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Trichloroethylene Issue Paper 2:

Interactions of Trichloroethylene, Its Metabolites, and Other Chemical Exposures

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DISCLAIMER

This issue paper does not represent and should not be construed to represent any agency determination or policy. This issue paper has not been externally reviewed. The information is being provided to assist the National Academy of Sciences in their review of the scientific issues surrounding trichloroethylene health risks.

CONTENTS

LIST OF ABBREVIATIONS AND ACRONYMS

ALDH2	Aldehyde dehydrogenase 2
BCA	Bromochloroacetate
BDCA	Bromodichloroacetate
CH	Chloral hydrate
CT	Carbon tetrachloride
CYP2E1	Cytochrome P450 2E1
DBA	Dibromoacetate
DCA	Dichloroacetic acid
DCVC	S-(1,2-dichlorovinyl)-L-cysteine
DCVCS	DCVC sulfoxide
DLPC	Dilinoleoylphosphatidylchloline
DMR-2	Differentially methylated region-2
DT	Distal tubular
GSH	Glutathione
GST	Glutathione-S-transferase
HA	Hepatoadenoma
HC	Hepatocellular carcinoma
IGF-II	Insulin-like growth factor-II
IR	Insulin receptor
LPS	Lipopolysaccharide
MOA	Mode of action
NAS	National Academy of Sciences
PBPK	Physiologically based pharmacokinetic
PK	Pharmacokinetics
PPAR	Peroxisome proliferator-activated receptor
PPC	Polyenylphosphatidylcholine
PT	Proximal tubular
TCA	Trichloroacetic acid
TCE	Trichloroethylene
TCOH	Trichloroethanol
TNF	Tumor necrosis factor

PREFACE

Publication of these issue papers is a part of EPA's effort to develop a trichloroethylene (TCE) human health risk assessment. These issue papers were developed to provide scientific and technical information to the National Academy of Sciences (NAS) for use in developing their advice on how to best address the important scientific issues surrounding TCE health risks. As such, these papers discuss a wide range of perspectives and scientific information (current through Fall 2004) on some of these important issues, highlighting areas of continuing uncertainty and data that may be relevant. They are intended to be useful characterizations of the issues, not a presentation of EPA conclusions on these issues. The papers have undergone internal review within EPA, but they have not been externally reviewed. The concepts presented in these papers will eventually be addressed in EPA's revised risk assessment of TCE, after the advice from the NAS, along with comments from the EPA Science Advisory Board and the public, as well as recently published scientific literature, have been incorporated.

AUTHORS AND CONTRIBUTORS

Many individuals contributed to the completion of this set of tichloroethylene (TCE) issue papers. The TCE Risk Assessment Team identified the topics covered by the papers and prepared them for submission to the National Academy of Sciences. The authors wish to thank Dr. Peter Preuss, Dr. John Vandenberg, Mr. David Bussard, Mr. Paul White, Dr. Bob Sonawane, Dr. Hugh Barton, Dr. Aparna Koppikar, Mr. David Bayliss, Dr. William Wood, and Dr. Ila Cote for their input and comments.

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THE TCE ISSUE PAPERS

BACKGROUND

In August 2001, a draft, *Trichloroethylene (TCE) Health Risk Assessment: Synthesis and Characterization,* was released for external review. This draft assessment drew on 16 "state-of-the-science" papers published as a supplemental issue of *Environmental Health Perspectives* (Volume 108, Supplement 2, May 2000). Subsequent to its release, EPA's 2001 draft assessment underwent a peer review by a panel of independent scientists through EPA's Science Advisory Board (SAB), which provided a peer review report in December 2002. In addition, the public submitted more than 800 pages of comments to EPA during a 120-day public comment period.

There are a number of important issues that EPA will need to examine as it moves forward in revising the draft TCE assessment. These include issues raised not only in the SAB peer review and public comments, but also by new scientific literature published since the release of the state-of-the-science papers and EPA's 2001 draft assessment. Some of this research is specific to the study of TCE or its metabolites while some of it describes advances in scientific fields more generally but which have potential relevance to characterizing the human health risks from TCE.

In February 2004, EPA held a symposium so that authors of some of the TCE-specific research that had been published since the release of the draft assessment could present their findings in more detail. This symposium represented only a limited cross section of recently published research, but was reflective of the breadth of new relevant science that EPA will consider in revising the assessment (the presentation slides and a transcript of the meeting are available separately on EPA's website and have already been sent to the NAS).

In 2004, EPA, in cooperation with a number of other federal agencies, initiated a consultation with the National Academy of Sciences (NAS) to provide advice on scientific issues related to the health risk assessment of TCE. It was recognized that a review by an NAS panel of the important scientific issues would be beneficial and informative to clarify the state-of-the-science as EPA moves forward in completing its health risk assessment. A charge was developed for the NAS through an Interagency Workgroup led by the White House Office of Science and Technology Policy.

PURPOSE OF THE TCE ISSUE PAPERS

Although EPA will need to address all of the issues identified in the charge to the NAS panel in updating its assessment, EPA would like to focus the NAS panel's attention on a subset of issues that EPA believes to be most critical in developing a revised risk assessment, as summarized in four issue papers developed by EPA staff:

1. Issues in trichloroethylene pharmacokinetics;
2. Interactions of trichloroethylene, its metabolites, and other chemical exposures;
3. Role of peroxisome proliferator-activated receptor agonism and cell signaling in trichloroethylene toxicity; and
4. Issues in trichloroethylene cancer epidemiology.

Each paper provides an overview of the science issues, a discussion of perspectives on those issues (including the SAB and public comments), and an outline of some of the recently published scientific literature. The pharmacokinetics issue paper also summarizes results from a recent collaboration with the U.S. Air Force on TCE pharmacokinetics, as well as EPA's planned approach for further refinement of the pharmacokinetic modeling of TCE and its metabolites. These scientific areas were selected because they are (a) critical to the hazard and/or dose-response characterization of TCE; (b) scientifically complex and/or controversial; and (c) areas in which substantial important scientific literature has been recently published. The input from the NAS on the topics described in the issue papers, as well as other topics put forth in the charge to the NAS, should help to strengthen EPA's revised TCE assessment.

NEXT STEPS

The advice from the NAS, along with comments already received from the EPA SAB and the public, as well as recently published scientific literature, will be incorporated into a revised EPA risk assessment of TCE, strengthening its scientific basis. Because of the substantial amount of new information and analysis that is expected, the revised draft of the assessment will undergo further peer review and public comment prior to completion.

1. INTRODUCTION

As described in the pharmacokinetics issue paper, exposure to trichloroethylene (TCE) results in a complex internal mixture of parent compound and its metabolites. Understanding which components of this mixture are toxicologically important is a critical step in characterizing the potential human health hazard of TCE. First, this information provides insights into potential modes of action (MOAs) for TCE toxicity, as discussed in a separate issue paper on peroxisome proliferator-activated receptor (PPAR) agonism. Second, as discussed in the pharmacokinetics issue paper, identification of the active agent or agents is essential for the selection of dose metrics to be used in deriving quantitative estimates of risk. It is important to note that this information on TCE metabolites also provides insights into possible effects from co-exposures to chemicals other than TCE. In particular, exposure studies have shown that humans exposed to TCE also may be exposed to some TCE metabolites, such as trichloracetic acid (TCA) and dichloroacetic acid (DCA), and to substances that share metabolites, metabolic pathways, and actions or targets of toxicity with TCE, such as perchloroethylene or ethanol. Therefore, EPA is seeking advice from the NAS on the following questions/issues:

1. What TCE metabolites, or combinations of metabolites, may be plausibly involved in the toxicity of TCE?

    a. Have TCA, DCA, or their combination been demonstrated to be the active agent(s) of TCE liver toxicity and/or carcinogenesis?

    b. Have either glutathione conjugation products and their bioactivation or other metabolites such as formic acid been demonstrated to be the active agents of TCE kidney toxicity and/or carcinogenesis?

    c. Is there sufficient information to reach conclusions as to the active agents of other endpoints of TCE toxicity?

2. What chemical co-exposures may plausibly modulate TCE toxicity?

    a. What can be concluded about the potential for common drinking water contaminants such as other solvents and/or haloacetates to modulate TCE toxicity?

    b. What can be concluded about the potential for ethanol consumption to modulate TCE toxicity?

    c. Is there sufficient information to quantify the effects of interactions from co-exposures?

This issue paper summarizes and outlines some of the recently published scientific literature identified by EPA that may be informative to these questions. Section 2 outlines recently published scientific literature on the toxicity of individual TCE metabolites and mixtures of TCE metabolites as they occur together in situ. New literature has also been published on interactions between TCE and common co-contaminants, as well as on the effects of ethanol on metabolic and toxicological systems that may be relevant to TCE toxicity, both of which are outlined in Section 3. Although there may not be definitive answers to the questions stated above given the current state of the science, input from the National Academy of Sciences (NAS) regarding the interpretation and characterization of the existing evidence, as well as the identification of research needs and data gaps, would help to strengthen the U.S. Environmental Protection Agency's (EPA's) revised assessment.

2. TOXICITY OF TCE AND ITS METABOLITES INDIVIDUALLY OR AS MIXTURES

Extensive metabolic studies show that exposure to TCE results in internal exposure to a complex mixture to metabolites (see the issue paper on TCE pharmacokinetics). Therefore, one of the first steps in determining the effects of TCE exposure is to examine common targets and actions of TCE metabolites. These compounds are not only produced by TCE metabolism but also occur as co-exposures from other environmental pollutants and are products of metabolism of other chemicals; so data on TCE metabolites can also help to identify the potential modulating effects from co-exposures. TCE exposure has been associated with a wide array of adverse health effects, including neurotoxicity, immunotoxicity, developmental toxicity, liver toxicity, kidney toxicity, endocrine effects, and several forms of cancer (see the Environmental Health Perspectives Supplement "TCE Health Risks" [Vol 108, Suppl 2, May 2000] and EPA's 2001 draft assessment for additional details). Most of the information on TCE metabolites is related to liver and kidney toxicity. For instance, one of the major issues in liver toxicity is the relative importance of TCA and DCA. For kidney toxicity, the roles of different glutathione conjugation products and their bioactivation have been the primary area of investigation, although an alternative hypothesis related to formic acid has been proposed.

Recently published information that may be relevant to a comparison of toxicity profiles among TCE, its metabolites, and mixtures of metabolites is outlined in this section. The major TCE metabolites with the most new information are DCA, TCA, chloral hydrate (CH), S-1(1,2-dichlorovinyl)-L-cysteine (DCVC), and trichloroethanol (TCOH); these are discussed in Section

2.1. Studies of mixtures of metabolites are covered in Section 2.2. Many of the new studies have focused on potential MOAs as well as toxicity. As with studies of TCE, techniques such are gene arrays, knockout mice, and phenotypic profiles of induced tumors have been employed to study the toxicity of TCE metabolites. Most of the focus of studies of TCA toxicity has been on its potential actions as a peroxisome proliferator; these are addressed in a separate issue paper on PPAR/cell signaling.

2.1. RECENTLY PUBLISHED TOXICITY INFORMATION ON INDIVIDUAL TCE METABOLITES

2.1.1. Dichloroacetic Acid

Because of the difficulty in directly detecting DCA following TCE exposure (discussed in the pharmacokinetics issue paper), direct evidence for the formation of DCA from TCE is limited. Although some have suggested that DCA levels after TCE exposure are too low to play a role in liver tumor induction, others have noted that DCA-induced toxicity has been observed at exposure levels for which DCA cannot be detected in vivo (Bull et al., 2002). However, toxicity information on DCA also may be informative as to the question of DCA's contribution to TCE's toxicity through a comparison of toxicity profiles. Most new studies of DCA have focused on its ability to induce liver tumors in rodents. The phenotype of liver tumors induced by DCA and the shape of the dose-response curve for that induction have been recently studied for insight into the MOA. Carter et al. (2003) report the dose-response of histopathologic changes occurring in the livers of mice exposed to DCA at 0.05–3.5 g/L for 26–100 weeks. They indicate that all classes of premalignant lesions and carcinomas (altered hepatic foci, large foci of cellular alteration, adenomas, and carcinomas) were found at both lower and higher doses and that foci and adenomas demonstrated neoplastic progression with time. The authors suggest that these data are consistent with the conclusion that nongenotoxic mechanisms, such as negative selection, are relevant to DCA carcinogenesis at lower doses where DCA genotoxicity has not been observed.

The findings are consistent with those of DeAngelo et al. (1999), who report that, based on hepatocellular carcinoma multiplicity, a no-observed-effect level for hepatocarcinogenicity could not be determined for DCA (lowest dose tested was 0.05 g/L DCA or 8 mg/kg/day) and that hepatic peroxisome proliferation was significantly increased only for 3.5 g/L DCA treatment at 26 weeks. The authors concluded that induction of liver cancer by DCA does not appear to be conditional on peroxisome induction or chemically sustained cell proliferation.

DCA has been shown to produce liver tumors in mice and rats and to affect glycogen status in hepatocytes. DCA has been reported to suppress apoptosis at levels that also induce liver tumors and to cause decreases in insulin and increases in glycogen (Bull, 2004; Bull et al.,

2004). The potential relationship of these effects to PPAR are discussed in a separate issue paper.

A number of studies have examined effects of DCA on carbohydrate handling in an attempt to understand how such effects lead to carcinogenesis. Lingohr et al. (2001) report that DCA treatment in drinking water for 2 weeks led to reduced serum insulin levels with the decrease persisting for at least 8 weeks in mice. In livers of mice treated with DCA for up to 52 weeks, insulin receptor (IR) protein levels and protein kinase B expression were significantly depressed. In contrast to normal liver, IR protein was elevated in DCA-induced liver tumors relative to that in liver tissue of untreated animals and to an even greater extent when compared to adjacent normal liver in treated animals. Mitogen-activated protein kinase phosphorylation was also increased in tumor tissue relative to normal liver tissue and tissue from untreated controls. The authors suggest that normal hepatocytes down-regulate insulin-signaling proteins in response to the accumulation of liver glycogen caused by DCA and that the initiated cell population, which does not accumulate glycogen and is promoted by DCA treatment, responds differently from normal hepatocytes to the insulin-like effects of this chemical.

Further studies by Lingohr et al. (2002) report that the in vivo glycogenic effect of DCA was dose related and independent of insulin. The authors suggest that the accumulation of hepatocellular glycogen induced by DCA was not the result of decreased glycogenolysis but was the result of a PI3K-dependent mechanism that does not involve PKB/Akt and is, at least in part, different from the classical insulin-stimulated glycogenesis pathway. The authors noted that PI3K is also known to regulate cell proliferation and apoptosis in hepatocytes, and that understanding these mechanisms may be important to understanding DCA-induced carcinogenesis.

Another group of studies has examined the effect of DCA on the activities of metabolic enzymes. Schultz et al. (2002) report that elimination of DCA is controlled by liver metabolism, which occurs by the cytosolic enzyme glutathione (GST)-S-transferase-zeta. GST-zeta is identical to an enzyme that is part of the tyrosine catabolism pathway known as maleylacetoacetate isomerase. In this pathway, GST-zeta plays a critical role in catalyzing the isomerization of maleylacetoacetate to fumarylacetoacetate. Disruption of tyrosine catabolism has been linked to increased cancer risk in humans.

Bull (2004) reports that TCE inhibits GST-zeta. Tzeng et al. (2000) report that DCA is a mechanism-based inactivator of GST-zeta and is biotransformed to electrophilic metabolites that covalently modify and thereby inactivate the enzyme. Schultz et al. (2002) report that studies of the elimination of intravenous doses of DCA indicate young mice were the most sensitive to changes in DCA elimination after drinking water treatment. The authors suggest that, in young mice, inactivation and resynthesis of GST-zeta is a highly dynamic process and

that exogenous factors that deplete or reduce GST-zeta levels will decrease DCA elimination and may increase the carcinogenic potency of DCA.

Like TCE, studies of gene expression have been conducted for DCA. Thai et al. (2003) report changes in gene expression in mouse livers after a tumorigenic dose of 2 g/L DCA in drinking water for 4 weeks. There were 24 genes with altered expression, of which 15 were confirmed by Northern blot analysis. Of the 15 genes, 14 revealed expression suppressed two- to fivefold. These included the following: MHR 23A, cytochrome P450, 2C29, CYP 3A11, serum paraoxonase/arylesterase 1, liver carboxylesterase, alpha-1 antitrypsin, ER p72, GST-pi 1, angiogenin, vitronectin precursor, cathepsin D, plasminogen precursor (contains angiostatin), prothrombin precursor and integrin alpha 3 precursor. An additional gene, CYP 2A4/5, had a twofold elevation in expression. Furthermore, in ancillary Northern analyses of total RNA isolated from DCA-induced hepatocellular carcinomas (from earlier reported studies of mice treated with 3.5 g/L DCA for 93 weeks), many of the same genes (11 of 15) noted above showed a similar alteration in expression. The authors suggested that the genes identified with altered expression were involved in cell growth, tissue remodeling, apoptosis, cancer progression, and xenobiotic metabolism.

A body of work has also been developed regarding the hypothesis that toxicity of TCE and its metabolites may arise from its effects on the methylation status of DNA. Ge et al. (2001) report that DCA and TCA induced DNA hypomethylation in mouse liver and a temporal association for DNA hypomethylation and cell proliferation. Tao et al. (2000) have reported DCA and TCA to induce the hypomethylation of the protooncogenes c-myc and c-June in mouse liver. Tao et al. (2004) report that the DNA and the differentially methylated region-2 (DMR-2) of the insulin-like growth factor-II (IGF-II) gene are hypomethylated in mouse liver tumors that were initiated by N-methyl-N-nitrosourea and promoted by 20 mmL/L DCA or TCA (46 weeks) to a greater degree than in noninvolved liver tissues. In noninvolved liver and the total liver, the DMR-2 of the IGF-II gene was hypomethylated to the same extent by DCA and TCA. Hypomethylation was noted after 46 weeks of exposure with the IGF-II gene hypomethylated in all cells evaluated. The authors suggest that hypomethylation of DNA and the IGF-II gene found in noninvolved liver would appear to be the result of a more prolonged activity and not cell proliferation, while hypomethylation of tumors could be an intrinsic property of the tumors.

2.1.2. Chloral Hydrate

CH is widely used as a sedative and hypnotic in pediatric medicine. It is also a byproduct of water chlorination and a metabolite of TCE. Because CH is one of the first metabolites from oxidative metabolism of TCE, with subsequent metabolism to TCA and TCOH, and because a large fraction of TCE metabolism appears to go through CH, information on CH toxicity may be

informative regarding which metabolites may contribute to TCE toxicity. Similarities in toxicity may indicate that common downstream metabolites may be toxicologically important, and differences may indicate the importance of other metabolic pathways. Recent studies have emerged that describe the chronic toxicity of CH and the effects of diet and peroxisome proliferation. There are similarities and differences in targets of toxicity between CH and TCE.

George et al. (2000) exposed male B6C3F1 mice and male F344/N rats to CH in drinking water for 2 years. Lifetime exposures to CH (up to 162.6 mg/kg/day) failed to increase the prevalence (percentage of animals with a tumor) or the multiplicity (tumors/animal) of hepatocellular neoplasia, nor was an increase in neoplasia observed at any other organ site in rats. In mice, enhanced neoplasia was observed only in the liver. Prevalence and multiplicity of hepatocellular carcinoma were increased only for the high-dose group. Hepatoadenoma (HA) prevalence and multiplicity were significantly increased at all CH concentrations (up to 146.6 mg/kg/day). Altered foci of cells were evident in all doses tested in the mouse, but no significant differences were observed over the control values. Hepatocellular necrosis was minimal and did not exceed that seen in untreated rats and mice. CH exposure did not alter serum chemistry and hepatocyte proliferation in rats and mice or increase hepatic palmitoyl CoA oxidase in mice at any of the time periods monitored. It was concluded that CH was carcinogenic (hepatocellular neoplasia) in the male mouse, but not in the rat, following a lifetime exposure in drinking water. Based on the increased HA and combined tumors at all CH doses tested, a no-observed-adverse-effect level was not determined.

Leakey et al. (2003) studied the effects of CH exposure to male B6C3F1 mice with dietary control used to manipulate body growth in a 2-year bioassay. Mice under dietary control received variably restricted feed allocations to maintain their body weights on a predetermined "idealized" weight curve predictive of a terminal background liver tumor incidence of 15–20%. This paradigm was also associated with a decreased variation in liver-to-body weight ratios. A statistically significant dose response to CH was observed in the dietary controlled group but not in the ad libitum-fed test groups. Terminally adjusted liver tumor incidence in both the dietary controlled and ad libitum-fed mice exposed to CH was reported with a statistically significant dose response observed only in the dietary controlled mice. However, the overall incidence of tumors was smaller after dietary control. The dose response for tumor formation was positively correlated with markers of peroxisomal proliferation in the dietary controlled mice only.

Seng et al. (2003) report that both dietary control and caloric restriction slightly reduced the acute toxicity of high doses of CH and potentiated the induction of hepatic enzymes associated with peroxisome proliferation compared with B6C3F1 mice fed ad libitum. TCA was the major metabolite in serum in all three diet groups. Although the area under the curve values for serum TCA were slightly greater in the dietary controlled and caloricallly restricted groups

than in the ad libitum-fed groups, this increase did not appear to completely account for the potentiation of hepatic enzyme induction by dietary restriction.

Ho et al. (2003) reported the toxicological effects and cell death mechanisms of CH in rats and human Chang liver cells and lymphocytes. Monitoring of urinary 8-epi-PGF2alpha and serum levels of TNF-alpha served as an index of lipid peroxidation and cytokine stimulation. A single intraperitoneal injection of 100 mg/kg CH in rats led to a nearly fivefold increase in urinary 8-epi-PGF2alpha on day 1 and a mild decrease on days 2 and 3. The same treatment also induced significantly higher amounts of serum TNF-alpha on day 2 (about sevenfold). Concurrent treatment with CH and vitamin E simultaneously reduced the effect. A greater cytotoxic effect from CH exposure was reported in human Chang liver cells than in comparison with lymphocytes. However, apoptosis features were observed in human lymphocytes but not in Chang liver cells after CH treatment.

2.1.3. DCVC

The MOA for TCE-induced renal toxicity is not well understood. DCVC is formed as a downstream metabolite to glutathione (GSH) conjugation of TCE and is a selective proximal tubular nephrotoxicant. Lash et al. (2003) report DCVC to be the penultimate nephrotoxic metabolite of TCE, although Dow and Green (2000) have suggested that the TCE metabolites TCA and TCOH interact with vitamin B12 through a free radical mechanism inducing a B12 deficiency that leads to excessive formic acid. Information on DCVC toxicity may help inform the plausibility of its role in TCE renal toxicity and provide information regarding possible MOAs. Most new studies have focused on kidney effects from short-term DCVC exposures.

Vaidya et al. (2003a) report that with acute high exposures, tissue repair in the kidney is inhibited after DCVC exposure in mice, resulting in lethality. However, Vaidya et al. (2003b) report that prior exposure to DCVC is protective from lethal injury following a second DCVC exposure. Administration of mercuric chloride, a nephrotoxicant with no effect on beta-lyase activity, also resulted in protection from a lethal dose of DCVC. Total glutathione was unchanged at any time after the lethal dose of DCVC was administered, obviating the role of glutathione in protection from either pretreatments. In both cases, augmented and sustained tissue repair was induced by the priming dose.

Cummings et al. (2000) studied the activities of several GSH-dependent enzymes, expression of cytochrome P450 isoenzymes, and time- and concentration-dependent cytotoxicity of TCE and DCVC in primary cultures of proximal tubular (PT) and distal tubular (DT) cells from rat kidney. Of the cytochrome P450 isoenzymes studied, only CYP4A expression was detected. CYP4A mRNA and protein expression were higher in primary cultures of DT cells

than in PT cells and were increased in DT cells by ciprofibrate treatment. TCE cytotoxicity was much less than that of DCVC.

Lash et al. (2001) report that DCVC causes apoptosis and enhances cell proliferation in hot cells at environmentally relevant doses and at earlier time points and lower concentrations than necrosis in primary cultures of human PT (hot) cells. The hot cells from males were modestly more sensitive to DCVC than those from females. A small increase was also noted in the percentage of cells in S-phase after a 4-hour treatment with as little as 10 µM DCVC, suggesting that cell proliferation was stimulated.

Although metabolism of DCVC by the cysteine conjugate beta-lyase is the most studied bioactivation pathway, DCVC may also be metabolized by the flavin-containing monooxygenase to yield DCVC sulfoxide (DCVCS). Lash et al. (2003) report that confluent hot cells incubated with as little as 10 µM DCVCS for 24 hours exhibited morphological changes, although at least 100 µM DCVCS was required to produce marked changes. Acute cellular necrosis did not occur until 48 hours with at least 200 µM DCVCS, indicating that this is a high-dose, late response. The extent of necrosis was similar to that observed with DCVC. In contrast, apoptosis occurred as early as 1 hour with as little as 10 µM DCVCS, and the extent of apoptosis was much less than that observed with DCVC. Mitochondrial function was maintained with DCVCS concentrations up to 100 µM, consistent with hot cells only being competent to undergo apoptosis at early time points and relatively low concentrations. Marked depletion (>50%) of cellular GSH content was observed only with 500 µM DCVCS. The authors suggest that formation of DCVCS plays a significant role in TCE-induced renal cellular injury in hot cells.

2.1.4. Trichloroethanol

As mentioned above, TCOH is formed as a downstream metabolite to oxidative metabolism of TCE and appears in measurable quantities in both blood and urine. Information on TCOH toxicity therefore may be directly applicable to TCE toxicity to the extent that TCOH or its metabolites are the active agents. In conjunction with the hypothesis that formic acid formation is the MOA for TCE kidney effects, Green et al. (2003) examined the chronic toxicity of TCOH in male Fischer rats in drinking water (0, 0.5, and 1.0 g/L) for 52 weeks. The rats excreted large amounts of formic acid in urine reaching a maximum after 12 weeks and thereafter declining to reach an apparent steady state at 40 weeks. Urine from treated rats was more acidic throughout the study, and urinary methylmalonic acid and plasma N-methyltetrahydrofolate concentrations were increased. The authors suggested that the changes were an indication of acidosis, vitamin B12 deficiency, and impaired folate metabolism, respectively. TCOH treatment resulted in kidney damage over the duration of the study, which was characterized by increased urinary N-acetyl-beta-D-glucosaminidase activity, protein excretion (from 4 weeks);

increased basophilia, protein accumulation, and tubular damage (from 12 to 40 weeks); increased cell replication (at week 28); and evidence in some rats of focal proliferation of abnormal tubules at 52 weeks.

2.2. STUDIES ON METABOLITE INTERACTIONS

Although some inferences can be drawn about common targets of toxicity and actions between TCE and its metabolites from the study of individual chemicals, there may be cases where TCE-induced responses are due to multiple active agents and/or MOAs. Recently, studies have emerged that attempt to directly examine how co-exposures and variations in relative concentration between metabolites may affect toxicity. Effects of exposure to TCE metabolites after initiation with other carcinogens have also been studied. Most of the recent information has been related to combinations of TCA and DCA.

TCA is active as a liver carcinogen in mice, and as discussed in the pharmacokinetics issue paper, a measurable fraction of metabolism of TCE ends up as TCA in mice, suggesting that TCA has the potential to contribute to the carcinogenic response to TCE in the liver. Unlike TCA, DCA is active as a liver carcinogen in both mice and rats, but it has not been possible to directly determine whether it is produced at carcinogenic levels because DCA is rapidly metabolized. (A more detailed discussion of pharmacokinetic issues related to the production of DCA from TCE are discussed in the issue paper on TCE pharmacokinetics) There is, however, indirect evidence that DCA is formed from TCE in vivo. Pretreatment with either DCA or TCE inhibits the cytosolic enzyme GST-zeta, which is thought to be responsible for DCA metabolism, while TCA pretreatment does not (Schultz et al., 2002).

Biomarkers for DCA and TCA have also been used to investigate whether the liver tumor response to TCE seen in mice is completely attributable to TCA or whether other metabolites, such as DCA, are involved. Bull et al. (2002) report that DCA produces tumors in mice that display a diffuse immunoreactivity to a c-Jun antibody, whereas TCA-induced tumors do not stain with this antibody. A comparison of tumor phenotypes induced by TCE shows that such tumors have characteristics of both DCA- and TCA-induced tumors (i.e., a mixture of phenotypes). When given in various combinations, DCA and TCA produced a few tumors that were c-Jun+, many that were c-Jun-, but a number with a mixed phenotype that increased with the relative dose of DCA. Furthermore, mutation frequencies showed that TCE-induced tumors were significantly different from those induced by TCA, and DCA-induced tumors were intermediate between values obtained with TCA and TCE. In particular, mutations of the H-ras protooncogene were examined in DCA-, TCA-, and TCE-induced tumors. The mutation frequency detected in tumors induced by TCA was significantly different from that observed in TCE-induced tumors (0.44 vs. 0.21, $p < 0.05$), whereas that observed in DCA-induced tumors

(0.33) was intermediate between values obtained with TCA and TCE but not significantly different from TCE. No significant differences were found in the mutation spectra of tumors produced by the three compounds. The presence of mutations in H-ras codon 61 appeared to be a late event, but ras-dependent signaling pathways were activated in all tumors. The authors suggest that these data are not consistent with the hypothesis that all liver tumors induced by TCE were produced by TCA.

Bull (2004) and Bull et al. (2004) report that responses to mixtures of DCA, TCA, and carbon tetrachloride (CT) in male mice show that interactions may result in additive or inhibitory effects, but no significant evidence of synergy was observed. Given alone, TCA and DCA produced liver tumors in mice with the phenotypic characteristics that are distinct in several respects and that are each effective as carcinogens at doses that do not produce cytotoxicity. CT was characterized as promoting the growth of liver tumors through cytotoxicity that produces a generalized growth stimulus in the liver reflected in a reparative hyperplasia. Thus, the authors concluded that CT is relatively nonspecific in its promotion of initiated cells within the liver and has a different MOA than TCA and DCA. Mice were initiated by vinyl carbamate and then promoted by DCA, TCA, CT, or the pair-wised combinations of the three compounds. As the dose of CT was increased, so did the number of tumors per animal with mean tumor size decreasing. When administered alone in the drinking water, DCA increased both tumor number and tumor size in a dose-related manner. With TCA treatment, a plateau in tumor number was reached by 24 weeks at a high dose. DCA treatment did not produce a plateau in tumor number within the experimental period, but the numbers observed at the end of the experimental period (i.e., 36 weeks) were similar to TCA and to doses of 50mg/kg CT. The authors concluded that the tumor numbers observed at the end of the experiment were consistent with the assumption that the administered dose of the tumor initiator, vinyl carbamate, was the major determinant of tumor number and that treatments with CT, DCA, and TCA primarily affected tumor size.

More complex activity was observed between the three compounds as mixtures. At 24 weeks, DCA produced a decrease in tumor numbers promoted by TCA, but the numbers were not different from those for TCA alone at 36 weeks. The reason for this result became apparent at 36 weeks of treatment, where a dose-related decrease in the size of tumors promoted by TCA resulted from DCA co-administration. However, the low dose of TCA decreased the number of tumors produced by a high dose of DCA (2 g/L), but higher doses of TCA (2 g/L) produced the same number as observed with DCA alone. DCA inhibited the growth rate of CT-induced tumors. TCA substantially increased the numbers of tumors observed at early time points when combined with CT, but this was not observed at 36 weeks. The lack of an effect at 36 weeks was attributable to the fact that more than 90% of the livers consisted of tumors and the earlier effect

was masked by coalescence of the tumors. Thus, TCA significantly increased tumor numbers in CT-treated mice.

Latendresse and Pereira (1997) report that after initiation with N-methyl-N-nitrosourea, the foci of altered hepatocytes and tumors occurring in the animals promoted with DCA were eosinophilic and positive immunohistochemically for TGF-alpha, c-jun, c-myc, CYP 2E1, CYP 4A1, and GST-pi. The DCA lesions also were essentially negative for c-fos and TGF-beta, but nontumor hepatocytes were consistently TGF-beta-positive. In contrast, tumors promoted by TCA were predominantly basophilic, lacked GST-pi, and stained variably; usually, more than 50% of the tumor hepatocytes were essentially negative for the other biomarkers.

3. EFFECTS FROM CO-EXPOSURES ON TCE TOXICITY

Interactions between carcinogens in chemical mixtures found in the environment have been a concern for several decades. Humans exposed to TCE may be exposed to chemicals both from their environment and from the consumption of therapeutic drugs and alcohol that may overlap with TCE in terms of active agents, pharmacokinetics, pharmacodynamics, and/or target tissue toxicity. Therefore, it is important to characterize whether and how these co-exposures may modulate the toxicity of TCE. An important subissue is the degree to which such modulation of toxicity can be quantified. Several approaches have been employed to study these interactions, including examination of tumor phenotype, gene expression, and development of physiologically based pharmacokinetic (PBPK) models to predict any possible synergy, antagonism, and additivity of effects or pharmacokinetics.

For TCE, because there is exposure to a “chemical mixture” internally due to its metabolism to multiple chemical species, information on TCE metabolite toxicity described above may be directly relevant to other co-exposures as well if they change either directly or indirectly the proportions of metabolites produced. For example, the work of Bull et al. (2004), Bull (2004), and Bull et al. (2002) cited in Section 2.2 on the contributions of DCA and TCA to liver tumors may be informative regarding the impact of co-exposures because the phenotype of tumor was reported to be dependent on the proportion of the two chemicals administered.

The draft TCE assessment included a brief discussion on extrinsic and intrinsic factors that may affect TCE toxicity. With regard to extrinsic factors, exposure data suggest that co-exposures of TCE, TCA, and DCA at environmental levels are not uncommon (Wu and Schaum, 2000). TCE co-exposures to solvents like perchloroethylene may have similar actions and targets that can affect TCE toxicity because they share common metabolites. Alcohol consumption has been noted to affect TCE toxicity. In the draft TCE assessment, the effects of ethanol exposure

on CYP2E1 induction and function, peroxisome induction, and targets of toxicity (e.g., the liver) were also briefly discussed. TCE exposure was cited as potentially increasing the toxicity of methanol and ethanol not only by altering their metabolism to aldehydes but also by their detoxification (e.g., similar to the "alcohol flush" reported for those who have an inactive aldehyde dehydrogenase allele) (Wang et al., 1999). Another example of potential interactions cited in the draft TCE assessment is that of TCE and DEHP (a peroxisome proliferator), which were reported to have synergistic effects with regard to prenatal loss, pup weight changes, and anaopthalmia (Narotsky 1995; Narotsky et al. 1995).

In its review, the SAB identified the potential effects on TCE toxicity by these extrinsic factors as an area for further expansion in EPA's revised assessment. Widespread background environmental exposure to TCE metabolites (DCA or TCA) and to other solvents like perchloroethylene that share common metabolites or actions of TCE can affect TCE health risk. The formation of TCE's toxic metabolites also can be affected by co-exposures.

A number of studies have recently emerged that examine the effects of co-exposures to common water contaminants and other solvents (e.g., perchloroethylene), described in Section 3.1. Relevant studies of the actions of ethanol on targets and actions ascribed to TCE and its metabolites are also described in Section 3.2. Such studies may help determine the effects of alcohol on susceptibility to TCE-induced effects. The intent of this portion of the issue paper is to identify current information that may aid in understanding the effects of potential co-exposures on toxicity. For more detailed information on the individual toxicity profiles of substances such as alcohol or perchloroethylene, assessments of these individual chemicals should be consulted because they are not summarized here.

3.1. STUDIES OF OTHER SOLVENTS, HALOACETATES, OR COMMON DRINKING WATER CONTAMINANTS

Chloroform is a chlorine disinfection byproduct found in drinking water as well as DCA and TCA and is also a mouse liver carcinogen. Pereira et al. (2001) studied the effect of chloroform on DCA and TCA-induced hypomethylation and expression of the c-myc gene and on their promotion of liver and kidney tumors in B6C3F1 mice. DCA, TCA, and to a lesser extent chloroform decreased the methylation and increased the mRNA expression of the c-myc gene. Co-administering chloroform prevented only DCA- and not TCA-induced hypomethylation and increased mRNA expression of the gene. The effect of chloroform on tumor promotion by DCA and TCA was determined in female and male B6C3F1 mice initiated on day 15 of age with N-methyl-N-nitrosourea. Liver tumors promoted by DCA and TCA were predominantly basophilic except for those in DCA-treated female mice that were eosinophilic. Only DCA promoted foci of altered hepatocytes, and they were eosinophilic in both sexes.

Chloroform prevented DCA but not TCA promotion of liver foci and tumors. In male mice, TCA promoted kidney tumors while DCA promoted kidney tumors only when co-administered with chloroform. Hence, chloroform prevented the hypomethylation and increased mRNA expression of the c-myc gene and the promotion of liver tumors by DCA, while enhancing DCA promotion of kidney tumors. The authors conclude that concurrent exposure to two carcinogens, chloroform and DCA, resulted in less than additive activity in one organ and synergism in another organ.

Other haloacetates produced in the bromination or chlorination of drinking water may affect TCE toxicity, and these effects may be concentration dependent. Kato-Weinstein et al. (2001) report that, as bromide concentrations increase, brominated haloacetates such as bromodichloroacetate (BDCA), bromochloroacetate (BCA), and dibromoacetate (DBA) appear at higher concentrations than the chlorinated haloacetates DCA or TCA in drinking water. Mice were administered DBA, BCA, and BDCA in drinking water at concentrations of 0.2–3 g/L. Both BCA and DBA caused liver glycogen accumulation similar to that of DCA, but TCA and low concentrations of BDCA reduced liver glycogen content, especially in the central lobular region. The high concentration of BDCA produced a pattern of glycogen distribution similar to that in DCA-treated mice but may have been attributable to the metabolism of BDCA to DCA. All dihaloacetates reduced serum insulin levels. Conversely, trihaloacetates had no significant effects on serum insulin levels. Dibromoacetate was the only brominated haloacetate that consistently increased acyl-coenzyme A oxidase activity and rates of cell replication in the liver.

Veeramachaneni et al. (2001) reported effects in male rabbits exposed to drinking water containing chemicals at concentrations typical of ground water near hazardous waste sites (the exposure mixture contained arsenic, chromium, lead, benzene, chloroform, phenol, and TCE). Even at 45 weeks after last exposure to drinking water pollutants, mating desire/ability, sperm quality, and Leydig cell function were subnormal. However, while the exposure levels are relevant to human environmental exposures, design of this study precludes a conclusion as to what combination of the seven toxicants, or individual toxicant, caused the effects.

Dobrev et al. (2001) attempted to investigate the pharmacokinetic interactions among TCE, perchloroethylene, and methylchloroform (MC) in rats to calculate defined “interaction thresholds.” A PBPK model was developed to test multiple mechanisms of inhibitory interactions, i.e., competitive, noncompetitive, or uncompetitive, when each was regarded as an inhibitor of the others’ metabolism, and concluded that the data were consistent with competitive inhibition. Using this inhibition model, the increase in TCE blood concentrations were calculated for different exposure levels of PERC and MC.

Dobrev et al. (2002) extended this work to humans by developing an interactive human PBPK model to predict the individual kinetics of TCE, perchloroethylene, and MC from

exposure to differently constituted chemical mixtures of the three solvents. The mixture model was used to explore the general pharmacokinetic profile of two common biomarkers of exposure, peak TCE blood levels, and total amount of TCE metabolites generated in rats and humans. Increases in the TCE blood levels led to higher availability of the parent compound for GSH conjugation, a metabolic pathway that may be associated with kidney toxicity/carcinogenicity. The simulated change in production rates of toxic conjugative metabolites exceeded 17% for a corresponding 10% increase in TCE blood concentration, indicating a nonlinear risk increase due to combined exposures to TCE.

Thrall and Poet (2000) investigated the pharmacokinetic impact of low-dose co-exposures to toluene and TCE in male F344 rats in vivo using a real-time breath analysis system coupled with PBPK modeling. The authors report that, using the binary mixture PBPK to compare the measured exhaled breath levels from high- and low-dose exposures with the predicted levels under various metabolic interaction simulations (competitive, noncompetitive, or uncompetitive inhibition), the optimized competitive metabolic interaction description yielded an interaction parameter Ki value closest to the Michaelis-Menten affinity parameter (K_M) of the inhibitor solvent, indicating that competitive inhibition is the most plausible type of metabolic interaction between these two solvents.

3.2. CO-EXPOSURES WITH ETHANOL/INTERACTIONS, SIMILARITIES OF EFFECTS AND TARGETS

Brautbar and Williams (2002) suggest in their review of solvent risk (including TCE) that laboratory testing that is commonly used by clinicians to detect liver toxicity may not be sensitive enough to detect early liver hepatotoxicity from industrial solvents and that the final clinical assessment of hepatotoxicity and industrial solvents must take into account synergism with medications, drugs of use and abuse, alcohol, age, and nutrition. Although many of these factors may be important, the focus here is on the effects of ethanol because of the amount of new literature on its effects. Previous reports indicated that ethanol pretreatment affects the pharmacokinetics and toxicity of TCE at concentrations as low as 500 ppm TCE (Nakajima et al., 1988). However, in general, the question of whether and how these “extrinsic factors” may modulate TCE health risks, and the degree to which it can be quantified, remains an important issue for EPA to address and for which NAS input would strengthen EPA’s revised assessment.

The field of research on ethanol effects on toxicity and carcinogenesis is an active one. A symposium at the 2002 Research Society on Alcoholism meeting in San Francisco (Badger et al., 2003) included presentations on intracellular CYP2E1 transport, oxidative stress, cytokine release, alcoholic liver disease, free radicals, adducts, autoantibodies resulting from ethanol metabolism, and the possible role of ethanol metabolism in carcinogenesis. New studies have

also examined the effects of ethanol on blood flow, induction of metabolism, Kupffer cell function, and PPAR alpha (PPARα) expression that may affect TCE toxicity.

Baraona et al. (2002a, b) report that nitric oxide mediated the stimulatory effects of ethanol on blood flow with ethanol markedly enhancing portal blood flow (twofold increase), with no changes in the hepatic, splenic, or pancreatic arterial blood flows. In addition, it quadrupled the coronary blood flow, doubled the renal flow, and increased cardiac output by 38%, with no significant changes in pulmonary, cerebral, or testicular flows. Ethanol consumption increased the cytosolic activity of the inducible nitric oxide synthase and induced microsomal cytochromes P450 capable of producing both nitric oxide and superoxide. Chronic, but not acute, ethanol administration increased peroxynitrite (a potent oxidant and nitrating agent) hepatotoxicity by enhancing concomitant production of nitric oxide and superoxide.

Examinations of the effects and interactions among ethanol exposure, PPARα, and the various enzymes involved in metabolism of TCE, such as CY2E1 and aldehyde dehydrogenase, also have been carried out. McCarver et al. (1998) suggest that the presence of an insertion mutation is associated with greater CYP2E1 metabolic ability in humans, but only among individuals who were either obese or had recently consumed ethanol.

Oneta et al. (2002) report that chronic ethanol consumption results in the induction of hepatic cytochrome P4502E1 (CYP2E1) in humans, which is believed to play an important role in the pathogenesis of alcoholic liver disease. They suggest that the amount and duration of alcohol intake associated with CYP2E1 induction is not known, but limited information is available on the disappearance of CYP2E1 following alcohol withdrawal. In their study, five healthy male volunteers received ethanol daily (40 g/day) over 4 weeks as did five alcoholics 1, 3, 8, and 15 days following ethanol withdrawal and five patients with nonalcoholic liver disease. A significant CYP2E1 induction occurred 1 week following ingestion of 40 g ethanol per day and increased further after 4 weeks. The disappearance of CYP2E1 was found to be significant 3 days following ethanol withdrawal and further decreased up to day 8. Thereafter, no significant change occurred and CYP2E1 activities were comparable with those in patients with nonalcoholic liver disease. The authors conclude that their data show a significant and quick induction of CYP2E1 activity even at moderate alcohol consumption, which may be of importance in the pathogenesis of alcoholic liver disease; of ethanol, drug, and vitamin A interactions; and in alcohol-associated carcinogenesis.

Daiker et al. (2000) report ethanol to be a potent inducer of the cytochrome P450 2E1 (CYP2E1) enzyme. A liquid diet containing 4.1% ethanol induced hepatic CYP2E1 activity fourfold in female CD-1 mice.

Proper function of PPARα is essential for the regulation of hepatic fatty acid metabolism. Fatty acid levels are increased in the liver during the metabolism of ethanol and should activate

PPARα. However, Fischer et al. (2003) report that ethanol feeding impairs fatty acid catabolism in the liver in part by blocking PPARα-mediated responses in C57BL/6J mice. WY-14,643 (WY), a PPARα agonist, restored the DNA-binding activity of PPARα/retinoid X receptor alpha, induced mRNA levels of PPARα target genes, stimulated the rate of fatty acid beta-oxidation, and prevented fatty liver in ethanol-fed animals. The authors suggest that impairment of PPARα function during ethanol consumption contributes to the development of alcoholic fatty liver, which can be overcome by WY. Galli et al. (2001) report that the transcriptional and DNA-binding activity of PPARα is inhibited by ethanol metabolism.

Wan et al. (2001) report that ethanol-induced CYP2E1 expression in rats is accompanied by inhibition of the expression of the PPARα gene and the reduction in polyunsaturated fatty acid content. They also report that the expression of PPARα and RXRα genes was activated in the livers of CYP2E1-null mice and suggest this is a compensatory effect for the absence of CYP2El. In addition, the expression of PPARα target genes, which included the liver fatty acid-binding protein, malic enzyme, and CYP4A1 genes, was induced, indicating the activation of PPARα-mediated pathways in CYP2E1-deficient mice. Ethanol inhibited the expression of some of the PPARα target genes in wild-type mouse livers, and the inhibitory effect of ethanol was particularly prominent in the CYP2E1-null mice. Morphologically, centrilobular fat accumulation was detected in the ethanol-fed CYP2E1-null mouse livers, suggesting that inhibition of PPARα-mediated pathways might be responsible for the ethanol-induced fatty liver in CYP2El-null mice. In addition, the expression of CYP2E1 was not changed in the PPARα-null mice. The authors suggest that both CYP2E1 and ethanol can regulate PPARα-mediated fatty acid homeostasis and that CYP2E1-induced lipid peroxidation might play a major role in lipid metabolism with PPARα becoming important only when the CYP2E1 level is low and polyunsaturated fatty acids increase.

Crabb et al. (2001) suggest that aldehyde dehydrogenase 2 (ALDH2) is not part of the battery of lipid-metabolizing enzymes and proteins regulated by PPARα. Treatment of rats with the PPARα ligand clofibrate repressed expression of ALDH2 in rats fed either stock rodent chow or a low-protein diet. Consistent with the transfection data, expression of ALDH2 protein was not different in PPARα-null mice. Treatment of the mice with the PPARα agonist WY slightly decreased the level of ALDH2 protein in both wild-type and PPARα-null mice, suggesting that the effect of WY was not mediated by the receptor.

The study of effects of ethanol toxicity includes not only target organs (e.g., liver) but also target cell types in the liver. Cottalasso et al. (2003) report that the various types of liver cells are differently affected by chronic ethanol exposure, which highlights the importance of studying each type of sinusoidal cell. Wheeler et al. (2001) present a review highlighting new

data in support of the hypothesis that Kupffer cells play a pivotal role in hepatotoxicity due to ethanol by producing oxidants via NADPH oxidase.

Activation of Kupffer cells by lipopolysaccharide (LPS) after ethanol feeding results in enhanced tumor necrosis factor-alpha (TNFα), leading to liver injury. Cao et al. (2002a, b) report that dilinoleoylphosphatidylcholine (DLPC) decreases LPS-induced TNFα generation by Kupffer cells of ethanol-fed rats by blocking p38, ERK1/2, and NF-kappaB activation and also decreases TNFα induction by acetaldehyde, a toxic metabolite released by ethanol oxidation. This effect is augmented in Kupffer cells of ethanol-fed rats, with upregulation of CYP2E1 by ethanol. The authors suggest that since increased TNFα generation plays a pathogenic role in alcoholic liver disease, the DLPC action on Kupffer cells may explain, in part, its beneficial effects on liver cell injury after ethanol consumption. Mak et al. (2003) report that polyenylphosphatidylcholine (PPC), a mixture of polyunsaturated phosphatidylcholines extracted from soybeans, attenuates hepatocyte apoptosis induced by ethanol feeding of rats. DLPC, at a dose contained in PPC, reproduces the antiapoptotic actions of PPC.

Morio et al. (2000) report that chronic ethanol consumption in rats is associated with increased incidence of hepatic and pulmonary infections. Hepatic and alveolar macrophages from control animals were found to exhibit distinct morphologic and functional properties. Thus, hepatic macrophages were highly vacuolated and appeared larger and more irregular in shape than alveolar macrophages. These cells also displayed greater phagocytic activity and random migration. In contrast, lung macrophages produced more superoxide anion and nitric oxide, and exhibited enhanced chemotactic activity toward the complement fragment C5a. Administration of ethanol to rats for 9–12 weeks resulted in decreased chemotaxis and superoxide anion production by alveolar macrophages, and cell adhesion molecule expression was reduced in hepatic macrophages. Nitric oxide production and inducible nitric oxide synthase protein expression were decreased in both macrophage populations. These effects were not observed after 3–6 weeks of ethanol administration to rats. The authors suggest that changes in macrophage functioning may play a role in decreased host defense following chronic ethanol exposure.

4. SUMMARY

The information presented in this issue paper illustrates the complex nature of interactions between TCE and its metabolites and other co-exposures as reported in the new literature. Information on TCE metabolites, either separately or in combination, may enhance understanding of TCE toxicity, particularly with regard to active agents and potential MOAs.

Extrinsic factors, such as co-exposures to other environmental contaminants or substances such as ethanol, appear to be capable of altering patterns of toxicity from TCE exposure, although the degree to which these alterations can be quantified remains an open issue. NAS advice on how best to address these issues, particularly with regard to the interpretation of new data, would help to strengthen EPA's revised risk assessment.

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