



HSIA

halogenated
solvents
industry
alliance, inc.

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Dr. John Budroe
Chief, Air Toxicology and Risk Assessment Section
Air, Community, and Environmental Research Branch
Office of Environmental Health Hazard Assessment
1515 Clay Street, 16th Floor
Oakland, CA, 94612

Re: Draft Inhalation Cancer Unit Risk Factor for Perchloroethylene

Dear Dr. Budroe:

The Halogenated Solvents Industry Alliance, Inc. (HSIA) represents producers and users of perchloroethylene (PCE), a solvent commonly used in dry cleaning and vapor degreasing. HSIA offers these comments on OEHHA's public review draft Technical Support Document for the derivation of an inhalation potency factor for PCE.

HSIA supports the use of sound, peer-reviewed scientific information in the development of toxicity values. In this regard, we note that the Public Review Draft departs significantly from the current consensus position reflected in the Toxicological Review of Tetrachloroethylene (Perchloroethylene) In Support of Summary Information on the Integrated Risk Information System (IRIS) published in February 2012 by the US Environmental Protection Agency (EPA) (the "IRIS Assessment"). That assessment derived an inhalation unit risk factor of 3×10^{-7} per $\mu\text{g}/\text{m}^3$, based on male mouse hepatocellular tumor data from a bioassay sponsored by the Japan Industrial Safety Association (JISA).¹

The OEHHA Public Review Draft, on the other hand, derives a unit risk factor of 6.1×10^{-6} per $\mu\text{g}/\text{m}^3$, based, in part, on an increase in mononuclear cell leukemia (MCL) in one strain of rats. Use of this endpoint was not recommended by the peer review panel convened by the National Research Council (NRC) of the National Academy of Sciences for the express purpose of reviewing the EPA IRIS Assessment.² Rather, the NRC panel recommended that the mouse hepatocellular tumors be used for cancer risk estimation.

¹ JISA, Carcinogenicity Study of Tetrachloroethylene by Inhalation in Rats and Mice, Hadano, Japan (1993).

² NRC, Review of the Environmental Protection Agency's Draft IRIS Assessment of Tetrachloroethylene, Washington, DC: National Academies Press (2010).

We urge OEHHA to adopt the approach taken by EPA following peer review by the National Academy of Sciences. Our comments make three principal points:

- MCL Lacks Relevance for Humans
- OEHHA Inappropriately Minimizes the Uncertainty Associated with the Glutathione Conjugation Pathway in Metabolism of PCE
- OEHHA's Choice of Total PCE Metabolism as the Dose Metric in PBPK Modeling is Inappropriate

1. MCL Lacks Relevance for Humans

As noted in the OEHHA Public Review Draft, there has been ongoing controversy on the importance of MCL as a potential toxicity endpoint for PCE. In its review of the draft IRIS assessment in 2010, the majority of the NRC panel recommended against the use of MCL data from F344 rats to calculate a cancer slope factor for regulatory use. In the final IRIS Assessment, EPA concurred with that majority opinion and used hepatocellular tumors in male mice from the 1993 JISA study for the estimation of potential cancer risk. EPA also provided a potency factor based on the male and female F344 rat MCL data from the JISA study, but did not recommend its use.

HSIA urges OEHHA to reconsider recommending a cancer potency value based on the MCL data. Its propensity to develop spontaneous MCL shows that the F344 strain does not reflect either the general human population or any significant sensitive sub-population. Mononuclear cell leukemia is an extremely common spontaneous neoplastic disease of the aging F344 rat. Consistent features are splenomegaly, anemia, thrombocytopenia and leukemic infiltration of the spleen, liver lung, and in advanced stage, of several other organs. The incidence is variable but has been increasing progressively with time and can exceed 70% in controls in some studies. This compares with background incidence of less than 1% in other strains of commonly used laboratory rats. The incidence in F344 rats is modulated by a variety of factors not clearly related to carcinogenicity. Corn oil gavage, for example, has been shown consistently to reduce the incidence of MCL in male, but not female, controls.

The neoplastic mononuclear cells appear to be derived from large granular lymphocytes (LGLs) and the alternative name of LGL leukemia has been suggested. The tumor cell is of the NK type in most, if not all, cases. LGL leukemia, although uncommon, does occur in man. There are two types: T-LGL leukemia which has a chronic course characterized by neutropenia, recurrent infections, splenomegaly and accompanying rheumatoid arthritis, and the much rarer NK-LGL leukemia which has an acute course, more pronounced splenomegaly, and thrombocytopenia. The latter type appears to resemble more closely the disease in the F344 rat than the former. The etiology of human LGL leukemia is

unknown. There is some evidence that viral infection may play a role but no evidence that a chemically-related increased of MCL in the F344 rat is indicative of potential to induce LGL leukemia in man.

The NRC position, shared by many others, is that the incidence of MCL in F344 rats is unsuitable for the calculation of cancer potency values for man. Although there have been a number of carcinogenicity studies of PCE in the rat involving several different rat strains, evidence of increases in MCL has only been seen in two studies in the F344 rat but not in other strains. As a strain-specific effect, it is not relevant for evaluation of human cancer risk. The evidence available from a number of large epidemiology studies of PCE-exposed individuals also supports this conclusion.

Thomas *et al.* (2007) evaluated the significance of MCL findings in multiple NTP bioassays that used the F344 rat. They examined the incidence of leukemia in 2-year bioassays that included untreated male and female F344 rats from 1971 to 1998 and found that background tumor incidence increased substantially, from 7.9% to 52.5% in males and from 2.1% to 24.2% in females, over that period. Their analysis also found that MCL responses are highly variable and subject to substantial modulation by dietary factors. As noted in the NRC review of the draft IRIS assessment, “NTP has decided to stop using its F344/N rat colony in its bioassays for reasons that include the high background rate of MCL”:

“Given the potential relevance of the F344 rat LGLL [MCL] to the rare human NK-LGLL and in light of the factors that complicate definitive interpretation of chemical-induced increases in LGLL (*i.e.*, that spontaneous LGLL in F344 rat occurs at a high and variable incidence, is capable of being modulated by dietary factors such as corn oil, and has little evidence to support a mode of action [MOA]), it is proposed . . . to adopt a “weight-of-evidence” approach when statistically identified increases in LGLL occur with exposure to a given compound.”³

In terms of weight of evidence, it is important to recognize that MCL was not induced by PCE in long-term studies in Sprague-Dawley (inhalation, Rampy *et al.*, 1978;⁴ oral, Maltoni and Cotti, 1986⁵) or Osborne-Mendel (oral, NCI, 1977⁶) rats, although the NCI study

³ Thomas, J, Haseman, JK, Goodman, JI, Ward, JM, Loughran, TP, Jr, Spencer, PJ, A Review of Large Granular Lymphocytic Leukemia in Fischer 344 Rats as an Initial Step toward Evaluating the Implication of the Endpoint to Human Cancer Risk Assessment, *Toxicol Sci* 99: 3-19 (2007).

⁴ Rampy, LW, Quast, JF, Balmer, MF, Leong, BKJ, Gehring, PJ, Results of a Long-term Inhalation Toxicity Study on Rats of a Perchloroethylene (Tetrachloroethylene) Formulation, Midland, MI: Dow Chemical (1978).

⁵ Maltoni, C and Cotti, G, Results of Long-term Carcinogenicity Bioassays of Tetrachloroethylene on Sprague-Dawley Rats Administered by Ingestion, *Acta Oncol* 7: 11-26 (1986).

is weakened in that respect by poor survival. Overall, there were no indications in rat or mouse long term studies that PCE can induce any other forms of lymphohematopoietic disease. The moderate MCL response limited to the highly susceptible F344 rat strain provides no indication that PCE is leukemogenic in humans.

“The NRC (2010) peer review panel agreed that there was little information on the mode of action of tetrachloroethylene-induced rat MCL incidence. The panel, however, had differing opinions about the human relevance of rat MCL. Some of the reviewers judged that more research was needed to establish the relevance of the rat MCL to assessing human cancer hazard or risk. Some reviewers believed that available data were adequate to establish the human relevance of the rat MCL. In the context of quantitative assessment, a majority of the NRC (2010) panel judged that uncertainties associated with MCL were too great to support their selection over other tumor types.”⁷

The NRC panel judged that “the use of the MCL data could be justified only if it is EPA’s policy to choose the most conservative unit risk when considering options but that such justification should be distinguished as a policy decision, not a scientific one. They believed that a more scientifically defensible approach would be to use the data set that has the least uncertainty rather than the dataset that yields the highest estimate of risk.” OEHHA’s arguments regarding strength of evidence notwithstanding, it is clear that the weight of the evidence does not justify use of the F344 rat MCL data for risk assessment.

2. OEHHA Inappropriately Minimizes the Uncertainty Associated with the Glutathione Conjugation Pathway in Metabolism of PCE

During its review of the draft PCE IRIS assessment, the NRC criticized EPA’s PBPK model for not reflecting the contribution of the GSH conjugation pathway. EPA had taken the position that the available data on GSH-dependent metabolism were from *in vitro* studies or constituted measurements of urinary excretion products which do not necessarily represent toxic species *in vivo*. The NRC agreed that the available data for the GSH pathway were more limited than for the CYP-dependent oxidative pathway, yet recommended that “better justification is necessary to rule out modeling the GSH pathway.” In response to the NRC comment, EPA developed a “harmonized” PBPK model that included consideration of the GSH pathway, but conceded that “the GSH conjugation pathway in humans remains highly

⁶ NCI (National Cancer Institute), Bioassay of Tetrachloroethylene for Possible Carcinogenicity (NCI-CGTR-13; DHEW Publication No. (NIH) 77-813), Bethesda, MD: National Institutes of Health (1977), http://ntp.niehs.nih.gov/ntp/htdocs/LT_rpts/tr013.pdf.

⁷ IRIS Assessment, 4-271.

uncertain and/or variable, and that additional data are needed to better quantify that pathway in humans.” As a consequence, “*the [IRIS] assessment does not rely on quantitative estimates of GSH pathway metabolism provided by the new PBPK model.*”⁸

An important consideration in evaluating the role of the GSH pathway in PCE toxicity is the exposure dose. Mice have been shown to metabolize PCE to trichloroacetic acid (TCA) to a greater extent than rats; human activity is reported to be even lower than that in rats. In both rats and humans, saturation of this CYP-dependent oxidation of PCE is reported to occur at exposure concentrations of 100 ppm or greater, raising the potential of exposure-dependent metabolite patterns.⁹ It should be noted that the F344 rats were exposed to PCE concentrations of 200 and 400 ppm in the NTP (1986) bioassay and 50, 200, or 600 ppm in the JISA (1993) study. In addition to potential impacts on metabolite patterns at/around CYP saturation, consideration must be given to the relevance of bioassay exposure levels to most human exposures.

As with oxidative metabolism, the primary pathway for metabolism of PCE, *in vitro* studies of GSH conjugation in mice, rats, and humans have shown considerable intra- and interspecies variability. Reported conjugation rates also differ by several orders of magnitude between laboratories. However, in order for GSH conjugation to be relevant in humans, there must be a significant capacity to form glutathione conjugates. In our view, this has not been demonstrated.

The notion of a high proportion of PCE being metabolized via the glutathione conjugation pathway is based largely upon the trichloroethylene (TCE) work of Lash and co-workers utilizing a questionable analytical technique.¹⁰ The technique is based on an indirect method developed by Reed¹¹ and involves liquid chromatographic (LC) separation followed by derivatization and UV detection. Substantial and credible information from three other laboratories (Dekant,¹² Green¹³ and Rusyn and co-workers¹⁴) using radioactivity, GC/MS or

⁸ *Id.* [emphasis added].

⁹ World Health Organization (WHO), WHO Guidelines for Indoor Air Quality: Selected Pollutants, The WHO European Centre for Environment and Health, Bonn, Germany (2010).

¹⁰ Lash, L, Lipscomb, J, Putt, D, Parker, J, Glutathione Conjugation of Trichloroethylene in Human Liver and Kidney: Kinetics and Individual Variation, *Drug Metab Dispos* 27: 351-359 (1999); Lash, L, Putt, D, Brashear, W, Abbas, R, Parker, J, Fisher, J., Identification of S-(1,2-dichlorovinyl)glutathione in the Blood of Human Volunteers Exposed to Trichloroethylene, *J Toxicol Environ Health A* 56: 1-21 (1999).

¹¹ Reed, DJ, Babson, JR, Beatty, PW, Brodie, AE, Ellis, WW, and Potter, DW, High Performance Liquid Chromatography Analysis of Nanomole Levels of Glutathione, Glutathione Disulfide, and Related Thiols and Disulfides, *Anal. Biochem.* 106:55-62 (1980).

¹² Dekant, W, Bioactivation of Halogenated Alkenes by Glutathione Conjugation a Mechanistic Explanation for

LC/MS methods indicate a *very* low level of metabolic capacity via the glutathione conjugation pathway. According to these researchers, the extent of metabolism via the glutathione conjugation pathway in humans is lower than the already low levels in rodents, in direct contrast to the Lash findings. In our view, the incorrect assumption of a high rate of formation of GSH metabolites in humans can lead to the false interpretation of rodent toxicity and carcinogenicity data, both qualitatively and quantitatively.

In describing EPA's results with the harmonized model, OEHHA did acknowledge the high level of uncertainty/variability associated with the GSH pathway:

“[t]he prediction range of the dose-metric estimates was narrow for both PCE AUC [area under the curve] (<20%) and oxidation (<1.5-fold). In contrast, the dose metrics for the GST conjugation pathway in mice and humans displayed significantly higher variability. In the human model, the MCMC analysis produced an apparently bimodal distribution with an approximately 3000-fold difference between the two posterior modes. Chiu and Ginsberg (2011) [the EPA PBPK developers] were not able to determine how much of the spread was due to model uncertainty or population variation”

Yet, “[i]n spite of the unresolved issues related to PCE's GST metabolism, OEHHA considers the Chiu and Ginsberg model to be the best available methodology for estimating dose metrics in the dose-response assessment.” HSIA recommends that OEHHA reconsider the role of the GSH conjugation pathway in the metabolism and potential toxicity of PCE.

3. OEHHA's Choice of Total PCE Metabolism as the Dose Metric in PBPK Modeling is Inappropriate

Acknowledging the uncertainties associated with the toxicokinetics and toxicodynamics of PCE, OEHHA considered four dose metrics in its dose-response assessment. These included:

their Renal Carcinogenicity, J Cancer Res Clin Oncol 116:1063 (1990).

¹³ Green, T, Dow, J, Ellis, MK, Foster, JR, Odum, J, The Role of Glutathione Conjugation in the Development of Kidney Tumours in Rats Exposed to Trichloroethylene, Chem Biol Interact 105: 99-117 (1997).

¹⁴ Kim, S, Kim, D, Pollack, G, Collins, L, Rusyn, I, Pharmacokinetic Analysis of Trichloroethylene Metabolism in Male B6C3F1 Mice: Formation and Disposition of Trichloroacetic Acid, Dichloroacetic Acid, S-(1,2-dichlorovinyl)glutathione and S-(1,2-dichlorovinyl)-L-cysteine, Toxicol Appl Pharmacol 238: 90-99 (2009), <http://dx.doi.org/10.1016/j.taap.2009.04.019>; Yoo HS, Bradford BU, Kosyk O, Shymonyak S, Uehara T, Collins L, Bodnar WM, Ball LM, Gold A, Rusyn I, Comparative Analysis of the Relationship between Trichloroethylene Metabolism and Tissue-Specific Toxicity among Inbred Mouse Strains: Liver Effects, J Toxicol Env Hlth Part A, 78: 15-31 (2015).

- Total PCE metabolism comprising the sum of oxidative and GST pathway metabolism in the liver and kidney, plus oxidation in the lung,
- Pathway-specific metabolism, using either oxidative or GST pathway metabolism separately for one or more tissues,
- PCE blood concentration expressed as area under the concentration curve (AUC), and
- Applied air concentration

OEHHA chose total metabolism as its dose metric, asserting that it “accounts for known metabolic differences across species and provides a dose adjustment for saturation effects in the oxidative pathway, but also involves assuming that carcinogenic potency is directly proportional to the rates of metabolic production in the two pathways.” HSIA submits that there are no data to support a role for metabolites of PCE in the generation of MCL in Fisher F344 rats and that, as affirmed by EPA and the NRC, a role for GSH-derived metabolites in renal or hepatocellular tumors is still controversial and associated with a high degree of uncertainty and variability.

In its review of the draft IRIS assessment, the NRC addressed this issue in considerable detail. The panel noted that EPA’s use of “total metabolism” as the dose metric for carcinogenicity reflected primarily the oxidative CYP metabolic pathway due to the “large differences in the flux of the metabolism between it and the GSH pathway.” It recommended that “EPA explore the possibility of adding the GSH pathway to a harmonized PBPK model. If such modeling is determined to be infeasible, total metabolism can be used as a reasonably conservative dose metric.”

As can be seen in its response to NRC’s recommendation, EPA did in fact create a harmonized model which looked at three different metabolic pathways (oxidation, GSH-conjugation with further β -lyase metabolism, and GSH-conjugation with further β -lyase-independent metabolism). EPA found, however, that due to high uncertainty/variability in the human database, the GSH pathway could not be used:

“The PBPK modeling analysis showed that the GSH conjugation pathway in humans remains highly uncertain and/or variable, and that additional data are needed to better quantify that pathway in humans Therefore, the assessment does not rely on quantitative estimates of GSH pathway metabolism provided by the new PBPK model. Instead, the quantitative risk estimates presented in the revised assessment rely on estimates of blood tetrachloroethylene, oxidation of tetrachloroethylene, and route-to-route extrapolation from this new model. These dose metric estimates from the new

model are robust and consistent with prior models and, thus, insensitive to model choice.”¹⁵

In the final IRIS Assessment, EPA used the total rate of oxidative metabolism in the liver as the dose metric for PCE-induced liver tumors and the blood level of PCE, expressed as the area under the curve (AUC), for all others, including MCL. HSIA agrees with EPA that the currently available data on the role of GSH conjugation in PCE toxicity do not support using total metabolism as the dose metric for dose-response analysis. We urge OEHHA to reconsider its selection. In addition, given the lack of any defined mechanism linking PCE and MCL in F344 rats, there is no justification for OEHHA’s decision to select total PCE metabolism as the dose metric for development of a potency factor based on that endpoint.

Respectfully submitted,

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¹⁵ IRIS Assessment, A-21 (Comment A.3.7).