West Virginia University
SCHOOL OF MEDICINE

January 4, 2018

Gerald W. Bowes, Ph.D.
Manager, Cal/EPA Scientific Peer Review Program
Office of Research, Planning and Performance
State Water Resources Control Board
1001 I Street
Sacramento, CA 95814

Dear Dr. Bowes:

Please find attached, a pdf copies of my review of the "Public Health Goals (PHG) for Cis- and Trans-1,2-dichloroethylene in drinking water" as two separate documents. I have included in these documents, my review of the immunotoxicology data provided in the document itself as well as the original data presented in the literature citations. Also, included is a recommendation to you, regarding the appropriateness of your proposed ADD.

Thank you for the opportunity served the citizens of the State of California.

Sincerely,

John B Barnett

Digitally signed by John B Barnett
DN: cn=John B Barnett, o=West Virginia University, ou=Micro, Immunol & Cell Biol, email=jbarnett@hsc.wvu.edu, c=US
Date: 2018.01.04 07:32:24 -05'00'

John B. Barnett, Ph.D.
Professor and Chair

DEPARTMENT OF MICROBIOLOGY, IMMUNOLOGY AND CELL BIOLOGY

PO Box 9177 | 2095 Health Sciences Center North
Morgantown, WV 26506-9177

(O) 304-293-2649 (F) 304-293-7823

http://medicine.hsc.wvu.edu/micro/

Evaluation of the Public Health Goals (PHG) for Cis-1,2-dichloroethylene in drinking water.

The California Environmental Protection Agency (CalEPA) has proposed that PHG for exposure levels via drinking water for Cis-1,2-dichloroethylene (cis-1,2-DCE) be modified based on data obtained on the immunotoxicity of these compounds. The characteristics of cis-1,2-DCE are provided in PHG proposal provided to this reviewer. The document reviews the previous data on kidney weight that is to be retained. The document also provides for a review of the data that formed the bases of setting the PHG of 50 parts per billion (ppb) for cis-1,2-DCE.

The previous standard, to be retained, is based on kidney weight observed in a 90-day gavage study in rats by McCauley et al., 1990. The data regarding increases in kidney weight are summarized in Tables 1 and 2 of the proposal. Following these data is a discussion of the calculations performed and exposure assumptions that formed the bases of the acceptable daily dose (ADD) that a human could consume for an entire lifetime without adverse health effects as well as the calculations that allowed the determination of the 'public health-protective concentration (C)'. The significance of the kidney weight data is outside the expertise of this reviewer and no comments will be provided on these data. However, no data or reports on the immunotoxicity of cis-1,2-DCE were provided or found in a PubMed search of the literature. Given this lack of data, the CalEPA appears to have no alternative but to rely on the kidney weight data to set the ADD for cis-1,2-DCE.

In reviewing the sum-total of data several limitations were noted. Most importantly, there were no developmental studies reported. Prenatal exposure often produces different outcomes than those seen with adult, or even, neonatally-exposed animals. This becomes especially important with lipophilic compounds, such as DCE, which are predicted to readily cross the placenta (PHG; Cis- and Trans-1,2-dichloroethylene; March 2006, page 14). Similarly, on the opposite spectrum, there were no studies on aged animals to determine possible effects on the elderly. This presents at least three data deficiencies that are problem from a regulatory perspective. These deficiencies are 1) lack of any immunotoxicology data, 2) lack of developmental studies in which immunotoxicology was an end point, and 3) lack of studies on the aged, with immunotoxicology endpoints. This leaves the only course of action to insert an additional 'uncertainty factor' into the calculation. Thus, some consideration should be made to include in the ADD calculation, a '*Database deficiency factor*' for the lack of data described above, i.e., $\sqrt{10}$, as indicated in Appendix III.

Submitted by:
John B Barnett, Ph.D.. Professor and Chair
Department of Microbiology, Immunology and Cell Biology
West Virginia University, Morgantown, WV 26508

Evaluation of the Public Health Goals (PHG) for Trans-1,2-dichloroethylene in drinking water.

The California Environmental Protection Agency (CalEPA) has proposed that PHG for exposure levels via drinking water for trans-1,2-dichloroethylene (trans-DCE) be modified based on data obtained on the immunotoxicity of these compounds. The characteristics of trans-DCE are provided in PHG proposal provided to this reviewer. The document reviews the previous data on kidney weight that is to be retained. The document also provides for a review of the new immunotoxicology data provided that formed the bases of setting the PHG of 50 parts per billion (ppb) for trans-DCE.

The previous standard, to be retained, is based on kidney weight observed in a 90-day gavage study in rats by McCauley et al., 1990. The data regarding increases in kidney weight are summarized in Tables 1 and 2 of the proposal. Following these data is a discussion of the calculations performed and exposure assumptions that formed the bases of the acceptable daily dose (ADD) that a human could consume for an entire lifetime without adverse health effects as well as the calculations that allowed the determination of the 'public health-protective concentration (C)'. The significance of the kidney weight data is outside the expertise of this reviewer and no comments will be provided on these data.

The remaining comments are in regard to the immunotoxicity data used to determine a toxicity endpoint for trans-DCE.

Two immunotoxicity studies were consulted by CalEPA to determine the toxicity endpoint of trans-DCE. Munson et al, 1982 measured the IgM antibody response to sheep erythrocytes (SRBC) using the antibody forming cell assay in CD-1 outbred mice exposed to trans-DCE. As described in the PHG, there was a 'trend' toward suppression at the highest dose. In addition to the tables provided in the PHG, this reviewer assessed the original literature report (Munson *et al., 1982*). The humoral immune response effects were assayed using a hemolytic plaque assay and the data is reported as the number of antibody-forming cells (AFC), which the author's purport has become a de facto assay for testing compounds for immunotoxicity. The rational for using this assay is that SRBC are multivalent antigens, which induce a robust immune response. The advantages of this assay is that generally shows good reproducibility and has a negligible false-positive rate for immunotoxicity, i.e., any repeatable significant decrease in AFC caused by a xenobiotic is definitely immunotoxic. Although not discussed in the article or the PHG, the likely assumption for this choice is that there must be a primary antibody response before there can be development of memory cells which are the bases of long-lasting immune protection. However, this assay only measures the primary immune response and, as the test was performed, only the IgM isotype of antibody is assayed and only one function of antibody, i.e., the ability to activate the lytic complement pathway. Thus, it is impossible to determine whether other important immune functions, such as isotype switching to the more important IgG isotype or formation of memory cells were affected. These studies were also conducted prior to our current understanding of the important interactions between the innate immune response, which would include natural killer cells, and the acquired immune response. That is, it is no longer appropriate to consider these two arms of the immune system to be independent entities but more synergistic in their functions. These studies also do not test for possible exacerbation of allergies or induction of autoimmune disease.

It also does not appear that the immunotoxicology studies were repeated to determine their reproducibility.

The study performed by Shopp et al. (Table 8, PHG) was a 90d study of trans-DCE administered via drinking water (Shopp *et al., 1985*). In addition to the AFC assay discussed above, this study also included determination of hemagglutination titers to SRBC and the response of spleen cells to immune cell-specific mitogens, lipopolysaccharide (LPS) from *Salmonella typhosa* for B cells and Concanavalin A (Con A) for T cells. It also does not appear

that the studies were repeated to determine the reproducibility of the studies which may have clarified the odd decrease in the number of AFC per spleen in females at the 23 mg/kg-day dose level (Table 8, PHG) while other doses, including a higher concentration, showed no difference. As previously reported, the males were more sensitive to the effects of trans-DCE with significantly fewer AFC at 175 and 387 m/kg-day (Shopp, *et al., 1985*). Other humoral immune response assays reported by Shopp et al. (Shopp, *et al., 1985*), such as the serum hemagglutination assay showed no difference. Mitogenic activity induced by LPS and Con A, showed no decrement at the doses tested. Neither of the mitosis assays induced by LPS or Con A are particularly sensitive to immunotoxic compounds, therefore, these negative tests should be interpreted with caution.

The original data in Barnes et al (Barnes *et al., 1985*) wherein they document several parameters, e.g., organ weights, serum and liver chemistry, etc., due to exposure to trans-DCE was examined. With few exceptions, they report basically no effect. What is distressing about this report is the lack of any attempt to follow up on their most basic findings. For example, females showed a dose-responsive decrease in thymus weight, and the reason for this decrease was not tested by flow cytometry nor was there any histopathology to corroborate the findings. I agree that these data should not be used for the calculations of the ADD.

In reviewing the sum-total of trans-DCE immunotoxicology data several limitations were noted. Most importantly, there were no developmental studies reported. Immunotoxicological effects due to prenatal exposure are often different than those seen with adult, or even, neonatally exposed animals. This becomes especially important with lipophilic compounds, such as DCE, which are predicted to readily cross the placenta (PHG; Cis- and Trans-1,2-dichloroethylene; March 2006, page 14) and the findings of Ruckart et al (Ruckart *et al., 2014*) reporting low mean birth weights in children born of mothers exposed to trans-DCE. It is agreed that the developmental studies by Hurtt et al., (Hurtt *et al., 1993*) are not relevant because the doses used are many times higher than the doses used in the immunotoxicology studies and because the tests reported are not as sensitive as the AFC test. Similarly, on the opposite spectrum, there were no studies on aged animals to determine possible effects on the elderly. These data deficiencies present a problem from a regulatory perspective, which then leaves the only course of action to insert an additional 'uncertainty factor' into the calculation. The choice of the antigenic challenge, T-dependent SRBC does not allow for determination of effects on T-independent antibody responses as well as numerous other immunological effects, as previously discussed. Although the reports by Loveless et al. (Loveless *et al., 2007*) and Ladics (Ladics, *2007*) did purport that the AFC assay using SRBC was "a well-validated and highly predictive test for immunotoxicity." It should be noted that some of the authors on the Loveless et al. (Loveless, *et al., 2007*) as well as the Ladics (Ladics, *2007*) reports are investigators that have been using this technique for years, were trained in the same laboratory (Munson) and are thus, potentially biased in favor of this technique. In addition, the only other test used for this validation was an ELISA assay using the same antigen (SRBC), again, precluding tests for effects on the T-independent response. No substantive contemporary experiments on the innate immune response, the cell-mediated immune response, allergic sensitization or autoimmune induction were reported. Further, the level of sensitivity of the AFC assay has not been compared to more contemporary assays.

The caveats identified above aside, it is agreed that the immunotoxicology data is of sufficient quality to be used for the determination of the ADD for trans-DCE. However, some consideration should be made to include in the ADD calculation, a *'Database deficiency factor'* for the lack of development immunotoxicity data, i.e., $\sqrt{10}$, as indicated in Appendix III.

Submitted by:
John B Barnett, Ph.D.. Professor and Chair
Department of Microbiology, Immunology and Cell Biology
West Virginia University, Morgantown, WV 26508

References

Barnes, D. W., Sanders, V. M., White, K. L., Jr., Shopp, G. M., Jr. and Munson, A. E. (1985). Toxicology of trans-1,2-dichloroethylene in the mouse. *Drug Chem Toxicol* **8**(5), 373-92, 10.3109/01480548509041065.

Hurtt, M. E., Valentine, R. and Alvarez, L. (1993). Developmental toxicity of inhaled trans-1,2-dichloroethylene in the rat. *Fundam Appl Toxicol* **20**(2), 225-30.

Ladics, G. S. (2007). Primary immune response to sheep red blood cells (SRBC) as the conventional T-cell dependent antibody response (TDAR) test. *J Immunotoxicol* **4**(2), 149-52, 10.1080/15476910701337357.

Loveless, S. E., Ladics, G. S., Smith, C., Holsapple, M. P., Woolhiser, M. R., White, K. L., Jr., Musgrove, D. L., Smialowicz, R. J. and Williams, W. (2007). Interlaboratory study of the primary antibody response to sheep red blood cells in outbred rodents following exposure to cyclophosphamide or dexamethasone. *J Immunotoxicol* **4**(3), 233-8, 10.1080/15476910701385687.

Munson, A. E., Sanders, V. M., Douglas, K. A., Sain, L. E., Kauffmann, B. M. and White, K. L. (1982). IN VIVO ASSESSMENT OF IMMUNOTOXICITY. *Environ.Health Perspect.* **43**, 41-52 1982.

Ruckart, P. Z., Bove, F. J. and Maslia, M. (2014). Evaluation of contaminated drinking water and preterm birth, small for gestational age, and birth weight at Marine Corps Base Camp Lejeune, North Carolina: a cross-sectional study. *Environmental health : a global access science source* **13**, 99, 10.1186/1476-069x-13-99.

Shopp, G. M., Jr., Sanders, V. M., White, K. L., Jr. and Munson, A. E. (1985). Humoral and cell-mediated immune status of mice exposed to trans-1,2-dichloroethylene. *Drug Chem Toxicol* **8**(5), 393-407, 10.3109/01480548509041066.