TCE Meeting Presentation Supporting Material for P. Dugard
Paul Dugard
to:
Marc Rigas
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Please respond to Paul Dugard
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Dear Dr Rigas:

Please find attached material that may form part of my presentation on May 10. This was originally part of HSIA's comments submitted during the public review of the IRIS draft and it has been abstracted for the convenience of the panel members.

Thank you.

Paul Dugard

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Toxicological Review of Trichloroethylene
In Support of the IRIS Database (draft of October 2009)
Comments of the Halogenated Solvents Industry Alliance, Inc.

Incorporating the Comments of
Prof. Dr. Wolfgang Dekant, University of Wurzburg, Germany
and
Lorenz Rhomberg, PhD, FATS, Gradient Corp.

Submitted by: Paul H. Dugard

General

EPA is to be congratulated for comprehensively covering the extensive literature relating to trichloroethylene (TCE) epidemiology and toxicology. Unfortunately, the review of the large amount information has, too often, been unbalanced with study results selected to support an EPA position without due consideration of contradictory evidence. In all cases where the end-point is significant, EPA should give equal weight to evidence, pro and con. What follows are comments on several of the more significant endpoints with detailed comments provided by Prof. W. Dekant. A separate submission includes a version of the draft IRIS document showing annotations by Prof. Dekant. Dr . Rhomberg provides a series of concerns, both general and specific, regarding the derivation of RfC and RfD values.

One approach that EPA could have used to organize and analyze data to aid interpretation would have been to employ the type of framework recommended by IPCS and ILSI. The framework approach is ideal for assessing mode of action, but other complex issues can often be addressed in this manner also.

Taken together, our comments show that RfC and RfD values selected by EPA are unreasonably low and that critical information regarding cancer induction by TCE has been misinterpreted.

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1. Kidney Toxicity and Carcinogenicity

1.1 General: EPA has followed a recommendation of the NRC in the review of the 2001 IRIS draft released in 2006 to accord greater weight to kidney toxicity and tumorigenesis than to liver responses in the mouse. In general, we support the change in emphasis recommended by the NRC but EPA has now applied unbalanced and incorrect interpretations to the data from epidemiological and toxicity studies to generate unfounded concerns about exposure to TCE and effects on the kidney.

1.2 Metabolism of TCE Relevant to Kidney Toxicity and Carcinogenicity (see the comments of Prof. Dekant for full technical detail): Under the assumed mode of action (MoA) for TCE (see critique below), products of the glutathione conjugation pathway are deemed to be responsible

for kidney toxicity and carcinogenicity. In this hypothesis, the initial product in this path, DCVG, is converted to DCVC which, in turn, may be activated in the kidney or detoxified and excreted following acetylation. In a number of places in the IRIS document, EPA states that "Glutathione conjugation and subsequent bioactivation in humans appears to be 10- to 100-fold greater than previously thought." This notion of a high proportion of TCE being metabolized via the glutathione conjugation pathway is based upon the work of Lash and co-workers which depended upon a questionable analytical technique. If EPA had employed a critical evaluation of the evidence, the substantial and credible information from three other laboratories (Dekant, Green and Kim/Rusyn and co-workers) that indicate a very low level of metabolism of TCE via the glutathione conjugation pathway would have been preferred. The extent of metabolism of TCE via the glutathione conjugation pathway (and DCVC activation) in humans is lower than the already low levels in rodents.

The incorrect assumption of high rate of formation of DCVG in humans leads to false interpretations of rodent kidney toxicity and carcinogenicity, both qualitative and quantitative. Man would be presumed much more sensitive to kidney effects than rodents for a given external dose. For example, the admission that "the inclusion of PBPK reduces RfC and RfD by 300- to 400-fold" when kidney toxicity is the basis, is almost certainly the result of the erroneous estimates – if anything, the use of PBPK should lead to higher RfC and RfD values than those based on external dose**.**

It is essential that EPA reevaluates the extent of metabolism of TCE via the glutathione conjugation pathway in rodents and man.

1.3 Mode of Action for Kidney Toxicity and Carcinogenicity (for additional technical detail see the comments of Prof. Dekant): EPA considers that the formation of DCVC from TCE and its activation in kidneys of rats, mice and humans to be the cause of toxicity and, through genotoxicity, tumor formation. A balanced evaluation of the evidence simply does not support these opinions. The summary of Prof. Dekant's review is as follows:

From the known potency of DCVC administered directly to rats, the toxicity of TCE in chronic or long term experiments in rats cannot be explained solely on the extent of DCVC production and activation. The generation of a flood of formic acid through the kidney of rats exposed to TCE (by a mechanism fully understood) does lead to recognizable kidney damage. Although EPA dismisses formic acid because histopathological damage appears to be different between that seen for trichloroethanol (generates formic acid only – no DCVC component) and TCE, it appears highly likely that a combination of DCVC and formic acid damage underlies kidney toxicity in the rat. In mice, less formic acid is released following TCE administration and DCVC activation is greater in mouse kidney which suggests that DCVC may play a greater role in mouse kidney toxicity. Since DCVC is not a highly potent kidney toxicant, the very low levels generated in man are unlikely to cause kidney toxicity. Human experience supports this: Despite historical occupational exposures greater than 100 ppm on an 8 hour time-weighted-average with peak exposures reaching many thousand ppm, kidney disease has not been associated with TCE. Those studies in which markers of kidney damage have been studied have not provided clear evidence of an effect of TCE in man. The conclusion must be that kidney damage is highly unlikely to occur at current occupational exposure levels (ACGIH TLV is 10 ppm, 8 hour TWA) and of no concern for the general population.

EPA considers that kidney tumors in rats result from the genotoxicity following DCVC activation. The reasons to consider this to be improbable are 1) That DCVC, although positive in *in vitro* bacterial mutagenicity tests (following activation by endogenous bacterial enzymes or enhanced by exogenous rat kidney preparations), has not been found, in credible studies, to be anything more than weakly genotoxic *in vivo*. 2) Combining the weak genotoxicity with the low levels generated in rats does not indicate a primary role for generation of tumors by a genotoxic mechanism. 3) The single long term experiment involving direct administration of DCVC to rats did not generate tumors in a protocol which would have been expected to show induction of tumors by a genotoxic mechanism (Terracini and Parker, 1965). This study cannot be used to "prove the negative" (i.e. DCVC is not a kidney carcinogen) but, despite its age, was well designed and conducted. 4) DCVC activation in the mouse kidney is greater than in rat kidney but kidney tumors have not been induced by TCE in any study. A genotoxic mode of action might have been expected to induce tumors in mice.

On balance, rat kidney tumors are unlikely to have arisen via a genotoxic mechanism following TCE administration. Since tumors have only been induced at dose levels of TCE that cause frank kidney toxicity, and male rats have a recognized tendency to develop kidney tumors under circumstances of repeated damage-repair cycles, this seems to be the most plausible mode of action.

Whether the incidence of rat kidney tumors should be used to calculate human cancer risk is debatable, but if such calculations are employed, a non-linear MoA should be assumed.

<u>1.4 Use of Epidemiological Data to Calculate Cancer Potency:</u> The NRC (2006) review of the 2001 IRIS draft document stated that epidemiology studies available at that time were unsuitable for calculations of cancer slope factors to be used for regulatory purposes. Calculations using epidemiological data were considered acceptable for comparison with animal-based calculations only. The 2009 IRIS draft document derives a slope factor based upon the Charbotel et al. (2006) case control study of renal cell carcinoma and TCE exposure. This study is better than many TCE epidemiology studies but it must be questioned whether it provides a sufficiently robust starting point for calculation of a slope factor that will form the basis of regulations, setting vapor intrusion limits and other factors having significant impact on societal resources. The Charbotel et al (2006) study shows the significance of confounders on the outcome of the analysis and, with confounders taken into account, the elevation of incidence above unity is too small and uncertain to be used in a firm calculation. The dose response relationship reported in the study is heavily dependent upon the exposure assessment for a very small number of individuals and is therefore less robust than statistical evaluation would suggest. It is interesting that EPA takes the Charbotel et al (2006) study as the primary evidence for a causal relationship between TCE and renal cell carcinoma; Charbotel herself concludes that the study "…suggests an association between exposures to high levels of TCE and increased risk of RCC."
The supposed agreement between the cancer slope factors derived from rat data and the Charbotel study is not real. The slope factor derived from rat data involves an inter-species conversion based on the erroneous estimate of high DCVG production and activation of DCVC in humans and the slope factor is higher because of that. As discussed above, rat kidney tumors are most likely to have developed as a result of a non-genotoxic MoA and a non-linear dose response relationship, if any should be assumed.

It is recommended that a calculation of cancer slope factor based on Charbotel et al (2006) is used <u>only</u> for comparison with results from animal studies.

2. Mouse Liver Tumors, Mode of Action

2.1 General: It is well recognized that TCE induces mouse liver tumors in some strains of mouse but not in rats. There is no convincing support for hepatocarcinogenicity in epidemiology studies. It has been reasonably concluded that a product of oxidative metabolism of TCE is responsible for mouse liver tumors. The more significant metabolites generated from TCE via the oxidative pathway are either weakly genotoxic, at most, or non-genotoxic (see Moore and Harrington-Brock, 2000 for a comprehensive review). It is highly likely that mouse liver tumors are generated by a non-genotoxic mechanism and many consider that the major circulating metabolite of TCE, trichloroacetic acid (TCA) is responsible acting via factors associated with peroxisome proliferation mediated through PPARα. Acceptance of a PPARα-related MoA could lead to a conclusion that the mouse liver tumors are not relevant to man. At the very least a non-linear MoA for mouse liver tumors should be accepted if TCA operates via a PPARα-related MoA.

EPA has two main reasons for rejecting a PPARα-related mechanism that are specific for TCE and a general position regarding the interpretation of cases where PPARα and rodent liver tumors are linked. The two specific reasons are 1) That dichloroacetic acid (DCA) is a metabolite of TCE; it is a rodent liver carcinogen; it is a genotoxin and makes an unknown but possibly significant contribution to mouse liver tumors induced by TCE. 2) That TCA is not sufficiently potent as a mouse liver carcinogen to explain the number of mouse liver tumors generated by TCE. The general position taken by EPA's NCEA Washington Office is that even if rodent tumors are generated via a PPARα-related MoA, it has not been sufficiently well established that this MoA is not relevant to man and, since the mechanism is not fully understood, a linear dose response extrapolation is appropriate. Here is HSIA's perspective on these issues:

2.2 The Role of DCA in the Induction of Mouse Liver Tumors by TCE: As discussed by Prof. Dekant, the amount of DCA generated from TCE is very small or even non-existent. If this low level of production is combined with the weak genotoxic potential and the relatively low potency of DCA as a mouse liver carcinogen in its own right, there seems to be no justification for assuming DCA contributes significantly to mouse liver tumors induced by TCE. Bull et al (2002) report a clear difference in the phenotypes of tumors induced by DCA versus TCA. A proportion of DCA tumors contained c-Jun but none of the TCA tumors examined showed this character. Tumors from TCE treated animals were reported to show a mixture of TCA and DCA phenotypes with quite a high proportion relating to DCA. The problem with this study is that the TCE tumors are much later stage than those examined for TCA and DCA (79 weeks versus 52 weeks). It is well known that later stage tumors develop complex genetic composition; thus a contribution from DCA to tumor induction by TCE cannot be supported by this study. The only true conclusion that can be drawn is that there is no evidence that conversion of TCA to DCA occurs to affect the nature of tumors seen, and this can be applied to TCA derived from TCE – conversion of TCA to DCA is unlikely to be significant for induction of mouse liver tumors.

EPA's detailed analysis of liver weight increases suffers from the same overestimates of TCA bioavailability discussed in section 2.3.

There is no convincing reason to believe that DCA contributes to mouse liver tumor induction by TCE.

2.3 Potency of TCA as a Mouse Liver Carcinogen: As explained by Prof. Dekant, and as analyzed by Sweeney et al (2009), EPA has misinterpreted the bioavailability of TCA from drinking water, the mode of administration used in mouse carcinogenicity studies employed to establish the potency of TCA. Since the bioavailability of TCA falls with rising concentration in drinking water, not taking this into account leads to a lower estimate of potency of an internal dose of TCA than if correct values for bioavailability are employed. Using a correct estimate of the potency of TCA shows that sufficient TCA is generated from TCE to explain the incidence of mouse liver tumors (Fisher and Dugard, unpublished).

EPA should recalculate the potency of internal doses of TCA (based on an improved estimate of bioavailability in TCA drinking water studies) and reassess the role of TCA in the generation of mouse liver tumors by TCE.

2.4 MoA of TCA Hepatocarcinogenicity and Implications for Human Exposure to TCE: At the time of writing, release of the report of an NRC committee review of the draft IRIS support document for perchloroethylene is imminent. The issue of TCA MoA is expected to be addressed in that review. The evidence strongly indicates a PPARα-related MoA for the induction of mouse liver tumors by TCA and thus also by TCE. This would leave the issue of the implications of such a MoA for human exposures to TCE. At this time EPA's NCEA Washington Office is becoming increasingly isolated in its opinion that PPARα-related rodent liver tumors remain fully relevant to man and that linear dose-response extrapolations are appropriate. This isolation is apparent within EPA as well as from other regulatory federal agencies in the US and around the world.

It remains to be seen how this debate plays out, but the majority opinion among respected scientists seems to support a diminished concern regarding rodent liver tumors associated with a PPARα-related MoA.

3. Immunotoxicity

Two immunotoxicity studies have been used to support very low RfC and RfD values. The effect chosen from the study of Keil et al (2009) is a reduced thymus weight in mice seen at relatively low dose levels. This stands in contrast to a number of studies (immunotoxicity and other) in which no effect on thymus weight was evident in rats and mice following relatively high dose levels of TCE. The other study used to develop the reference values is the developmental immunotoxicity study reported by Peden-Adams et al (2006) in which effects were reported in mouse offspring following exposure of dams and, post-weaning, the pups to 1.4 ppm TCE in drinking water. The study appears to have been well conducted and stands as the only one of its kind. The reason for concern is that the effect is apparently seen at such a low dose which stands in contrast with the same effects seen only at relatively high dose levels in

adult rodents. It is important for the substantially higher sensitivity of fetus or pup to be confirmed in separate investigation.

After comments on other endpoints driving low reference values have been taken into account, it is possible that only these immunotoxicity studies would be left supporting very low RfD and RfC values. At this time, the findings do not appear to be sufficiently robust to carry that responsibility.

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