

# Response to Comments Pertaining to the Notice of Intent to List 1-Bromopropane as Causing Cancer under Proposition 65

## Office of Environmental Health Hazard Assessment California Environmental Protection Agency August 2016

The Office of Environmental Health Hazard Assessment (OEHHA) has determined that 1-bromopropane meets the criteria for listing under Proposition 65<sup>1</sup> via the authoritative bodies mechanism based on conclusions by the National Toxicology Program (NTP) that 1-bromopropane causes cancer, and on the scientific evidence relied on by NTP<sup>2</sup>. NTP is designated as an authoritative body for purposes of listing chemicals as causing cancer pursuant to Title 27, Cal. Code of Regulations, section 25306<sup>3</sup>. 1-Bromopropane will therefore be added to the Proposition 65 list as a chemical known to cause cancer.

OEHHA made this determination after reviewing public comments on the proposed listing of 1-bromopropane under Proposition 65. On July 10, 2015, OEHHA issued a Notice of Intent to List<sup>4</sup> (NOIL) 1-bromopropane under Proposition 65 as a chemical known to the state to cause cancer. The action was based on Proposition 65 statutory requirements<sup>5</sup> and on the authoritative bodies provision of the Proposition 65 implementing regulations, Section 25306. This document responds to public comments received on the Notice of Intent to List 1-bromopropane under Proposition 65.

Under Section 25306, a chemical has been “formally identified” as causing cancer by an authoritative body if: (1) the chemical has been included in a list of chemicals causing cancer published by the authoritative body; is the subject of a report which is published by the authoritative body and which concludes that the chemical causes cancer; or has been “otherwise identified” as causing cancer by the authoritative body in a document that indicates that the identification is a final action; and (2) if the list, report, or document meets specified criteria in Section 25306(d)(2).

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<sup>1</sup> The Safe Drinking Water and Toxic Enforcement Act of 1986 (codified at Health and Safety Code section 25249.5 *et seq.*) hereinafter referred to as Proposition 65 or the Act.

<sup>2</sup> National Toxicology Program (NTP, 2014). Report on Carcinogens, Thirteenth Edition, US Department of Health and Human Services, Public Health Service, NTP, Research Triangle Park, North Carolina. Available at URL: <http://ntp.niehs.nih.gov/pubhealth/roc/roc13/index.html>.

<sup>3</sup> All further references are to sections of Title 27 of the California Code of Regulations, unless otherwise indicated.

<sup>4</sup> Notice of Intent to List: 1-Bromopropane. Available at [http://www.oehha.ca.gov/prop65/CRNR\\_notices/admin\\_listing/intent\\_to\\_list/NOIL0710151bromopropane.html](http://www.oehha.ca.gov/prop65/CRNR_notices/admin_listing/intent_to_list/NOIL0710151bromopropane.html)

<sup>5</sup> Health and Safety Code section 25249.8(b)

OEHHA has reviewed the conclusions and statements in the 2014 NTP *Report on Carcinogens, Thirteenth Edition*<sup>6</sup>. OEHHA has determined that these conclusions and statements satisfy the Section 25306(d)(1) requirement. Specifically, 1-bromopropane has been included in a list of chemicals causing cancer published by the authoritative body; and it is the subject of a report published by the authoritative body that concludes that 1-bromopropane causes cancer; and the NTP *Report on Carcinogens* indicates this identification is a final action. Further, OEHHA has determined that the report meets the Section 25306(d)(2) requirements. Thus the NTP *Report on Carcinogens* satisfies the formal identification criteria in the Proposition 65 regulations for 1-bromopropane. In the 2014 *Report on Carcinogens*, NTP concludes that 1-bromopropane is “reasonably anticipated to be a human carcinogen” based on sufficient evidence of carcinogenicity from studies in experimental animals<sup>7</sup>. OEHHA is relying on NTP’s discussion of data and conclusions in the report that 1-bromopropane causes cancer. Evidence described in the report includes studies showing that 1-bromopropane increased the incidence of combined malignant and benign skin tumors in male rats and increased the incidence of combined malignant and benign lung tumors in female mice:

“In male rats, 1-bromopropane caused significant dose-related increases in the incidences of several types of benign and/or malignant skin tumors (keratoacanthoma; keratoacanthoma and squamous-cell carcinoma combined; and keratoacanthoma, squamous-cell carcinoma, basal-cell adenoma, and basal-cell carcinoma combined).”

“In female mice, 1-bromopropane caused significant dose-related increases in the incidence of benign and malignant lung tumors combined (alveolar/bronchiolar adenoma and carcinoma).”<sup>8</sup>

The evidence cited by NTP<sup>9</sup> in support of these conclusions was reviewed by OEHHA with regard to the sufficiency of evidence criteria in Section 25306(e)(2). Based on NTP’s conclusions and the data relied on by NTP in reaching those conclusions, OEHHA has determined that 1-bromopropane meets the sufficiency of evidence criteria in Section 25306.

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<sup>6</sup> National Toxicology Program (NTP, 2014). Report on Carcinogens, Thirteenth Edition, US Department of Health and Human Services, Public Health Service, NTP, Research Triangle Park, North Carolina. Available at URL: <http://ntp.niehs.nih.gov/pubhealth/roc/roc13/index.html>.

<sup>7</sup> *Ibid.*

<sup>8</sup> *Ibid.*

<sup>9</sup> *Ibid.*

The July 10, 2015 notice initiated a 30-day public comment period. Comments on the Notice of Intent to List were submitted on August 7, 2015 by Albemarle Corporation.

OEHHA reviewed all of the comments and accompanying materials submitted in the context of the regulatory criteria for listing chemicals under the authoritative bodies mechanism in Section 25306.

Comments relevant to the NOIL are summarized and numbered by topic, and responses follow below.

## 1. Sufficiency of Evidence Criteria

### 1.1 Comment:

“[R]ecent data suggest that 1-BP [*1-bromopropane*] 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.” (p. 1)

### Response:

The NTP *Report on Carcinogens* classified 1-bromopropane as “*reasonably anticipated to be a human carcinogen* based on sufficient evidence of carcinogenicity from studies in experimental animals”. In reviewing the studies in experimental animals, NTP found:

“In male rats, 1-bromopropane caused significant dose-related increases in the incidences of several types of benign and/or malignant skin tumors (keratoacanthoma; keratoacanthoma and squamous-cell carcinoma combined; and keratoacanthoma, squamous-cell carcinoma, basal-cell adenoma, and basal-cell carcinoma combined).”

“In female mice, 1-bromopropane caused significant dose-related increases in the incidence of benign and malignant lung tumors combined (alveolar/bronchiolar adenoma and carcinoma)”<sup>10</sup>.

The criterion NTP relied upon in making the determination that 1-bromopropane is “*reasonably anticipated to be a human carcinogen*” is the following:

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<sup>10</sup> National Toxicology Program (NTP, 2014). Report on Carcinogens, Thirteenth Edition, U.S. Department of Health and Human Services, Public Health Service, NTP, Research Triangle Park, North Carolina. Available at URL: <http://ntp.niehs.nih.gov/pubhealth/roc/roc13/index.html>.

“[T]here is sufficient evidence of carcinogenicity from studies in experimental animals, which indicates there is an increased incidence of malignant and/or a combination of malignant and benign tumors (1) in multiple species or at multiple tissue sites, or (2) by multiple routes of exposure, or (3) to an unusual degree with regard to incidence, site, or type of tumor, or age at onset”<sup>11</sup>.

NTP<sup>12</sup> also reviewed the available mechanistic information for 1-bromopropane, and concluded that there are a number of molecular effects caused by 1-bromopropane, “either directly or via reactive metabolites” that “typically are associated with carcinogenesis”. NTP<sup>13</sup> found that “[t]he mechanism(s) by which 1-bromopropane causes cancer is not known. However, exposure to 1-bromopropane has been shown to cause molecular alterations related to carcinogenicity, including genotoxicity (mutations and DNA damage), oxidative stress, glutathione depletion, and immunomodulation”.

With regard to the genotoxicity of 1-bromopropane, NTP<sup>14</sup> noted a number of positive findings. Specifically, 1-bromopropane has been shown to induce mutations in *Salmonella typhimurium*, mutations in cultured mammalian cells, and DNA damage in cultured human cells. There is also limited evidence of induction of DNA damage in leukocytes of 1-bromopropane-exposed humans. In addition, 1-bromopropane can bind to macromolecules, and forms S-propylcysteine-globin adducts in humans and animals. NTP further noted that “[s]everal reactive metabolites (or intermediates) of 1-bromopropane have been identified in rodents, including glycidol and  $\alpha$ -bromohydrin, and propylene oxide has been proposed as a metabolite”<sup>15</sup>. Both glycidol and propylene oxide are genotoxic carcinogens listed in the NTP *Report on Carcinogens as reasonably anticipated to be human carcinogens*<sup>16</sup>, and each is also listed as causing cancer under Proposition 65.

In summary, the designation of 1-bromopropane as “*reasonably anticipated to be a human carcinogen*” is based on the NTP’s findings of sufficient evidence of

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<sup>11</sup> NTP (2014). Report on Carcinogens Listing Criteria. Available at <http://ntp.niehs.nih.gov/pubhealth/roc/criteria/index.html>

<sup>12</sup> National Toxicology Program (NTP, 2014). Report on Carcinogens, Thirteenth Edition, U.S. Department of Health and Human Services, Public Health Service, NTP, Research Triangle Park, North Carolina. Available at URL: <http://ntp.niehs.nih.gov/pubhealth/roc/roc13/index.html>.

<sup>13</sup> *Ibid.*

<sup>14</sup> *Ibid.*

<sup>15</sup> *Ibid.*

<sup>16</sup> *Ibid.*

carcinogenicity from studies in experimental animals. NTP also reviewed multiple lines of evidence on the genotoxicity of the chemical that are indicative of genotoxic activity.

### 1.2 Comment:

“The rodent tumor data from the NTP 2-year inhalation study [*experiments in male mice; female mice; male rats; and female rats*] are not impressive.” “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.” (p. 2)

### Response:

Induction of tumors at the same sites, and with similar incidences across species and sex is not expected of carcinogens by the NTP *Report on Carcinogens*, or other authoritative bodies. Nor is tumor site concordance or comparable cancer potency across species and sex required in order to meet the sufficiency of evidence criteria in Section 25306(e)(2), which reads as follows:

“Sufficient evidence of carcinogenicity exists from studies in experimental animals. For purposes of this paragraph, “sufficient evidence” means studies in experimental animals indicate that there is an increased incidence of malignant tumors or combined malignant and benign tumors in multiple species or strains, in multiple experiments (e.g., with different routes of administration or using different dose levels), or, to an unusual degree, in a single experiment with regard to high incidence, site or type of tumor, or age at onset.”

The NTP found that 1-bromopropane causes significant dose-related increased incidences of combined malignant and benign skin tumors in male rats, and significant dose-related increased incidence of combined malignant and benign lung tumors in female mice. Thus, it is the observations of increased incidences of combined malignant and benign tumors induced by 1-bromopropane in male rats and female mice which satisfy the sufficiency of evidence criteria in Section 25306(e)(2).

## **2. Human relevance of animal tumor findings**

### 2.1 Comment:

“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.” (p. 2)

Response:

Listing of a chemical under Proposition 65 involves only identification that the chemical can cause cancer as specified in Section 25306(e)(2). As noted above, OEHHA has found that 1-bromopropane meets the criteria of this section. The assessment of human cancer risk takes place during a later phase of the Proposition 65 process, in the development of a “no significant risk level”<sup>17</sup>. Issues such as route of exposure and extent of absorption are addressed during this process.

We note that for the animal studies with 1-bromopropane, routes of exposure are similar to the routes through which humans may be exposed, as discussed by NTP. The NTP *Report on Carcinogens* states that occupational exposure to 1-bromopropane occurs primarily through inhalation or dermal contact, and is well absorbed following ingestion, inhalation, or dermal exposure<sup>18</sup>.

2.2 Comment:

“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.”  
“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 [*link*]. 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.” (pp. 2-3)

Response:

A high prevalence in humans of a tumor type seen in animals is not required for a chemical to be identified as a carcinogen. As noted previously (see response to Comment 1.2), tumor site concordance between laboratory animals and humans is neither required, nor expected. Indeed, scientific organizations that evaluate cancer-

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<sup>17</sup> Section 25701 *et seq.*

<sup>18</sup> National Toxicology Program (NTP, 2014). Report on Carcinogens, Thirteenth Edition, U.S. Department of Health and Human Services, Public Health Service, NTP, Research Triangle Park, North Carolina. Available at URL: <http://ntp.niehs.nih.gov/pubhealth/roc/roc13/index.html>.

causing chemicals concur that “tumors observed in animals are generally assumed to indicate that an agent may produce tumors in humans”<sup>19</sup>. This is supported by the fact that all human carcinogens that have been studied in experimental animals have been shown to cause cancer in one or more animal species. In addition, there are many chemicals that were first found to cause cancer in experimental animals and were later found to cause cancer in humans as well. As summarized in the Preamble to the *IARC Monographs*,

“Although this association cannot establish that all agents that cause cancer in experimental animals also cause cancer in humans, it is biologically plausible that agents for which there is *sufficient evidence of carcinogenicity* in experimental animals...also present a carcinogenic hazard to humans. Accordingly, in the absence of additional scientific information, these agents are considered to pose a carcinogenic hazard to humans”<sup>20</sup>.

### 3. Genotoxicity findings

#### 3.1 Comment:

“Determining whether 1-BP is mutagenic in the Ames *Salmonella* assay is an important step in classifying its potential cancer hazard to humans.” “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.” “Ablemarle 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 conducted the Ames test twice in multiple strains of bacteria with and without S9 metabolic activation and found 1-BP to be negative.” (pp. 3-4)

“The only readily identifiable difference between the two assays [*Barber et al. 1981 and BioReliance*] 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.” “Since the current Ames test [*Wagner, 2014*] used a super pure sample of 1-BP, and the Barber et al. (1981) study a less pure sample [99.85%], it is possible that Barber et al. experienced some interference with their Ames test from 2-BP, the most common

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<sup>19</sup> US EPA (2005). United States Environmental Protection Agency. Guidelines for carcinogen risk assessment. Washington, DC. Document number EPA/630/P-03/001F.

<sup>20</sup> IARC (2006). International Agency for Research on Cancer. IARC monographs on the evaluation of carcinogenic risks to humans: Preamble. World Health Organization. Lyon, France. Updated September 2015. Available from <http://monographs.iarc.fr/ENG/Preamble/index.php>.

contaminant in 1-BP preparations.” “Whether 2-bromopropane contamination affected Barber *et al.*’s results is unknown.” (pp. 4-5)

Response:

1-Bromopropane was classified as “*reasonably anticipated to be a human carcinogen*” based on sufficient evidence of carcinogenicity from studies in experimental animals, including significant dose-related increases in incidences of several types of benign and/or malignant skin tumors in male rats and significant dose-related increases in the incidence of benign and malignant lung tumors combined in female mice<sup>21</sup>. Evidence of mutagenicity in the *Salmonella* assay is not part of the sufficiency of evidence criteria in Section 25306.

In order for a chemical to be considered a carcinogen, it need not be a bacterial mutagen, as there are many different mechanisms of carcinogenesis, and a single chemical may act through multiple mechanisms. “The mechanism(s) by which 1-bromopropane causes cancer is not known. However, exposure to 1-bromopropane has been shown to cause molecular alterations related to carcinogenicity, including genotoxicity (mutations and DNA damage), oxidative stress, glutathione depletion, and immunomodulation”<sup>22</sup>. Moreover, in addition to the findings of mutations in *Salmonella typhimurium*, NTP<sup>23</sup> summarized other positive genotoxicity findings for 1-bromopropane, including induction of mutations in cultured mammalian cells and DNA damage in cultured human cells (see response to Comment 1.1 above).

3.2 Comment:

“Similar to the actual BioReliance Ames test result, the QSAR [*quantitative structure-activity relationship*] modeling program OECD [*Organisation for Economic Co-operation and Development*] Toolbox predicts that 1-BP will be negative in the Ames test.” “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.” (p. 4)

Response:

As stated above, evidence of mutagenicity in the *Salmonella* assay is not part of the sufficiency of evidence criteria in Section 25306, and a chemical need not be a bacterial mutagen to be a carcinogen.

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<sup>21</sup> National Toxicology Program (NTP, 2014). Report on Carcinogens, Thirteenth Edition, U.S. Department of Health and Human Services, Public Health Service, NTP, Research Triangle Park, North Carolina. Available at URL: <http://ntp.niehs.nih.gov/pubhealth/roc/roc13/index.html>.

<sup>22</sup> *Ibid.*

<sup>23</sup> *Ibid.*

We also note that five predictions were made in the Organisation for Economic Co-operation and Development (OECD) Toolbox by the commenter: three for 1-bromopropane and two for 2-bromopropane. According to the OECD Toolbox reports provided, only one of the predictions was made with high confidence: a prediction for 1-bromopropane as negative in an *in vitro* bacterial reverse mutation assay (e.g. Ames test). The other predictions (*in vitro* chromosome aberration and *in vivo* summary carcinogenicity for 1-bromopropane, and *in vivo* micronucleus assay and summary carcinogenicity for 2-bromopropane) were not made with adequate confidence, and should not be considered reliable predictions. Experimental data have demonstrated that 1-bromopropane induces positive genotoxicity findings in bacteria and mammalian cells, including mutations and DNA damage<sup>24</sup> (see response to Comment 1.1).

### 3.3 Comment:

“Further testing of 1-BP is required to disentangle its potential carcinogenicity to humans.” “Given the inconsistency in the two [*Ames Salmonella*] study results, further experimentation, possibly in the Comet Assay might shed further light on the carcinogenic potential of 1-BP in humans.” (p. 5)

### Response:

As discussed in the response to Comment 3.1, lack of genotoxicity does not equate with lack of carcinogenicity and evidence of genotoxicity is not part of the sufficiency of evidence criteria in Section 25306(2). The sufficiency of evidence criteria are met by the NTP findings in studies in experimental animals of increased incidences of combined malignant and benign tumors in multiple species. Listing under the authoritative bodies mechanism of Proposition 65 requires that the basis for an authoritative body’s formal identification of a chemical as causing cancer satisfy the scientific criteria of Section 25306(e). It does not require that a mechanism of carcinogenic action be identified.

1-Bromopropane has been tested in other genotoxicity assay systems, in addition to the two sets of *Salmonella* reverse mutation assays mentioned by the commenter. Evidence of genotoxicity summarized by NTP<sup>25</sup> includes induction of mutations in cultured mammalian cells and DNA damage in cultured human cells (see response to Comment 1.1). In reviewing the mechanistic information, NTP found that “1-bromopropane has been shown to cause molecular alterations related to carcinogenicity, including genotoxicity (mutations and DNA damage), oxidative stress,

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<sup>24</sup> National Toxicology Program (NTP, 2014). Report on Carcinogens, Thirteenth Edition, U.S. Department of Health and Human Services, Public Health Service, NTP, Research Triangle Park, North Carolina. Available at URL: <http://ntp.niehs.nih.gov/pubhealth/roc/roc13/index.html>.

<sup>25</sup> *Ibid.*

glutathione depletion, and immunomodulation”<sup>26</sup>. Thus there are a number of possible mechanisms of action, including genotoxicity, through which 1-bromopropane may induce tumors.

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<sup>26</sup> *Ibid.*