutation pattern in the VHL gene from renal tumours of trichloroethylene exposed patients and, in particular, none of the hot spot mutations reported by Brauch.

Conclusion - There is no evidence to show that DCVC is an in vivo mutagen. A single report describing a hot spot mutation in tumours from populations exposed to trichloroethylene appears not to be a reproducible finding.

Comparisons with other human carcinogens

Analogy has been drawn (by NTP) between vinyl chloride, vinylidene chloride, trichloroethylene and perchloroethylene in that they all cause liver cancer in mice. This is an entirely inappropriate analogy since vinyl chloride is a multi-species liver carcinogen with an

accepted genotoxic mode of action which involves a reactive epoxide and the formation of DNA adducts. Vinylidene chloride, the closest structural analogue to vinyl chloride, did not cause liver cancer in any species and tri- and perchloroethylene cause liver cancer in mice, but not rats, by non-genotoxic mechanisms involving peroxisome proliferation and increased cell division. It would be more appropriate to compare the biological properties of vinyl chloride, a known human carcinogen, with those of trichloroethylene.

Vinyl chloride is multi-species carcinogen causing liver cancer reproducibly in all rat, mouse and hamster studies. The tumours are induced at low dose levels and following limited exposures. It has a define mode of action based on the formation of a reactive epoxide which is genotoxic and alkylates DNA in vivo. There is clear unequivocal evidence of human cancer from multiple epidemiology studies with known exposure levels. Trichloroethylene on the other hand gives tumours in animals only after exposure to maximum tolerated dose levels. There is no site concordance between rats and mice and poor reproducibility between studies in the same species. All of the evidence suggests that trichloroethylene does not cause cancer by somatic mutation, but by toxicity and increased cell division induced by high dose levels. The evidence of human cancer is weak with an increase in renal tumours seen only in a small poorly characterised cluster study with no measurements of exposure. Evidence of kidney toxicity in humans is similarly confounded by poor characterisation of exposure and the fact that some of the assessments were made 20 years after exposure had ceased.

Thus, for vinyl chloride there is consistency in the animal studies, a clearly defined mode of action and an increase in human cancer linked to exposure. None of this consistency is present for trichloroethylene.

Overall Conclusions

There is a marked lack of consistency in all of the data sets, a poorly defined mode of action, and a general lack of any single unifying observation to suggest that trichloroethylene should

be included alongside those carcinogens which have been proven unequivocally to cause cancer in exposed human populations.

The Henschler and Brauch studies suggest areas of further investigation and follow-up but at the present time these studies are flawed by a lack of exposure data and confounded by the fact that the findings are not reproduced in other studies. At the present time a causal relationship cannot be made between exposure to trichloroethylene and increased incidences of human kidney cancer.

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