Date: September 4, 2018

Dear **Ministry of Health, Labour and Welfare Government of Japan,**
Chemical Risk Assessment Office, Chemical Hazards Control Division
Industrial Safety and Health Department, Labour Standards Bureau

The purpose of this letter is to briefly describe the history and procedural deficiencies of the American Conference of Governmental Industrial Hygienists' (ACGIH) decision to set a threshold limit value (TLV) of 0.1 ppm for workplace air exposure to 1-bromopropane. In 2011, the American Conference of Governmental and Industrial Hygienists (ACGIH) published the Notice of Intended Change (NIC) to lower the 1-bromopropane 8-hour TLV-TWA from 10 ppm to 0.1 ppm (ACGIH 2012). In 2012, the ACGIH reclassified 1-bromopropane as a "Confirmed Animal Carcinogen with Unknown Relevance to Humans" (ACGIH 2012, 2013). On January 31, 2014, the ACGIH Board of Directors issued their Annual Report indicating that the NIC for 1-bromopropane had been adopted. The 8-hour TLV-Time Weighted Average (TWA) for 1-bromopropane, as determined by the ACGIH, is now 0.1 ppm (ACGIH 2014; U.S. Army Public Health Command (USAPHC) 2014).

ACGIH lowered the TLV based on results from the following study:
Dose-Dependent Neurologic Abnormalities in Workers Exposed to 1-Bromopropane
Weihua Li, MD, Eiji Shibata, MD, PhD. Zhijun Zhou, MD. PhD, Sahoko lchihara, MD, PhD, Hai/an Wang, MD. PhD, Qiangyi Wang, MD, Jiefei Li, MD, Lingyi Zhang, MD, Kenji Wakai, MD, PhD, Yasuhiro Takeuchi, MD, PhD, Xuncheng Ding, MD, and Gaku lchihara, MD, PhD *Occupational and Environmental Medicine*, Volume 52, Number 8, August 2010. In November 2015, the group director for the Li et al. (2010) paper noted above stated that, "It is noted that ACGIH used this report to derive the TLV for 1-bromopropane." This statement is found on page 5 in the "DISPOSITION OF PEER REVIEW COMMENTS FOR TOXICOLOGICAL PROFILE FOR 1-BROMOPROPANE, Prepared by: SRC, Inc. 7502 Round Pond Road, North Syracuse, NY 13212. Prepared for the Agency for Toxic Substances and Disease Registry, U.S. Public Health Service, Henry Abadin, Work Assignment Manager.

Putative nerve damage in the legs and feet was the particular data point in Li et al. (2010) used by ACGIH to set the TLV at 0.1 ppm, as measured by the loss of vibration sense in the toes, in a cohort of Chinese factory workers. This adverse effect was believed by the authors to be measured at only 1.28 ppm 1-bromopropane. Albemarle's assumption is that the TLV of 0.1 ppm was estimated by dividing the 1.28 ppm Lowest Adverse Effect Level (LOAEL) for peripheral nerve damage by a safety factor of 12.8-fold.

Albemarle initiated the process of deconstructing the results of the Li et al. (2010) study with a discussion with Peter J. Dyck, M.D., Director of the Peripheral Nerve Research Laboratory at the Mayo Clinic, in Rochester Minnesota. Dr. Dyck informed us that the manual tuning forks used to take the measurements in Li et al. (2010) are not employed in the United States as a research measure of peripheral neurotoxicity because this methodology has been replaced by much more quantitative automated methods, *e.g.*, the CASE IV System.

Following the discussion with Dr. Dyck, Albemarle hired an expert consultant to review the Li et al. (2010), i.e. Masayuki Baba, M.D., Ph.D., Chairman, Department of Neurology, Aomori Prefecture Medical Center. In Dr. Baba's report to Albemarle, he noted that tuning forks vibrating at 64 Hertz (Hz) and not 128 Hz as used in Li et al. (2010) are used for clinical assessment. Dr. Baba noted, "Minimally, the study such as this paper should have at least used the 64 Hz tuning fork, or possibly the quantitative vibration esthesiometer. It is reckless to quantitatively evaluate by 128 Hz tuning fork, and is almost as ridiculous as treating the data obtained by stethoscope as that from cardiac echography."

Peripheral nerves that have been damaged do not conduct neural impulses at the same speed as do normal healthy nerves. A parameter termed distal latency (DL) represents the speed at which nerves are transmitting their impulses. Dr. Baba noted the following abnormality in the supposedly healthy normal control group from the Li et al. (2010) study, "However, the DL of control group in Table 3 is 6.7 ± 1.7 ms, and this value is considered as seriously pathologic prolongation. It is hard to believe that this is the data from healthy control subjects…"

In summary, tuning fork data are too variable to conduct research studies measuring damage to nerves in lower extremities. While 64 Hz tuning forks can be used for individual patient assessment, they are not advisable for use in taking the accurate measurements required in the research setting. Li *et al*. (2010) not only used 128 Hz tuning forks to make their primary measurement of peripheral neurotoxicity, but the clinical assessment was conducted improperly. Tuning fork measurements should be taken from the patient's toes to the observer's fingers because there are more nerve endings in the fingers, resulting in additional sensation time. Li *et al*. (2010) tuning forks went from the patient's toes to the observer's toes. "Control" values in Li *et al*. (2010) are within the range for patients with nerve damage. The LOAEL of 1.28 ppm in Li *et al*. (2010) does not represent a valid measurement of peripheral nerve damage. Li *et al*. (2010) should not be considered in establishing a new TLV for 1-BP.

Albemarle's full-length comment to The Agency for Toxic Substances and Disease Registry (ATSDR), a federal public health agency of the U.S. Department of Health based in Atlanta, Georgia, is found at the following citation:

https://www.regulations.gov/docketBrowser?rpp=25&so=DESC&sb=commentDueDate&po=0&D=ATSDR-2016-0003; **Comment on FR Doc # N/A;** See attached file(s) **Public Submission Posted:** 06/03/2016; **ID:** ATSDR-2016-0003-0006; **Submitter Name:** Carr Smith

Albemarle personnel and their academic colleagues published a Letter to the Editor describing problems with Li et al. (2010) in addition to the tuning fork measurement deficiencies at the following citation:

C.J. Smith, G.T. Johnson, R.D. Harbison, Y. Zhu, R.V. Lee, M. Banasik, and T. Stedeford (2011). "Dose-dependent neurologic abnormalities in workers exposed to 1-bromopropane'", *Journal of Occupational and Environmental Medicine*, Vol. 53, pp.707-708.

Evaluation of Studies Related to Cancer Risk

In addition to Li et al. (2010), one other major new study directly related to the reduction of the 1-bromopropane TLV from 10 ppm to 0.1 ppm has been conducted and is cited as follows: National Toxicology Program (NTP). US Department of Health and Human Services. 2011. NTP technical report on the toxicology and carcinogenesis studies of 1-bromopropane (CAS No. 106-94-5) in F344/N rats and B6C3F1 mice (inhalation studies). National Toxicology Program, Technical report series TR-564, 2011.

The National Toxicology Program (NTP) uses a classification scheme for carcinogens that differs from that employed by the International Agency for Research on Cancer (IARC). The NTP and IARC carcinogen classifications are as follows:
NTP - Known to be human carcinogens
NTP - Reasonably anticipated to be human carcinogens
IARC Group 1: Carcinogenic to humans
IARC Group 2A: Probably carcinogenic to humans
IARC Group 2B: Possibly carcinogenic to humans
IARC Group 3: Unclassifiable as to carcinogenicity in humans
IARC Group 4: Probably not carcinogenic to humans

The NTP classification of "Known to be human carcinogens" is directly equivalent to the IARC Group 1: "Carcinogenic to humans" classification. The other NTP carcinogen category, "NTP Reasonably anticipated to be human carcinogens" is equivalent to the "IARC 2A: Probably carcinogenic to humans," with some overlap with the "IARC 2B: Possibly carcinogenic to humans." NTP classifies 1-bromopropane as "Reasonably anticipated to be a human carcinogen." IARC classifies 1-bromopropane somewhat lower than the NTP classification as an IARC Group 2B: Possibly carcinogenic to humans.

Several lines of evidence suggest that the IARC Group 2B classification of "Possibly carcinogenic to humans" for 1-BP is better supported than the somewhat stronger NTP classification of "Reasonably anticipated to be a human carcinogen." Our research group at Albemarle has statistically and mechanistically analyzed the entire NTP 2-year rodent cancer database of 594 bioassays resulting in successful completion of final NTP reports for 479 chemicals or chemical mixtures, with an additional three chemicals described in two Report on Carcinogens (RoC) reports. A six paper series has been published. Analysis of the entire NTP database demonstrated that only tumorigenic results rising to the level of "Clear" evidence are sufficiently robust to enable demonstration of biologically plausible statistical correlations. Only two neoplasticity results for 1-bromopropane demonstrate "Clear" evidence: bronchioloalveolar carcinomas (BACs) in female mice and adenomas of the large intestine in female rats.

Pulmonary tumors induced in "mice only" are not relevant to human lung cancer.
A significant body of circumstantial evidence strongly suggests that when bronchioloalveolar carcinomas are induced in mice only (not also in rats) that the tumors are not indicative of increased lung cancer risk in human workers exposed at real-world levels of 1-bromopropane.

Six Albemarle Publications Analyzing the Entire NTP Database

Smith CJ and Anderson SP. High discordance in development and organ site distribution of tumors in rats and mice in NTP 2-year inhalation studies. *Toxicology Research and Application* 2017; Volume 1: 1–22.

Smith CJ and Perfetti TA. Tumor Site Concordance and Genetic Toxicology Test Correlations in NTP Two-Year Feed Studies. *Toxicology Research and Application,* Volume 1: 1-12. DOI: 10.1177/2397847317739942

Smith CJ and Perfetti TA (January 9, 2018). Tumor Site Concordance and Genetic Toxicology Test Correlations in NTP Two-Year Gavage, Drinking Water, Dermal, and Intraperitoneal Injection Studies. *Toxicology Research and Application.* https://doi.org/10.1177/2397847317751147

Smith CJ, Perfetti TA, Ko GM, Garg R. Ames mutagenicity, structural alerts of carcinogenicity, Hansch molecular parameters (ClogP, CMR, MgVol), tumor site concordance/multiplicity, and tumorigenicity rank in 2-year NTP studies. *Toxicology Research and Application* 2018; 2: 1–14. DOI: 10.1177/2397847318759327

Smith CJ and Perfetti TA. Comparison of carcinogenicity predictions by the Oncologic expert system with NTP 2-year rodent study tumorigenicity results. *Toxicology Research and Application*. Volume 2: 1–11. DOI: 10.1177/2397847318771128

Smith CJ and Perfetti TA. The 'False Positive' Problem in the NTP 2-Year Rodent Cancer Study Database. *Toxicology Research and Application*. Volume 2: 1–13. DOI: 10.1177/2397847318772839

Although workers can be exposed dermally or via ingestion of contaminated food, inhalation is the route of administration of concern for vapor degreasing operations. Albemarle conducted a statistical analysis of all NTP inhalation studies to better understand the relevance of pulmonary tumors in rats and mice to human lung cancer. The citation for the analysis of the NTP inhalation studies is the following:

Smith CJ and Anderson SP. High discordance in development and organ site distribution of tumors in rats and mice in NTP 2-year inhalation studies. Toxicology Research and Application 2017; Volume 1: 1–22.

In order to better understand the results of the analysis of the inhalation studies, it should be noted that rats and mice are much closer to one another on the phylogenetic evolutionary tree that either are to humans. Humans and rodents separated from a common ancestor about 80 million years ago, with rats and mice diverging from one another between 12 and 24 million years ago (Gibbs et al. 2004). As phylogenetic distance between mammalian species increases,

genes affecting target tissue microanatomy and biochemistry, tumor susceptibility, tumor suppression, apoptosis, DNA repair, immune surveillance and metabolism can differ.
Also, metabolic rate differences can affect cellular proliferation rates. Since rats and mice are closer on the evolutionary tree than either rodent species is to a human, high discordance between rats and mice in two-year bioassays lowers confidence in the quality of the extrapolation from rodent results to human cancer risk.

Gibbs RA, Weinstock GM, Metzker ML, et al. Genome sequence of the Brown Norway rat yields insights into mammalian evolution, Nature 428(6982) (2004) 493-521.

NTP tested 58 compounds for 2 years via inhalation in rats and mice. 11/58 (19%) of agents tested were negative in the Ames assay test and showed lung tumors in mice only.
0/58 agents tested in mice induced a tumor outside the lungs when a rat did not induce a tumor outside the lung for the same chemical. In contrast, 16/58 agents tested in rats induced a tumor outside the lung when a mouse did not develop a tumor outside the lung for the same chemical
For a given chemical inhaled, mice are much more likely than rats to develop a lung tumor, and rats are much more likely to develop a tumor outside the lung. For the same inhalation chemical exposure, the risk of a mouse developing a pulmonary tumor is a poor predictor of a rat developing a pulmonary tumor. The microanatomy of the mouse lung provides a ready explanation for the high degree of discordance in lung tumor development between mice and rats, and by extension, the poor modeling of human lung cancer risk provided by mouse lung tumors. Pulmonary tumors in mice are bronchioloalveolar carcinomas, usually developing in the periphery of the lung. The precursor cells of bronchioloalveolar carcinomas are Type 2 and Clara cells. Tissues lining the airways of rats and humans possess many fewer precursor cells that can be transformed to the bronchioloalveolar carcinomas that frequently develop in mice.
When pulmonary tumors form in both rats and mice from an inhalation exposure, the level of concern is significantly higher than when pulmonary tumors form in mice only.
In the particular case of 1-bromopropane, not only do pulmonary tumors only form in mice, but they only form in female mice. The clinical irrelevance of the female mouse lung tumors leaves the female rat intestinal adenomas as the endpoint of interest from the NTP 1-bromopropane study.

What is the mechanism of action (MOA) underlying the neoplastic potential of 1-BP? There are several possibilities. First, 1-bromopropane contains one structural alert of carcinogenicity, i.e. the electrophilic bromine atom that can interact with nucleophilic sites on DNA via a nucleophilic substitution reaction. The N7-position of the guanine residue is the most nucleophilic site on the DNA bases and is a favored site of reaction for almost all small, freely-diffusible alkylating agents.
An Overview of Chemical Processes That Damage Cellular DNA ...
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2806061/

Second, given the presence of a single structural alert of carcinogenicity, is 1-bromopropane mutagenic? The NTP expert panel on 1-bromopropane based their decision on potential mutagenicity on the Barber et al. (1981) study conducted non-GLP at Eastman Kodak.

Barber et al. (1981) reported Ames mutagenicity in the absence of metabolic activation by S9, i.e. that 1-BP was a direct acting mutagen. However, a major conclusion from Albemarle's analysis of the entire NTP database is that, "Recent Ames assays conducted under GLP conditions and employing the relevant OECD protocols should be given priority over historical Ames data conducted under non-GLP conditions and using older, non-standard protocols."

Smith CJ and Perfetti TA. The 'False Positive' Problem in the NTP 2-Year Rodent Cancer Study Database. *Toxicology Research and Application*. Volume 2: 1–13. DOI: 10.1177/2397847318772839

In contrast with the much older, non-GLP results from Eastman Kodak, Albemarle contracted with BioReliance Corporation and conducted two Ames tests, with and without metabolic activation, in a closed system accounting for the volatility of 1-bromopropane, and reported no increase in mutagenicity.

Summary of BioReliance Ames Test Results on 1-Bromopropane Conducted Under Contract to Albemarle

1-Bromopropane (N-propyl bromide) is Not Mutagenic in an Ames Assay Using Closed Test Conditions and is Clastogenic in an *in vitro* Chromosome Aberration Assay

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Poster Presented
European Environmental Mutagenesis and Genomics Society, August 2016

Abstract 04, Ames Salmonella Mutagenicity Assay of Volatile 1-Bromopropane in a Closed System

Carr Smith, Steve Anderson, Indu Kheterpal, Joseph Miller, Sylvia Jacobi

Abstract
1-Bromopropane (1-BP) (CASRN 106-94-5) is an important industrial chemical. The National Toxicology Program (NTP) has classified 1-BP as "reasonably anticipated to be a human carcinogen." NTP has stated that 1-BP is a direct-acting mutagen in *Salmonella* strains TA100 and TA1535. 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 *uvr*A in both the presence and absence of metabolic activation. 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. In contrast, under similar closed assay conditions 1-BP

was clastogenic in the *in vitro* chromosome aberration assay conducted using human peripheral blood lymphocytes (HPBL). The *in vitro* mammalian chromosome aberration assay was conducted using standard procedures as per OECD testing guideline 473. At this time, the mechanism of action underlying the reported tumorigenicity of 1-BP in rodents remains unclear.

Keywords: 1-bromopropane, Ames test, Chromosome Aberration, Analytical verification
1-BP Ames Test Highlights
Tubes capped to account for sample volatility
Doses taken up to point of bacterial cytotoxicity and then backed down as per protocol
Assay conducted with and without S9 metabolic activation
Negative mutagenicity in *Salmonella* strains TA98, TA100, TA1535 and TA1537, and in *Escherichia coli* WP2 *uvr*A in both the presence and absence of metabolic activation

Final Ames Assay and
In Vitro Chromosome Aberration Assay GLP Reports
https://www.regulations.gov/#!documentDetail;D=EPA-HQ-OPPT-2015-0084-0021
This link will lead the reader to Albemarle's public comments to USEPA regarding the draft risk assessment of 1-bromopropane.
Please see Appendix III for final BioReliance Ames test report; Appendix IV for final BioReliance Analytical Protocol report; and Appendix V for final BioReliance *in vitro* chromosome aberration report.
Two replications

Other Recent GLP Tests of Mutagenicity of 1-Bromopropane

Two other recent GLP tests of mutagenicity are negative. Israel Chemicals Ltd. (ICL) contracted and tested the mutagenicity of 1-bromopropane in the Big Blue ® Transgenic Rodent Mutation (TGR) assay. ICL matched the doses used in the assay to the doses used in the NTP 2-year inhalation study. The result was negative, i.e. not mutagenic. Similarly, EnviroTech International (ETI) also contracted and tested the mutagenicity of 1-BP in the Big Blue ® Transgenic Rodent Mutation (TGR) assay. This assay was also negative, i.e. not mutagenic. ETI submitted the final report of this assay to USEPA.

Overall Summary of 1-Bromopropane Toxicity

At some unknown higher dose level, 1-bromopropane is neurotoxic to humans. However, the Li et al. (2010) LOAEL of 1.28 ppm is an experimental artifact resulting from an improper attempt to measure peripheral neuropathy with a 128 Hz tuning fork. The 0.1 ppm TLV set by ACGIH based on this study is incorrect. 1-bromopropane contains a "structural alert of carcinogenicity," i.e. the electrophilic bromine atom can interact with nucleophilic sites on DNA via a nucleophilic substitution reaction. The evidence suggests that 1-bromopropane is not a mutagen. However, 1-bromopropane is clastogenic, i.e. positive in the *in vitro* chromosome aberration assay. Two neoplasms were clearly induced via 2-years of inhalation in rats and mice: bronchioloalveolar carcinoma lung tumors in female mice only; and intestinal adenomas in female rats only.
The weight-of-the-evidence strongly suggests that the female mouse lung tumors are not related to cancer risk in human workers exposed to real-world levels of 1-bromopropane. Risk

assessment should be conducted based on the intestinal adenomas in female rats that were induced at the 500 ppm dose level.

Conclusions

The ACGIH TLV of 0.1 ppm for 1-bromopropane should not be considered in establishing other exposure limits. Improper classification of 1-bromopropane as a direct acting mutagen inappropriately influenced the interpretation of positive tumor results in the 2-year NTP study. Development of intestinal adenomas in female rats at 500 ppm is consistent with either a DNA-interaction mechanism related to electrophilic reaction with nucleophilic sites on DNA; or with increased cell proliferation amplifying the background mutation rate due to cytotoxicity-induced cellular repair processes. Regardless of mechanism of action, the neoplastic potential of 1-bromopropane is moderate.

Albemarle continues to conduct analysis on the toxicity of 1-bromopropane for consideration for publication in the peer-reviewed literature. Albemarle appreciates the opportunity to continue to provide these analyses to assist a 'best science' evaluation of the toxicity of 1-bromopropane.

Sincerely,

Bromo Carbon Association (Japan)