

**Response to Comments Pertaining to the Request for Relevant Information on  
β-Myrcene as Causing Cancer under Proposition 65**

**Office of Environmental Health Hazard Assessment  
California Environmental Protection Agency  
February 2014**

On February 10, 2012, the Office of Environmental Health Hazard Assessment (OEHHA) issued a Request for Relevant Information concerning the possible addition of β-myrcene to the Proposition 65<sup>1</sup> list of chemicals known to cause cancer. The consideration of β-myrcene for listing is based on the authoritative bodies provision<sup>2</sup> of the Proposition 65 implementing regulations and the National Toxicology Program's (NTP) identification of β-myrcene as causing cancer. This document responds to comments received in response to the Request for Relevant Information.

The NTP concluded that β-myrcene causes cancer in the 2010 report entitled *Toxicology and Carcinogenesis Studies of β-Myrcene (CAS No. 123-35-3) in F344/N Rats and B6C3F1 Mice (Gavage Studies)*.

NTP concluded:

“Under the conditions of these 2-year gavage studies, there was *clear evidence of carcinogenic activity* of β-myrcene in male F344/N rats based on increased incidences of renal tubule neoplasms. ... There was *clear evidence of carcinogenic activity* of β-myrcene in male B6C3F1 mice based on increased incidences of hepatocellular adenoma, hepatocellular carcinoma, and hepatoblastoma.”<sup>3</sup>

In the “Conclusions” section of the “Summary”, NTP stated:

“We conclude that β-myrcene caused kidney cancers in male rats and liver cancer in male mice.”

Comments responsive to the Request for Relevant Information were submitted by F. Jay Murray and Gary M. Roberts on behalf of the Flavor and Extract Manufacturers

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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> Title 27, Cal. Code of Regulations, section 25306.

<sup>3</sup> NTP 2010. *Toxicology and Carcinogenesis Studies of β-Myrcene (CAS No. 123-35-3) in F344/N Rats and B6C3F1 Mice (Gavage Studies)*. Technical Report Series No. 557, NIH Publication No. 11-5898. U.S. Department of Health and Human Services, NTP, Research Triangle Park, NC.

Association; the International Fragrance Association, North America; the Juice Products Association; and the Renewable Citrus Products Association.

The comments are grouped and numbered by topic, and responses follow below.

## 1. Formal Identification Criteria

### 1a. NTP TECHNICAL REPORT

#### Comment:

“OEHHA does not have the authority to list  $\beta$ -myrcene as a carcinogen because the NTP did not ‘conclude’ that  $\beta$ -myrcene ‘causes cancer’ in animals.”

#### Response:

Under California law, chemicals are required to be listed via the authoritative bodies listing mechanism as known to cause cancer if they meet certain criteria specified in Title 27, California Code of Regulations, section 25306<sup>4</sup>. That regulation provides that a chemical is known to the state to cause cancer if a body considered authoritative has “formally identified” the chemical as causing cancer and if certain scientific criteria are met. OEHHA has determined that an authoritative body, NTP, has formally identified  $\beta$ -myrcene as causing cancer in its Technical Report, *Toxicology and Carcinogenesis Studies of  $\beta$ -Myrcene (CAS No. 123-35-3) in F344/N Rats and B6C3F1 Mice (Gavage Studies)* (NTP, 2010).

The NTP Technical Report on  $\beta$ -myrcene<sup>5</sup> concludes that the chemical causes cancer. On page 9 of the report the NTP report concludes that there is clear evidence of carcinogenic activity of  $\beta$ -myrcene in male rats and male mice.

“Under the conditions of these 2-year gavage studies, there was *clear evidence of carcinogenic activity* of  $\beta$ -myrcene in male F344/N rats based on increased incidences of renal tubule neoplasms. ... There was *clear evidence of carcinogenic activity* of  $\beta$ -myrcene in male B6C3F1 mice based on increased incidences of hepatocellular adenoma, hepatocellular carcinoma, and hepatoblastoma.”

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<sup>4</sup> All further references are to Title 27, California Code of Regulations, unless otherwise indicated.

<sup>5</sup> NTP 2010. *Toxicology and Carcinogenesis Studies of  $\beta$ -Myrcene (CAS No. 123-35-3) in F344/N Rats and B6C3F1 Mice (Gavage Studies)*. Technical Report Series No. 557, NIH Publication No. 11-5898. U.S. Department of Health and Human Services, NTP, Research Triangle Park, NC.

In the report “Summary” Conclusion section (page 5), NTP states: “We conclude that  $\beta$ -myrcene caused kidney cancers in male rats and liver cancer in male mice.”

These conclusions by NTP about the carcinogenic activity of  $\beta$ -myrcene, and the data in the report supporting the conclusions, are the basis for OEHHA’s determination that  $\beta$ -myrcene meets the criteria for listing pursuant to the authoritative bodies mechanism set out in Section 25306.

With regard to the formal identification criteria, this report meets both the “identification” and “formality” requirements of Section 25306. The identification requirements are met because  $\beta$ -myrcene is the subject of a report<sup>6</sup> which is published by the authoritative body (i.e., NTP) and which concludes that the chemical causes cancer. The formality requirements are met because the NTP specifically identifies  $\beta$ -myrcene as causing cancer in a report (1) that is peer reviewed in a public meeting, (2) is subject to public review and comment, and (3) is formally published by the NTP.

The conclusions of the NTP Technical Report on  $\beta$ -myrcene also satisfy the “sufficiency of evidence” criteria set out in Section 25306 (see Topic 2 below for discussion of the sufficiency of evidence criteria).

Comment:

“The NTP did not perform an analysis that extended beyond the conditions of its Technical Report data to evaluate whether  $\beta$ -myrcene causes cancer in animals.”

“...the NTP expressed four separate and limited conclusions about carcinogenic activity in one strain of mice and one strain of rats *under the conditions of its experiment.*”

Response:

The commenter is correct that the NTP Technical Report on  $\beta$ -myrcene expressed separate conclusions about four individual experiments. OEHHA notes that in the NTP Technical Report Series, original scientific work conducted by the NTP is presented and discussed, and NTP’s conclusions regarding the strength of the evidence for each study are presented.

Similar comments regarding the NTP Technical Report Series were raised several years ago by a member of the public at the September 25, 1998 meeting of the

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<sup>6</sup> NTP 2010. *Toxicology and Carcinogenesis Studies of  $\beta$ -Myrcene (CAS No. 123-35-3) in F344/N Rats and B6C3F1 Mice (Gavage Studies)*. Technical Report Series No. 557, NIH Publication No. 11-5898. U.S. Department of Health and Human Services, NTP, Research Triangle Park, NC.

Carcinogen Identification Committee (CIC). At that meeting, the CIC specifically considered and affirmatively retained as “authoritative” NTP Technical Reports, designating NTP without limitation or qualification as an authoritative body for the purposes of identifying chemicals as known to cause cancer.

To determine if the NTP Technical Report<sup>7</sup> sufficiently identifies  $\beta$ -myrcene as known to cause cancer for purposes of Proposition 65, OEHHA must apply the criteria in Section 25306 to the report. As explained in the preceding response, the NTP Technical Report satisfies the criteria in the regulation since it meets both the “identification” and “formality” requirements. Therefore, OEHHA is relying on the NTP Technical Report’s identification of  $\beta$ -myrcene as a chemical known to cause cancer.<sup>8</sup>

Comment:

“The Technical Report warns that its conclusions are not to be extrapolated ‘to other species, including characterization of hazards and risks to humans’ because doing so would require ‘analysis beyond the intent’ of the report.”

Response:

The Forward to the NTP Technical Report states:

“The studies described in the Technical Report series are designed and conducted to characterize and evaluate the toxicologic potential, including carcinogenic activity, of selected substances in laboratory animals (usually two species, rats and mice). ...Extrapolation of these results to other species, including characterization of hazards and risks to humans, requires analyses beyond the intent of these reports.”

The NTP Technical Report further explains under ‘Explanation of Levels of Evidence of Carcinogenic Activity’:

“Positive results demonstrate that a chemical is carcinogenic for laboratory animals under conditions of the study and indicate that exposure to the chemical has the potential for hazard to humans. ...the actual determination of risk to humans from chemicals found to be carcinogenic in laboratory animals requires a wider analysis that extends beyond the purview of these studies.”

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<sup>7</sup> NTP 2010. *Toxicology and Carcinogenesis Studies of  $\beta$ -Myrcene (CAS No. 123-35-3) in F344/N Rats and B6C3F1 Mice (Gavage Studies)*. Technical Report Series No. 557, NIH Publication No. 11-5898. U.S. Department of Health and Human Services, NTP, Research Triangle Park, NC.

<sup>8</sup> See also *ExxonMobil Corp v OEHHA* (2009) 169 Cal. App. 4<sup>th</sup> 1264, *CLFP v OEHHA* (Nov. 2011) Sacramento County Superior Court case # 34-2011-80000784.

Thus NTP is explaining that additional analyses are needed for inter-species extrapolation and characterization of hazards and risks (i.e., quantitative risk analysis), but that positive results demonstrate that the chemical is carcinogenic in laboratory animals and poses a potential cancer hazard to humans. Listing under Proposition 65 concerns only identification of chemicals that cause cancer, as provided in Section 25306.<sup>9</sup> Under Proposition 65, the quantitative assessment of human risk occurs during a later phase of the process during the development of a “No Significant Risk Level” for chemicals listed as causing cancer.

The NTP Technical Report on  $\beta$ -myrcene concludes that the chemical causes cancer, finding clear evidence of carcinogenic activity of  $\beta$ -myrcene in male rats and male mice, and stating “ $\beta$ -myrcene caused kidney cancers in male rats and liver cancer in male mice”. These conclusions satisfy the criteria for listing pursuant to the authoritative bodies mechanism set out in Section 25306.

#### *1b. NTP REPORT ON CARCINOGENS*

##### Comment:

$\beta$ -Myrcene has not been considered for listing in the NTP Report on Carcinogens. “If ‘sufficient evidence’ was a conclusion expressed explicitly or inferentially by the NTP in the Technical Report, the NTP would not need to undertake its thorough review of all relevant studies. Instead, it simply could add chemicals to the Report on Carcinogens based on its work in the Technical Report.”

##### Response:

The NTP Technical Report Series development process is a separate and distinct activity from the NTP Report on Carcinogens. Both the NTP Technical Reports and the NTP Reports on Carcinogens are reports that satisfy the “formal identification” provision of Section 25306. Further, the NTP Technical Report on  $\beta$ -myrcene satisfies the “sufficiency of evidence” criteria set out in Section 25306<sup>10</sup> (see Topic 2 below for discussion of the sufficiency of evidence criteria).

## **2. Sufficiency of Evidence Criteria Applied to $\beta$ -Myrcene**

##### Comment:

“The NTP did not make a “sufficient evidence” finding with regard to  $\beta$ -myrcene.”

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<sup>9</sup> See *AFL-CIO v Deukmejian* (1989) 212 Cal. App. 3d. 425

<sup>10</sup> See *CLFP v OEHHA* (Nov. 2011) Sacramento County Superior Court case # 34-2011-80000784

“NTP has never ‘conclude[d]’ that ‘sufficient evidence of carcinogenicity exists from studies in experimental animals’ within the meaning of section 25306 for  $\beta$ -myrcene.”

“The 1986 EPA Guidelines for Carcinogenic Risk Assessment provide that a “sufficient evidence” determination cannot be based on the results of individual animal studies considered in isolation, but must be based on a broader review of relevant data. ... The NTP has not yet performed that overall analysis for  $\beta$ -myrcene, and thus its Technical Report does not contain a ‘sufficient evidence’ determination required to support an authoritative body listing...”

“The data suggesting carcinogenic activity for  $\beta$ -myrcene come from NTP Technical Report No. 557 (TR-557). There are only two findings of ‘clear evidence of carcinogenic activity’: kidney tumors in male rats and liver tumors in male mice. ”

Response:

OEHHA has determined that NTP has formally identified  $\beta$ -myrcene as a chemical that causes cancer, and that the basis for this formal identification by NTP meets the sufficiency of evidence criteria for “as causing cancer” as laid out in Section 25306(e)(2):

“... ‘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.”

NTP found that there is clear evidence of the carcinogenicity of  $\beta$ -myrcene in multiple species and experiments, i.e., the study in male rats and the study in male mice. NTP’s conclusion of clear evidence for the carcinogenic activity of  $\beta$ -myrcene in male rats is based on an increased incidence of combined malignant and benign renal tubule tumors (which are rare in male F344/N rats) in treated animals as compared to vehicle controls. NTP’s conclusion of clear evidence for the carcinogenic activity of  $\beta$ -myrcene in male mice is based on increased incidences of malignant and combined malignant and benign liver tumors in treated animals as compared to vehicle controls. Thus, the findings by NTP satisfy the sufficiency of evidence criteria in Section 25306.

## 2a. EVIDENCE IN MALE RATS

### Comment:

The commenters raised concern that “[t]he evaluation of the potential carcinogenicity of  $\beta$ -myrcene [in male rats] was limited to the low- and mid-dose levels because the high dose caused such excessive mortality that NTP decided not to even present the histopathological data for the high dose group of male rats.”

The commenters further noted, “At the middle and low doses, a statistically significant increase in renal tubule adenomas and combined adenomas and carcinomas was observed among the male rats. No statistically significant increase in renal tubule carcinomas alone was seen at either dose level. In addition, a clear dose-response relationship was not evident for any renal tubule neoplasm since there was very little difference between the results at the low and middle doses.”

### Response:

Contrary to the statement by the commenters, the NTP did present the histopathological data for the high dose group of male rats in Table A1 (Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Gavage Study of  $\beta$ -Myrcene) and Table A4 (Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Gavage Study of  $\beta$ -Myrcene) of the report<sup>11</sup>.

In the male rat study all the high-dose animals died early, with deaths occurring primarily between 60 and 85 weeks of  $\beta$ -myrcene administration. The NTP attributed the deaths in the high dose group to renal toxicity, and concluded that the maximum tolerated dose had been exceeded in the high dose group. For this reason the NTP did not consider the tumor incidence data from the high-dose group when evaluating the evidence of carcinogenicity in the male rat study.

Both renal tubule adenomas and renal tubule carcinomas were observed in the low- and mid-dose groups. NTP found that the incidence of combined renal tubular adenoma and carcinoma (by single and step-sections combined) for the low-dose group ( $p < 0.001$ ) and the mid-dose group ( $p < 0.001$ ) were statistically significantly increased compared to the incidence in controls by pairwise comparison. A statistically significant trend ( $p < 0.001$ ) in incidence was also observed across the control, low- and mid-dose groups.

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<sup>11</sup> NTP (2010). *Toxicology and Carcinogenesis Studies of  $\beta$ -Myrcene (CAS No. 123-35-3) in F344/N Rats and B6C3F1 Mice (Gavage Studies)*. Technical Report Series No. 557, NIH Publication No. 11-5898. U.S. Department of Health and Human Services, NTP, Research Triangle Park, NC.

Comment:

The commenters state that kidney tumors in male rats “occur at a high background incidence, are suspect due to genetic predisposition and are of doubtful relevance for cancer hazard identification.”

Response:

The background incidence of kidney tumors in male F344/N rats is not high, and OEHHA is not aware of any data that support the commenters’ claim that rats are genetically predisposed to develop kidney tumors. No renal tubular adenomas or carcinomas were observed in the control males in the NTP F344/N rat study of  $\beta$ -myrcene. In addition, NTP historical control data compiled from 5 sets of corn oil gavage studies conducted in F344/N male rats during the same time period (2002-2005) and in the same laboratory (Battelle Columbus Laboratory) as the  $\beta$ -myrcene study indicate a very low incidence of renal tubular adenoma or carcinoma combined in vehicle controls (2/249, or 0.8%)<sup>12</sup>. Other analyses of the background incidence of renal tubular tumors in Fischer rats include that of Haseman et al. (1998)<sup>13</sup>, which found that renal tubular adenomas and carcinomas in F344/N rats occurred at very low incidences in 27 feed and 18 inhalation studies conducted by NTP before 1997: 0.7% adenoma rate and 0.2% carcinoma rate, feed studies; 1.0% adenoma rate and 0.1% carcinoma rate, inhalation studies.

Comment:

The commenters state:

“Although NTP discussed the relationship between kidney toxicity and kidney tumors observed in male rats under the conditions of the  $\beta$ -myrcene study, NTP never indicated whether it considered these results [in male rats] to be relevant for the purposes of hazard identification.”

Response:

With regard to the relevance to cancer hazard identification of the kidney tumors seen in male rats treated with  $\beta$ -myrcene, NTP concluded that that the increased incidences of renal tubular neoplasms in male rats provide “clear evidence of carcinogenic activity” of  $\beta$ -myrcene. This is a clear cancer hazard-identification statement.

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<sup>12</sup> [http://ntp.niehs.nih.gov/ntp/Historical\\_Controls/NTP2000\\_2011/RatsGavCornOil.pdf](http://ntp.niehs.nih.gov/ntp/Historical_Controls/NTP2000_2011/RatsGavCornOil.pdf)

<sup>13</sup> Haseman J, Hailey J, Morris R (1998). Spontaneous Neoplasm Incidences in Fischer 344 Rats and B6C3F1 Mice in Two-Year Carcinogenicity Studies: A National Toxicology Program Update. *Toxicol Pathol*, 26(3):428–441.

Comment:

The commenters argue that

“...there is every possibility that the significant increase in kidney tumors in male rats is explained by the  $\alpha$ 2u-globulin mechanism, a mechanism known to be species- and sex-specific. The renal pathology reported in male F344/N rats in the NTP bioassay of  $\beta$ -myrcene is similar to that of other substances tested in NTP bioassays (e.g., *d*-limonene, pinene) that have been associated with kidney tumors. ...these findings are widely considered irrelevant to humans because the tumors are unique to the male rat.”

“Many chemicals have been found to cause kidney tumors in male rats, but not in female rats or male or female mice. Examples include *d*-limonene, 1,4-dichlorobenzene, and isophorone. This phenomenon reflects the sensitivity of the male rat kidney to chronic progressive nephropathy (CPN). And, in the NTP bioassay of  $\beta$ -myrcene chronic progressive nephropathy was pronounced in male rats.”

Response:

In its discussion of the  $\beta$ -myrcene kidney tumor findings in male rats, NTP identified three distinct non-neoplastic renal lesions that were observed in treated males:  $\alpha$ 2u-globulin nephropathy, CPN, and nephrosis, which colocalized with the renal tubule necrosis seen in the outer stripe of the outer medulla in the 3-month exposure study. NTP also discussed neoplastic and non-neoplastic lesions observed in the  $\beta$ -myrcene study in female rats, noting that the incidence of renal tubule adenoma was marginally increased (0/50, 2/50, 1/50, 3/50 for control, low-, mid-, and high-dose groups respectively), that the renal tubule adenoma incidence in high-dose females was above the historical control range, and that dose-related increases in CPN and nephrosis were also observed in  $\beta$ -myrcene-treated female rats. NTP went on to discuss the similarities and differences between the kidney effects seen in  $\beta$ -myrcene- and *d*-limonene-treated rats, noting that while *d*-limonene also induced renal tubule neoplasms and  $\alpha$ 2u-globulin nephropathy in male rats, nephrosis was not seen in the *d*-limonene studies, nor was renal tubule necrosis seen in the outer stripe of the outer medulla with *d*-limonene treatment<sup>14</sup>. NTP concluded that “several lines of evidence suggest that  $\beta$ -myrcene might cause nephrotoxicity by a mechanism other than, or in addition to,  $\alpha$ 2u-globulin nephropathy,” and that “the presence of renal neoplasms in female rats also suggests a mechanism of carcinogenesis that may be related to the nephrosis **and is distinct from**

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<sup>14</sup> NTP (1990) *Toxicology and Carcinogenesis Studies of d-Limonene (CAS No. 598-27-5) in F344/N Rats and B6C3F1 Mice (Gavage Studies)*. Technical Report Series No. 347, NIH Publication No. 90-2802. U.S. Department of Health and Human Services, NTP, Research Triangle Park, NC.

**the  $\alpha$ 2u-globulin mechanism.”** (emphasis added) Thus, NTP’s discussion indicates that i) there are key differences between the effects of  $\beta$ -myrcene and *d*-limonene on the rat kidney, ii) the effects of  $\beta$ -myrcene in the rat kidney are not sex-specific, and iii) the species- and sex-specific  $\alpha$ 2u-globulin mechanism cannot account for the increased incidences in kidney tumors observed in male rats treated with  $\beta$ -myrcene. NTP took this information into account when concluding that  $\beta$ -myrcene showed “clear evidence of carcinogenic activity” in male rats.

Comment:

The commenters argue:

“In female rats there was no significant increase in any tumor at any dose level, and NTP categorized the evidence of carcinogenic activity as “equivocal.” Thus the rat data is not a sufficient basis on which to move forward with an authoritative body listing.”

Response:

The basis for OEHHA’s determination that  $\beta$ -myrcene meets the criteria for listing pursuant to section 25306 is the NTP’s conclusion that there is clear evidence of carcinogenic activity of  $\beta$ -myrcene in male rats and male mice. OEHHA is not relying on the NTP’s conclusion regarding equivocal evidence of carcinogenic activity in female rats.

With regard to the NTP study in female rats<sup>15</sup>, OEHHA notes the following:

- The incidence of renal tubule adenoma in female rats was 0/50, 2/50, 1/50, 3/50 for control, low-, mid-, and high-dose groups respectively.
- In discussing the female rat study, NTP stated the renal tubule adenoma incidence in high-dose females was above the historical control range, and that dose-related increases in CPN and nephrosis were observed.
- NTP stated “the presence of renal neoplasms in female rats also suggests a mechanism of carcinogenesis that may be related to the nephrosis and is distinct from the  $\alpha$ 2u-globulin mechanism.”
- The NTP (2010)<sup>16</sup> concluded “[t]here was *equivocal evidence of carcinogenic activity* of  $\beta$ -myrcene in female F344/N rats based on increased incidences of renal tubule adenoma.”

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<sup>15</sup> NTP (2010). *Toxicology and Carcinogenesis Studies of  $\beta$ -Myrcene (CAS No. 123-35-3) in F344/N Rats and B6C3F1 Mice (Gavage Studies)*. Technical Report Series No. 557, NIH Publication No. 11-5898. U.S. Department of Health and Human Services, NTP, Research Triangle Park, NC.

<sup>16</sup> NTP (2010). *Toxicology and Carcinogenesis Studies of  $\beta$ -Myrcene (CAS No. 123-35-3) in F344/N Rats and B6C3F1 Mice (Gavage Studies)*. Technical Report Series No. 557, NIH Publication No. 11-5898. U.S. Department of Health and Human Services, NTP, Research Triangle Park, NC.

## 2b. EVIDENCE IN MALE MICE

### Comment:

The commenters raised concern that “[t]he evaluation of the dose-response relationship [in male mice] is complicated by the fact that there were only two exposed groups assessed for potential carcinogenicity since there were no histopathological data at the high-dose due to excessive mortality.”

### Response:

The NTP did present the histopathological data for the high dose group of male mice in Table C1 (Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Gavage Study of  $\beta$ -Myrcene) and Table C4 (Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Gavage Study of  $\beta$ -Myrcene) of the report<sup>17</sup>.

In the NTP male mouse study, survival of high-dose male mice was significantly less than that of vehicle controls. Survival differences became apparent after approximately week 47 of  $\beta$ -myrcene administration. NTP stated that the cause of early deaths in the high-dose group was uncertain. NTP concluded that the maximum tolerated dose had been exceeded in the high dose group. For this reason NTP did not consider the tumor incidence data from the high-dose group when evaluating the evidence of carcinogenicity in the male mouse study.

NTP found that the combined incidence of hepatocellular adenoma, hepatocellular carcinoma, and hepatoblastoma for the low-dose group ( $p = 0.003$ ) and the mid-dose group ( $p < 0.001$ ) were statistically significantly increased compared to the incidence in controls by pairwise comparison. A statistically significant trend ( $p < 0.001$ ) in incidence was also observed across the control, low- and mid-dose groups.

### Comment:

The commenters argue that the “statistically significant increases were seen at the mid-dose level of 500 mg/kg/day [*sic*]. At the low dose (250 mg/kg/day [*sic*]), the only statistically significant response was an increase in hepatocellular adenoma, a benign liver tumor.”

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<sup>17</sup> NTP (2010). *Toxicology and Carcinogenesis Studies of  $\beta$ -Myrcene (CAS No. 123-35-3) in F344/N Rats and B6C3F1 Mice (Gavage Studies)*. Technical Report Series No. 557, NIH Publication No. 11-5898. U.S. Department of Health and Human Services, NTP, Research Triangle Park, NC.

Response:

As discussed above, NTP found statistically significant increases by pairwise comparison with controls in the combined incidence of hepatocellular adenoma, hepatocellular carcinoma, and hepatoblastoma for the low-dose group ( $p = 0.003$ ) and the mid-dose groups ( $p < 0.001$ ), as well as a statistically significant trend ( $p < 0.001$ ). These findings by NTP for male mice, together with NTP's findings for male rats, satisfy the sufficiency of evidence criteria in Section 25306.

While not reaching statistical significance, the incidence of malignant liver tumors (hepatocellular carcinomas or hepatoblastomas) in the low-dose group (44%) was elevated as compared to that in controls (32%). NTP reported statistically significant increases in the low-dose group by pairwise comparison with controls for multiple hepatocellular adenoma ( $p < 0.01$ ), hepatocellular adenoma ( $p < 0.001$ ), and combined hepatocellular adenoma and carcinoma ( $p = 0.003$ ). In the high-dose group, NTP reported statistically significant increases by pairwise comparison with controls for multiple hepatocellular adenoma ( $p < 0.01$ ), hepatocellular adenoma ( $p < 0.001$ ), hepatocellular carcinoma ( $p = 0.004$ ), combined hepatocellular adenoma and carcinoma ( $p < 0.001$ ), hepatoblastoma ( $p = 0.041$ ), and combined hepatocellular carcinoma and hepatoblastoma ( $p < 0.001$ ). NTP also reported statistically significant trends for the incidence of hepatocellular adenoma ( $p < 0.001$ ), hepatocellular carcinoma ( $p = 0.003$ ), combined hepatocellular adenoma and carcinoma ( $p < 0.001$ ), hepatoblastoma ( $p = 0.027$ ), and combined hepatocellular carcinoma and hepatoblastoma ( $p = 0.002$ ). Hepatocellular carcinoma and hepatoblastoma are malignant tumors. These findings are consistent with a treatment-related progression of benign tumors to malignant tumors, and demonstrate positive dose-response relationships for the induction of malignant, benign, and combined malignant and benign liver tumors.

Comment:

The commenters state that liver tumors in B6C3F1 male mice “occur at a high background incidence, are suspect due to genetic predisposition and are of doubtful relevance for cancer hazard identification,” and note that “[t]he background incidence of hepatocellular tumors among the control males in this study was extremely high.”

The commenters also assert that the “predictive value of mouse hepatocellular tumors with respect to cancer risk has been repeatedly challenged. ...The European Food Safety Authority (EFSA) has concluded that ‘hepatic tumors in mice are generally considered as irrelevant for human risk assessment’ in mouse dietary administration study.... Beginning in 2000, the National Industrial Chemicals Notification and Assessment Scheme (NICNAS) of Australia has concluded that the liver tumors observed in B6C3F1 mice after prolonged exposure to a range of chemicals (e.g., p-

dichlorobenzene) are considered to be irrelevant to humans” and argue that “β-[m]yrcene mouse data should not form the basis for cancer hazard identification.”

Response:

NTP is aware of the high background incidence of liver tumors typically observed in studies with the male B6C3F1 mouse, and the widely recognized susceptibility of this mouse strain to hepatocarcinogenesis. The predictive value of the B6C3F1 mouse for use in carcinogenesis studies has been carefully considered and assessed by NTP. The B6C3F1 mouse continues to be strain selected for use in the NTP toxicology and carcinogenesis studies<sup>18</sup>.

The high incidence of liver tumors in untreated B6C2F1 mice and the variation between studies underscores the importance of the concurrent control group in assessing the significance of incidence data.

With regard to the commenters’ assertion that the incidence of hepatocellular tumors in control males in the β-myrcene study was ‘extremely high’, OEHHA notes that control data compiled from 6 sets of corn oil gavage studies conducted by NTP during the same time period (2002-2005) and in the same laboratory (Battelle Columbus Laboratory) as the β-myrcene studies showed very similar control incidence of hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma in male mice (208/300, or 69%) as was observed in the β-myrcene study (34/50, or 68%)<sup>19</sup>.

Although the sensitivity of mouse liver has been widely discussed, it has not led to discounting of hepatic tumors in mice. Mouse liver tumors are considered relevant for cancer hazard identification by the NTP and other bodies designated as authoritative for purposes of identifying chemicals as causing cancer under Proposition 65 (e.g., International Agency for Research on Cancer [IARC], U.S. Environmental Protection Agency [U.S. EPA]).

NTP has been designated as “authoritative” under Proposition 65 (section 25306 (I)(1)), and its determination regarding the carcinogenicity of β-myrcene serves as the basis for the proposed listing. Neither the European Food Safety Authority nor the National

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<sup>18</sup> King-Herbert A and Thayer K (2006). NTP Workshop: Animal Models for the NTP Rodent Cancer Bioassay: Stocks and Strains----Should we Switch? *Toxicol Pathol* 34:802-805.

<sup>19</sup> National Toxicology Program (NTP, 2011). *NTP Historical Controls for NTP-2000 Diet: Gavage, Corn Oil, Fischer F344/N Rats, Pathology Tables*, version: May 2011, U.S. Department of Health and Human Services, NTP, Research Triangle Park, NC. Available at [http://ntp.niehs.nih.gov/ntp/Historical\\_Controls/NTP2000\\_2011/RatsGavCornOil.pdf](http://ntp.niehs.nih.gov/ntp/Historical_Controls/NTP2000_2011/RatsGavCornOil.pdf) [Accessed: May 2013]

Industrial Chemical Notification Assessment Scheme of Australia are considered “authoritative” under Proposition 65.

Comment:

“Induction of hepatocellular tumors in mice by non-genotoxic compounds can be considered as irrelevant for human risk assessment [citing Holsapple et al., 2006 and Billington et al., 2010<sup>20</sup>]....Holsapple et al. (2006) concluded that in the case of chemicals displaying a phenobarbital-like P450 inducing mode of action, the observed hepatocarcinogenicity in rodents is not relevant to humans.”

Response:

Neither of the references cited above support the assertion that liver tumors induced in mice by nongenotoxic carcinogens are irrelevant for human risk assessment. The paper by Billington et al. addresses the question of whether studies in the mouse identify carcinogens not identified by studies in the rat. The paper by Holsapple et al. discusses the use of mode of action information to determine the relevance of rodent data to human risk assessment.

Neither NTP nor other bodies designated as authoritative for purposes of identifying chemicals as causing cancer under Proposition 65 (e.g., U.S. EPA) consider mouse liver tumors induced by nongenotoxic carcinogens as irrelevant for human risk assessment, absent clear evidence of a mode of action not relevant to humans<sup>21</sup>. NTP did not identify the mechanisms by which  $\beta$ -myrcene induced liver tumors in mice, stating:

“Further studies are needed to understand the mechanism of action of  $\beta$ -myrcene-induced toxicity and carcinogenesis in rats and mice.  $\beta$ -Myrcene and  $\alpha$ -limonene are not mutagenic or clastogenic...  $\beta$ -Myrcene may be metabolized by P450 to an epoxide, which may have the ability to alkylate DNA.”

Comment:

“It appears that, in at least one case, NTP has called into question the relevance of mouse liver tumors for purposes of hazard identification. In an NTP bioassay (NTP TR-

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<sup>20</sup> Holsapple, MP, Pitot HC, Cohen SM, Boobis AR, Klaunig JE, Pastoor T, Dellarco VL, Dragan YP (2006). Mode of action in relevance of rodent liver tumors to human cancer risk. *Toxicol Sci* 89:51-56  
Billington R, Lewis RW, Mehta JM, Dewhurst (2010). The mouse carcinogenicity study is no longer a scientifically justifiable core data requirement for the safety assessment of pesticides. *Crit Rev Toxicol* 40(1):35-49.

<sup>21</sup> U.S. Environmental Protection Agency (U.S. EPA, 2005a). Cancer Assessment Document. Evaluation of the Carcinogenic Potential of Benthialvalicarb-isopropyl. Cancer Assessment Review Committee, Health Effects Division, Office of Pesticide Programs. October 18, 2005.

190), *p*-nitrosodiphenylamine caused ‘positive’ findings of liver tumors in male mice and male rats...based on the results of the NTP bioassay [NTP Technical Report TR-190<sup>22</sup>], it is clear that the only reason for initially listing [*p*-nitrosodiphenylamine] as a carcinogen [in the 5<sup>th</sup> Annual Report on Carcinogen, and subsequently delisting it in the 6<sup>th</sup> Annual Report] was the rodent liver tumors, including the statistically significant increase in hepatocellular carcinoma in male mice.”

Response:

As discussed under Topic 1b above, the NTP Report on Carcinogens is a separate and distinct activity from the NTP Technical Report Series development process. OEHHA considers both the NTP Technical Reports and the NTP Reports on Carcinogens as reports that satisfy the “formal identification” provision of Section 25306.

The comment refers to findings from an early set of bioassays conducted by the National Cancer Institute (NCI) [on *p*-nitrosodiphenylamine] and published as a report in 1979. This NCI report classifies the evidence of carcinogenic activity in male mice, based on increases in hepatocellular carcinomas, as ‘positive’, and notes that

“...due to the large number of early deaths among high dose mice of both sexes, the statistical conclusion concerning carcinogenicity was based on comparisons between the low dose and control groups.”

This NCI report pre-dates the creation of NTP and the adoption of the five categories of evidence of carcinogenic activity used by NTP, which are defined in the NTP Technical Reports under ‘Explanation of Levels of Evidence of Carcinogenic Activity’ as follows:

“...two categories for positive results (**clear evidence and some evidence**), one category for uncertain findings (**equivocal evidence**), one category for no observable effects (**no evidence**), and one category for experiments that cannot be evaluated because of major flaws (**inadequate study**). These categories of interpretative conclusions were first adopted in June 1983 and then revised in March 1986 for use in the Technical Report series to incorporate more specifically the concept of actual weight of evidence of carcinogenic activity.”

There is no evidence that the delisting of *p*-nitrosodiphenylamine from the Annual Report on Carcinogens was the result of NTP questioning the relevance of mouse liver tumors for purposes of hazard identification. OEHHA notes that the 4<sup>th</sup> Annual Report on Carcinogens characterized the evidence of carcinogenicity of *p*-nitrosodiphenylamine in animals as “limited”, but then stated that “In view of an NCI/OTA correlative

interpretation, the evidence may be regarded as sufficient”<sup>23</sup>. The 5<sup>th</sup> Annual Report on Carcinogens characterized the evidence in animals for *p*-nitrosodiphenylamine as “inadequate”<sup>24</sup> and the 6<sup>th</sup> Annual Report on Carcinogens cited the reason for delisting as “insufficient evidence of carcinogenicity”<sup>25</sup>.

## 2c. GENOTOXICITY FINDINGS

### Comment:

β-Myrcene is not genotoxic. β-Myrcene has not shown any evidence of genotoxicity. A battery of genotoxicity studies was conducted by NTP. No mutagenicity was observed in any of several strains of *Salmonella typhimurium* or *E. coli* in two independent Ames tests conducted with and without activation. In addition, β-myrcene was negative in a micronucleus test in male and female mice administered β-myrcene by gavage for three months.

### Response:

OEHHA agrees that NTP did not find evidence of genotoxicity in its own testing, which consisted of tests for mutation in *Salmonella typhimurium* and *Escherichia coli*, as well as tests for increases in the frequency of micronucleated erythrocytes in mouse peripheral blood. NTP states that the mechanism of β-myrcene-induced carcinogenesis is not clear and discusses a number of possible non-genotoxic modes of action for β-myrcene in rats and mice<sup>26</sup>. OEHHA notes that lack of genotoxicity does not equate with lack of carcinogenicity and that evidence of genotoxicity is not part of the sufficiency of evidence criteria in section 25306.

### Comment:

“Interestingly, two publications have reported that β-myrcene protects against known genotoxic substances. ... β-Myrcene had a substantial protective effect against oxidant-induced genotoxicity, which is predominantly mediated by its radical scavenging activity.”

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<sup>23</sup> NTP (1985). Fourth Annual Report on Carcinogens. pp. 345-346 U.S. Department of Health and Human Services, Public Health Service.

<sup>24</sup> NTP (1989). Fifth Annual Report on Carcinogens. pp. 458-460 U.S. Department of Health and Human Services, Public Health Service, National Toxicology Program.

<sup>25</sup> NTP (1991) Sixth Annual Report on Carcinogens. p. 461, U.S. Department of Health and Human Services, Public Health Service, National Toxicology Program.

<sup>26</sup> NTP (2010). *Toxicology and Carcinogenesis Studies of β-Myrcene (CAS No. 123-35-3) in F344/N Rats and B6C3F1 Mice (Gavage Studies)*. Technical Report Series No. 557, NIH Publication No. 11-5898. pp. 61-63, U.S. Department of Health and Human Services, NTP, Research Triangle Park, NC.

Response:

$\beta$ -Myrcene may have radical scavenging activity and protect against oxidant-induced genotoxicity *in vitro*. However, these observations are not inconsistent with the chemical having carcinogenic activity. Indeed,  $\beta$ -myrcene has been shown to induce cancer in studies in male rats and mice<sup>27</sup>. The Proposition 65 listing would be based on the determination by NTP that there is clear evidence of the carcinogenicity of  $\beta$ -myrcene in male rats and male mice.

### 3 Other Comments

#### 3a. NTP REPORTS

Comment:

OEHHA may not base a listing solely on an NTP Technical Report since the NTP also publishes the NTP Report on Carcinogens (RoC), which involves a more comprehensive review of the chemicals that are included in the RoC.

Response:

The NTP Technical Report consists of the first-hand bench science evaluation of a chemical performed by the NTP itself. The work is conducted pursuant to Title 42, United States Code, Section 241(b)(1). The Secretary of the Department of Health and Human Services (DHHS) publishes the RoC pursuant to Section 241(b)(4). The timelines and processes for prioritizing chemicals for review for these two distinct activities differ. When NTP issues its Technical Reports, it is operating pursuant to a regulatory regime separate and apart of the publication of the RoC and vice versa. There is no statutory linkage of these distinct scientific efforts conducted by the NTP.

OEHHA reviews the NTP Technical Reports as they are issued to determine whether the NTP concludes that the chemical tested causes cancer and whether the evidence set out by NTP satisfies the scientific criteria for listing specified in Section 25306(e). The chemicals identified in the NTP RoC are evaluated by OEHHA for listing via the Authoritative Bodies mechanism against the same formality and scientific criteria specified in Section 25306.

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<sup>27</sup> NTP (2010). *Toxicology and Carcinogenesis Studies of  $\beta$ -Myrcene (CAS No. 123-35-3) in F344/N Rats and B6C3F1 Mice (Gavage Studies)*. Technical Report Series No. 557, NIH Publication No. 11-5898. pp. 61-63, U.S. Department of Health and Human Services, NTP, Research Triangle Park, NC

### *3b. REQUEST FOR REFERRAL TO THE CARCINOGEN IDENTIFICATION COMMITTEE (CIC)*

#### Comment:

The commenters “oppose listing  $\beta$ -myrcene as a carcinogen through the authoritative bodies process,” arguing that additional expert judgment and analysis of the male rat kidney tumor findings and the male mouse liver tumor findings is required, and stating that “[i]f California wishes to proceed with a listing evaluation of  $\beta$ -myrcene, it should do so by referring review of  $\beta$ -myrcene to the Carcinogen Identification Committee (CIC).”

#### Response:

Listings via review by the CIC are just one of the ways a chemical can be listed under Proposition 65. The statute’s four listing mechanisms are not hierarchical. Proposition 65 requires the listing of a chemical that meets the criteria for any of the four mechanisms.

NTP has been designated by the CIC as an authoritative body for the purpose of identifying chemicals as causing cancer under Proposition 65 (section 25306(m)(3)). OEHHA has determined that NTP has formally identified  $\beta$ -myrcene as causing cancer and that the evidence meets the scientific criteria specified in the regulation. OEHHA will therefore proceed to the next step in the authoritative bodies listing process by issuing a notice of intent to list  $\beta$ -myrcene.

### *3c. REQUEST FOR HOLDING OPEN THE RECORD*

#### Comment:

“We also request that OEHHA hold open the record until we are able to obtain a copy of the Sixth Annual Report on Carcinogens and evaluate this information.” This request is related to the comment appearing on page 14 and 15 concerning NTP’s decision to delist the chemical p-nitrosodiphenylamine in its Sixth Report on Carcinogens, which was published in 1991.

#### Response:

A formal public comment period will be provided with the Notice of Intent to List  $\beta$ -myrcene. The commenter is free to include information in subsequent comments from its review of the Sixth Annual Report on Carcinogens.