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Dear Ms. Barajas-Ochoa:

The purpose of this letter is to make OEHHA aware of some recent data relevant to the National Toxicology Program's designation of 1-bromopropane (1-BP) as "reasonably anticipated to be a human carcinogen." These recent data suggest that 1-BP may not be a genotoxic carcinogen as reported by NTP (NTP, 2013), and that to the extent that NTP relied upon the putative genotoxicity of 1-BP to develop its designation as "reasonably anticipated to be a human carcinogen," that designation might be overly robust.

In this letter the following points related to the potential human relevance of the rodent and *in vitro* genotoxicity studies will be developed:

- 1) The rodent tumor data from the NTP 2-year inhalation study are not impressive.
- 2) The lung tumors reported in only female mice are probably the tumor type most relevant to a human population exposed to volatile 1-BP.
- 3) The histological tumor type seen in only the female mice is bronchioloalveolar carcinoma, a tumor type that might possess limited relevance to human lung cancer.
- 4) Determining whether 1-BP is mutagenic in the Ames *Salmonella* assay is an important step in classifying its potential cancer hazard to humans.
- 5) NTP relied on the 35-year old Barber et al. (1981) study to posit that 1-BP is genotoxic in the Ames *Salmonella* mutagenicity assay.
- 6) Albemarle Corporation contracted with BioReliance in Rockville Maryland to conduct an Ames test using a closed system to account for the volatility of 1-BP.
- 7) BioReliance conducted the Ames test twice in multiple strains of bacteria with and without S9 metabolic activation and found 1-BP to be negative. The definitive assay doses selected were set after determining the cytotoxic dose level and slightly backing off as per OECD regulations.
- 8) Similar to the actual BioReliance Ames test result, the QSAR modeling program OECD Toolbox predicts that 1-BP will be negative in the Ames test.

- 9) In contrast with a negative Ames prediction for 1-BP in OECD Toolbox, 2-BP has tested positive in the Ames test (CCRIS, 2015).
- 10) Since the current Ames test used a super pure sample of 1-BP, and the Barber et al. (1981) study a less pure sample, it is possible that Barber et al. experienced some interference with their Ames test from 2-BP, the most common contaminant in 1-BP preparations.
- 11) Further testing of 1-BP is required to disentangle its potential carcinogenicity to humans.

In addition, three attachments are provided as accompanying documentation to this letter. First, please find attached the final Ames assay report from BioReliance stating that under the conditions of the assay that 1-BP was not mutagenic. Second, please find attached Gradient Corporation's "Comments on the Petition to Add n-Propyl Bromide to the List of Hazardous Air Pollutants Regulated Under § 112 of the Clean Air Act." Third, please find attached the printout from OECD Toolbox showing the predicted negative Ames result for 1-BP and measured positive Ames result for 2-BP.

Point One - The rodent tumor data from the NTP 2-year inhalation study are not impressive.

The National Toxicology Program (NTP) has classified 1-BP as "reasonably anticipated to be a human carcinogen" based on induction of tumors in rats and mice in a two-year inhalation study (NTP, 2013). Four categories of animals were exposed: male mice; female mice; male rats; and female rats. Dose-related skin neoplasms were observed in male rats only. Neoplasms of the large intestine were seen in both male and female rats, but in neither male nor female mice. Lung neoplasms were found in female mice only. Although dose-related neoplasms of the skin, large intestine and lung were reported, the incidence varied by sex and species. Therefore, the animal data are not particularly robust in that a consistent pattern by species, sex and tumor type is lacking.

Point Two - The lung tumors reported in only female mice are probably the tumor type most relevant to a human population exposed to volatile 1-BP.

The mice and rats exposed to 1-BP in the 2-year NTP inhalation study were exposed via whole body. Therefore, digestive tracts were exposed to 1-BP when the rodent licked their fur, and the skin was exposed directly. Also, the lungs were exposed as the rodent breathed. In a human occupational setting, the primary route of exposure would be to the lung via inhalation of the volatile 1-BP in workplace air.

Point Three - The histological tumor type seen in only the female mice is bronchioloalveolar carcinoma. A positive result in this lung tumor type might possess limited relevance to human lung cancer.

Humans present with a variety of lung cancers. In the United Kingdom the approximate breakdown by histologic types is as follows (<http://gpnotebook.co.uk/simplepage.cfm?ID=1959788590>):

- a) Squamous cell carcinoma (40%)
- b) Small cell carcinoma (20-30%)
- c) Large cell carcinoma (10-15%)
- d) Adenocarcinoma (20%)

There are two major subdivisions within the adenocarcinoma category – bronchial derived adenocarcinoma and bronchioloalveolar carcinoma. Of the two subcategories, bronchioloalveolar carcinoma is much less common than bronchial derived adenocarcinoma in humans (<http://med.umich.edu/rad/res/resources/bronchioloalveolarcellcarcinoma.htm>). Estimates vary but pure bronchioloalveolar carcinoma probably represents only about 4% of the cases of lung cancer in the Western world. In 2011, Boffetta et al. precisely estimated the proportion of bronchioloalveolar carcinoma attributable to cigarette smoking at 0.47 (95% CI 0.39-0.54). Based on the Boffetta et al. (2011) estimate, about 2% of lung cancer cases in the Western world are bronchioloalveolar carcinomas found in smokers.

Point Four - Determining whether 1-BP is mutagenic in the Ames *Salmonella* assay is an important step in classifying its potential cancer hazard to humans.

NTP reviewed the potentially important role played by the Ames test on page 36 of the NTP Report on Carcinogens (NTP, 2013b):

“DNA reactivity combined with *Salmonella* mutagenicity is highly correlated with induction of carcinogenicity in multiple species/sexes of rodents and at multiple tissue sites (Ashby and Tennant, 1991). A positive response in the *Salmonella* test was shown to be the most predictive *in vitro* indicator for rodent carcinogenicity (89% of the *Salmonella* mutagens are rodent carcinogens) (Tennant *et al.*, 1987; Zeiger *et al.*, 1990). Additionally, no battery of tests that included the *Salmonella* test improved the predictivity of the *Salmonella* test alone...”

Point Five - NTP relied on the 35-year old Barber et al. (1981) study to posit that 1-BP is genotoxic in the Ames *Salmonella* mutagenicity assay.

In the current experiment, 1-bromopropane was dosed to the bacterial cells in the liquid phase. In Barber *et al.* (1981), the vapor phase exposure was converted to a similar liquid phase exposure prior to bacterial cell contact. Barber *et al.* describe this process on page 43 of their manuscript under the subheading “Rate of Movement from Vapor Phase to Liquid Phase,” wherein they state that “The kinetics are complex as expected; however the rate of entry into the aqueous phase is rapid when compared with the 48-h incubation period.” Therefore, the issue from a dose-response perspective is how much 1-bromopropane is delivered over a given time period to the bacterial cells and not whether the initial phase of the 1-bromopropane is vapor or liquid.

The dose range of 1-bromopropane tested by Barber *et al.* (1981) was the equivalent of 600-2,490 micrograms per plate. In the current Ames assay, the dose levels tested were 50, 150, 500, 1500, 2000, 3000 and 5000 micrograms per plate. Therefore, the doses tested by Barber *et al.* (1981) are within the dose ranges tested here. This overlap in dose ranges tested eliminates the possibility that some interplay between cytotoxicity and mutagenicity is masking the ability to detect mutagenicity in the current assay but allowed detection in the 1981 experiment.

In summary, the current Ames assay on 1-bromopropane and the earlier assay conducted in 1981 both documented exposure to the bacterial cells and used a closed-system design to prevent volatile 1-bromopropane from escaping the confines of the assay. In both assays, the final phase of 1-bromopropane that contacted the *Salmonella* bacterial cells was the liquid phase, and in both cases was below the limit of cytotoxicity. The only readily identifiable difference between the two assays is that the current assay had access to a super pure 1-bromopropane sample documented as free from 2-bromopropane contamination and the earlier assay purchased 1-bromopropane with an unknown percentage of 2-bromopropane.

Point Six - Albemarle Corporation contracted with BioReliance in Rockville Maryland to conduct an Ames test using a closed system to account for the volatility of 1-BP.

BioReliance was selected by Albemarle because it is a world class contract lab with a particular reputation for competency in genetic toxicology testing (www.bioreliance.com).

Point Seven - BioReliance conducted the Ames test twice in multiple strains of bacteria with and without S9 metabolic activation and found 1-BP to be negative. The definitive assay doses selected were set after determining the cytotoxic dose level and slightly backing off as per OECD regulations.

The mutagenic potential of 1-BP is difficult to accurately assess because the substance is volatile at temperatures employed in standard assays and presents additional challenges in solubilizing in assay buffers. NTP has stated that 1-BP is a direct-acting mutagen in *Salmonella* strains TA100 and TA1535 when testing is conducted within a closed system to control for volatility. In the current study, a closed preincubation system was employed to test 1-BP for mutagenicity in *Salmonella* strains TA98, TA100, TA1535 and TA1537, and in *Escherichia coli* WP2 *uvrA* in both the presence and absence of metabolic activation. The concentration range tested was from 50 to 5000 µg per plate. In addition, chemical analysis of the assay tubes was conducted to ensure that the bacterial cells were exposed to the 1-BP as intended. Although 1-BP was toxic to the bacteria beginning at 3000 or at 5000 µg per plate, it did not cause a positive mutagenic response with any of the tester strains in either the presence or absence of Aroclor-induced rat liver S9. The Ames assays were repeated twice. Therefore, 1-BP is negative in the Ames test.

Point Eight - Similar to the actual BioReliance Ames test result, the QSAR modeling program OECD Toolbox predicts that 1-BP will be negative in the Ames test.

Point Eight - In contrast with a negative Ames prediction for 1-BP in OECD Toolbox, 2-BP has been shown to be positive in the Ames test.

The European Chemicals Agency (ECHA) has selected OECD Toolbox as its Quantitative Structure-Activity Relationship (QSAR) program of choice for the prediction of the biological activity (including toxicity) of a molecule from its chemical structure.

Point Nine - Since the current Ames test used a super pure sample of 1-BP, and the Barber et al. (1981) study a less pure sample, it is possible that Barber et al. experienced some interference with their Ames test from 2-BP, the most common contaminant in 1-BP preparations.

In 1981, E.D. Barber *et al.* conducted an Ames *Salmonella* assay on 1-bromopropane inside of BBL Gas-Pak® anaerobic incubation jars to prevent the volatile 1-bromopropane from escaping into the atmosphere. Barber *et al.* report the purity of their 1-bromopropane sample assayed at 99.85%. During the 1-bromopropane manufacturing process, the most common impurity produced is the Ames mutagen 2-bromopropane. 2-bromopropane has been shown to produce base-pair substitution mutations in a dose-response manner (Maeng & Yu, 1997; Yu *et al.*, 1999). Whether 2-bromopropane contamination affected Barber *et al.*'s results is unknown. Concern over the potential mutagenic effects of 2-bromopropane contamination led us to use a 1-bromopropane sample in the current study determined to be 99.99% pure. The principle impurities in the current 1-bromopropane sample were isopropyl bromide (65 ppm) and water (27 ppm).

Point Ten - Further testing of 1-BP is required to disentangle its potential carcinogenicity to humans.

The 2-year inhalation study conducted by NTP on 1-BP was the best effort that could have been reasonably conducted. Therefore, it is unlikely that the time and money required for conducting another study to address the same hypothesis will be conducted. To date, two major attempts to address the genotoxicity of 1-BP have been conducted – the Barber et al. study published in 1981 and the current study conducted at BioReliance. The evidence suggests that the BioReliance study was conducted on a more highly purified sample of 1-BP. Given the inconsistency in the two study results, further experimentation, possibly in the Comet Assay might shed further light on the carcinogenic potential of 1-BP in humans.

References

Barber ED, Donish WH, Mueller KR (1981). A procedure for the quantitative measurement of the mutagenicity of volatile liquids in the Ames *Salmonella*/microsome assay. *Mutation Research* 90(1): 31-48. (Support not reported. Authors affiliated with Eastman Kodak Company, NY)

Boffetta P, Jayaprakash V, Yang P, Asomaning K, Muscat JE, Schwartz AG, Zhang ZF, Le Marchand L, Cote ML, Stoddard SM, Morgenstern H, Hung RJ, Christiani DC. Tobacco smoking as a risk factor of bronchioloalveolar carcinoma of the lung: pooled analysis of seven case-control studies in the international lung cancer consortium (ILCCO). *Cancer Causes Control* (2011) Jan; 22(1):73-79.

CCRIS (Chemical Carcinogenesis Research Information System). [internet]. Bethesda (MD): National Library of Medicine (US); [last revision date January 15, 2008; cited 2015 August]. 2-bromopropane; CCRIS record number: 7919.

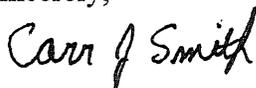
Gradient Corporation. Comments on the petition to add n-propyl bromide to the list of hazardous air pollutants regulated under § 112 of the clean air act. Prepared for Albemarle Corporation on May 7, 2015, bottom of page 4.

National Toxicology Program. US Department of Health and Human Services. Report on carcinogens, monograph on 1-bromopropane. September 2013.

National Toxicology Program. US Department of Health and Human Services. Report on carcinogens, monograph on 1-bromopropane. Genetic Toxicology, Page 36. September 2013.
National Toxicology Program. 1-bromopropane key points. Page 1. October 2014.

In summary, while the NTP rodent bioassay is probably the best experiment that can be done, the issue of whether 1-BP is a genotoxin, and thus the mode of action of the tumorigenicity of 1-BP in rodents remains unresolved. At your request, extensive documentation can be provided to demonstrate that 2-BP is a common manufacturing contaminant of 1-BP preparation. If you require any additional assistance or clarification, please do not hesitate to contact me.

Sincerely,

A handwritten signature in black ink that reads "Carr J. Smith". The signature is written in a cursive, slightly slanted style.

Carr J. Smith, Ph.D., DABT