

β-Myrcene

Should Not be Listed as a Proposition 65 Carcinogen

Pursuant to the Authoritative Bodies Listing Process

Comments of

Flavor and Extract Manufacturers Association

International Fragrance Association, North America

Juice Products Association

Renewable Citrus Products Association

Submitted to the
Office of Environmental Health Hazard Assessment

by

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April 10, 2012

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I. Introduction

β -Myrcene should not be listed as a carcinogen because both the mouse and rat data upon which the NTP identified carcinogenic activity under the conditions of NTP's study are of dubious relevance to cancer hazard identification. The NTP did not perform an analysis that extended beyond the conditions of its Technical Report data to evaluate whether β -myrcene causes cancer in animals. Thus, the Flavor and Extract Manufacturers Association, the International Fragrance Association, North America, the Juice Products Association and the Renewable Citrus Products Association ("Associations") oppose listing β -myrcene as a Proposition 65 carcinogen. In particular, the Associations oppose listing β -myrcene as a carcinogen through the authoritative bodies process. If California wishes to proceed with a listing evaluation of β -myrcene, it should do so by referring review of β -myrcene to the Carcinogen Identification Committee (CIC).

The data suggesting carcinogenic activity for β -myrcene come from NTP Technical Report No. 557 (TR-557).¹ There are only two findings of "clear evidence of carcinogenic activity": kidney tumors in male rats and liver tumors in male mice. Both involve tumors that occur at a high background incidence, are suspect due to genetic predisposition and are of doubtful relevance for cancer hazard identification. In both cases, these findings occurred in studies where there were only two dose levels available to evaluate carcinogenic potential because NTP decided not to present the histopathological data at the high dose due to excessive mortality. No other data exist

¹ National Toxicology Program (NTP). 2010. NTP Technical Report on the Toxicology and Carcinogenesis Studies of β -Myrcene (CASRN 123-35-3) in F344/N Rats and B6C3F1 Mice. (NTP TR-557). National Toxicology Program, Research Triangle Park, NC.

that support a finding of sufficient evidence of carcinogenicity. And, β -myrcene was not genotoxic in a battery of tests conducted by NTP.

The Associations request that OEHHA either proceed no further with the listing of β -myrcene or that the CIC review the data for β -myrcene before OEHHA takes any further regulatory action. The Associations also request the opportunity to meet with OEHHA to further discuss the legal and scientific issues raised by its evaluation of β -myrcene.

II. β -Myrcene rat data should not form the basis for cancer hazard identification

The NTP rat data shows an increase in kidney tumors in male rats, a species- and sex-specific response unique to male rats and of doubtful relevance to cancer hazard identification. 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.

In the NTP bioassay, male F344/N rats were administered 0, 250, 500, and 1000 mg/kg/day of β -myrcene by gavage for 5 days/week. The evaluation of the potential carcinogenicity of β -myrcene 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:

“Due to the early mortality in the 1 g/kg male rats, data from this group are not presented in this section.”²

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. For example, the incidence of renal tubule adenomas (single and step sections) was 0% (0/50), 24% (12/50), and 26% (13/50) at 0, 250 and 500 mg/kg/day, respectively. The incidence of renal tubule carcinomas (single and step sections) was 0% (0/50), 6% (3/50), and 2% (1/50) at 0, 250 and 500 mg/kg/day, respectively.

For male rats, the NTP Technical Report 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.” However, it should be recognized that NTP qualified its statements regarding the level of carcinogenic activity with the phrase “under the conditions of these 2-year gavage studies.” Importantly, NTP did not indicate whether it considered these results to be relevant for purposes of hazard identification.

Male kidney tumors are of questionable relevance for cancer hazard identification since they appear to be a species- and sex-specific response that is unique to the male

² National Toxicology Program (NTP). 2010. NTP Technical Report on the Toxicology and Carcinogenesis Studies of β -Myrcene (CASRN 123-35-3) in F344/N Rats and B6C3F1 Mice. NTP TR-557. NIH Publication No. National Toxicology Program, Research Triangle Park, NC, p. 39.

rat.^{3,4} As expected, in female rats, there was no statistically significant increase in renal tubule adenomas or carcinomas (or any other tumor) at any dose level of β -myrcene. NTP decided there was “equivocal evidence of carcinogenic activity” in female rats based on a slight (not statistically significant) increase in the incidence of benign kidney tumors compared to historical controls.

Many chemicals have been found to cause kidney tumors in male rats, but not in female rats or male or female mice.⁵ Examples include *d*-limonene, 1,4-dichlorobenzene and isophorone. This phenomenon reflects the sensitivity of the male rat kidney to chronic progressive nephropathy. And, in the NTP bioassay of β -myrcene, chronic progressive nephropathy was pronounced in male rats.

NTP discussed the possible relationship between the appearance of kidney tumors and the non-neoplastic lesions found in the kidneys:

“In the 2-year rat study, there was clear evidence of carcinogenic activity of β -myrcene in male rats based on the increased incidences of renal tubule adenoma or carcinoma. . . . β -myrcene administration also resulted in increased incidence and/or severity of a number of non-neoplastic renal lesions, including nephrosis and exacerbations of CPN in both sexes, and papillary mineralization in the males. The papillary mineralization had a linear appearance and was

³ Travlos GS, Hard GC, Betz LJ, Kissling GE (2012) Chronic Progressive Nephropathy in Male F344 Rats in 90-Day Toxicity Studies, Its Occurrence and Association with Renal Tubule Tumors in Subsequent 2-Year Bioassays. *Toxicol Pathol* 40: 473-481.

⁴ Swenberg JA, Lehman-McKeeman LD (1999). α_2 -Urinary globulin-associated nephropathy as a mechanism of renal tubule cell carcinogenesis in male rats. In *Species Differences in Thyroid, Kidney and Urinary Bladder Carcinogenesis* (Capen C. C., Dybing E., Rice J. M., Wilbourn J. D., eds.), pp. 95–118. IARC Publications No. 147, International Agency for Research on Cancer, Lyon.

⁵ Lock EA, Hard GC (2004) Chemically induced renal tubule tumors in the laboratory rat and mouse of the NCI/NTP database and categorization of renal carcinogens based on mechanistic information. *Crit Rev Toxicol* 34(3):211-299.

found in the loops of Henle in the medulla. This type of mineralization, which is considered a chronic manifestation of α 2u-globulin nephropathy, was also seen in NTP chronic studies of the structurally related compound α -limonene (NTP, 1990; Hard et al., 1993).

“Nephrosis was unique lesion in the 2-year study of β -myrcene in rats and was more severe in males than in females. The pathogenesis of this lesion is unknown, but the co-localization of this lesion with the renal tubule necrosis in the outer stripe of the outer medulla (in the 3-month study) and the proliferative nature of the nephrosis (as evidenced by the karyomegaly and tubule hyperplasia) suggest that it is an unusual response to repeated renal tubule epithelial cell injury, primarily in the P3 segment of the proximal tubules. Whether or not this unusual regenerative response could lead ultimately to neoplasia, either directly or through exacerbation of CPN, is not clear. Nephrosis was not seen in the α -limonene studies, nor was renal tubule necrosis seen in the outer stripe of the outer medulla (NTP, 1990).

“The mechanism of β -myrcene -induced renal carcinogenesis in male . . . rats is not clear. The observation of α 2u-globulin nephropathy and linear papillary mineralization in male rats suggests this syndrome as one potential mechanism of carcinogenesis. However, several lines of evidence suggest this syndrome as one potential mechanism of carcinogenesis. However, several lines of evidence suggest that β -myrcene might cause nephrotoxicity by a mechanism other than, or in addition to, α 2u-globulin nephropathy. The incidence and severity of linear papillary mineralization were greatest in the 0.25 mg/kg males but slightly decreased in the 0.5 mg/kg males; this response is consistent with the decrease in the incidences of hyaline droplet accumulation seen in the 3-month study. Additionally, there were dose-related increases in the incidence and severity of CPN and nephrosis in both the male and female rats. 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 α 2u-globulin mechanism.”⁶

Although NTP discussed the relationship between the kidney toxicity and kidney tumors observed in the male rats under the conditions of the β -myrcene study, NTP never indicated whether it considered these results to be relevant for purposes of hazard identification. In fact, NTP’s discussion is further evidence that expert judgment would be required to determine the relevance of the male kidney tumors beyond the conditions of the study for purposes of cancer hazard identification. Also, NTP’s

⁶ National Toxicology Program (NTP). 2010. NTP Technical Report on the Toxicology and Carcinogenesis Studies of β -Myrcene (CASRN 123-35-3) in F344/N Rats and B6C3F1 Mice. NTP TR 557. NIH Publication No. National Toxicology Program, Research Triangle Park, NC, p. 62.

mention of the “presence of renal neoplasms in female rats” in the context of exploring the mechanism of action should not be over interpreted. The presence of a few tumors in the female rats was not statistically significant and is not described by NTP as biologically meaningful. There was no statistically significant increase in any renal neoplasms in female rats and NTP concluded the evidence of carcinogenic activity in female rats was “equivocal,” as discussed in the next section. Thus, there is every possibility that the significant increase in kidney tumors in male rats is explained by the α_2 -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 β -myrcene is similar to that of other substances tested in NTP bioassays (e.g., *d*-limonene, pinene) that have been associated with kidney tumors. Although high doses of these substances have been shown to cause kidney tumors in male rats, these findings are widely considered irrelevant to humans because the tumors are unique to the male rat. In fact, *d*-limonene is considered by some researchers to be a potential chemopreventive agent.⁷ IARC classifies *d*-limonene as a Group 3 carcinogen: *not classifiable as to its carcinogenicity to humans*.⁸

⁷ Tsuda, H, Ohshima Y,; Nomoto H, Fujita K, Matsuda E, Iigo M, Takasuka N, Moore M (2004) Cancer Prevention by Natural Compounds. *Drug Metabolism and Pharmacokinetics* 19 (4): 245–63.

⁸ IARC (1999) Volume 73. Some Chemicals that Cause Tumors of the Kidney or Urinary Bladder in Rodents and Some Other Substances.

III. β -Myrcene mouse data should not form the basis for cancer hazard identification

A. The male mouse data demonstrated an increase in liver tumors in B6C3F1 mice, a strain with a high background rate of and high degree of susceptibility to liver tumors

The NTP bioassay of β -myrcene states: “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.”⁹ These statistically significant increases were seen at the mid-dose level of 500 mg/kg/day. At the low dose (250 mg/kg/day), the only statistically significant response was an increase in hepatocellular adenoma, a benign liver tumor.

The high dose level (1000 mg/kg/day), which was not properly selected, produced excessive mortality. There was a statistically significant decrease in survival among high dose males compared to controls, and the cause of deaths was uncertain.¹⁰ As in the case of the high dose male rats, NTP decided not to even present the histopathological data for the high-dose group of male mice due to high early mortality (i.e. acute toxicity).¹¹

The evaluation of the dose-response relationship 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. The background incidence of hepatocellular tumors among the control males in this study was extremely high. For example, the incidence of liver adenomas and carcinomas

⁹ TR-557 at 9.

¹⁰ TR-557 at 50.

¹¹ *Id.*

among the control male mice in the β -myrcene bioassay was 66%. In other words, approximately 2/3 of the control males had liver tumors. In effect, β -myrcene caused an increase in the incidence of liver tumors that most of the mice would have even if they weren't exposed to β -myrcene. This high background rate is a strong indicator that this strain is highly susceptible to liver tumors. Further, mouse liver tumors are of dubious relevance to cancer hazard assessment, as discussed in subsequent sections of this submission.

B. Mouse liver tumors require additional expert analysis in cancer hazard identification because of serious questions concerning sufficiency of such evidence

The predictive value of mouse hepatocellular tumors with respect to cancer risk has been repeatedly challenged.^{12,13} This is in part due to the fact that hepatocellular carcinoma in humans, particularly chemically-induced, is rare. In humans, the major risk factors associated with liver tumors are viral hepatitis, excessive alcohol consumption, and exposure to aflatoxin, in most cases accompanied by liver cirrhosis.

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.¹⁴ Gavage administration, which replicates actual

¹² Velazquez SF, Schoeny R, Rice GE, Cogliano VJ (1996). Cancer risk assessment: historical perspectives, current issues, and future directions. *Drug Chem Toxicol* 19(3):161-185.

¹³ Carmichael NG, Enzmann H, Pate I, Waechter F (1997). The significance of mouse liver tumor formation for carcinogenic risk assessment: results and conclusions from a survey of ten years of testing by the agrochemical industry. *Environ Health Perspect* 105(11):1196-1203.

¹⁴ EFSA (2011). European Food Safety Authority; EFSA Statement on the scientific evaluation of two studies related to the safety of artificial sweeteners (question no EFSA-Q-2011-00064, approved on 25 February 2011 by European Food Safety Authority). *EFSA J* 9(2):2089 [16 pp.]. doi:10.2903/j.efsa.2011.2089. Available at: <http://www.efsa.europa.eu/en/efsajournal/pub/2089.htm>.

exposures even less, also should be considered irrelevant. 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.¹⁵ During these evaluations NICNAS has emphasized that the high natural spontaneous incidence of liver tumors in this strain and sex of mice significantly affects the ability to interpret the results.

Induction of hepatocellular tumors in mice by non-genotoxic compounds can be considered as irrelevant for human risk assessment.^{16,17} In their evaluation of the mode of action with respect to the relevance of rodent liver tumors to human cancer risk, 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. Indeed, clinical use for over 80 years of phenobarbital, a known enzyme inducer in the rodent liver, has not been associated with an increased risk of tumor formation in the liver or any other organ in humans.¹⁸ It is generally well accepted that male and female B6C3F1 mouse liver tumors that arise

¹⁵ Commonwealth of Australia, 2000. National Industrial Chemical Notification and Assessment Scheme (NICNAS), December 2000, Commonwealth of Australia, 134 pp.

¹⁶ Holsapple, M.P., Pitot, H.C., Cohen, S.M., Boobis, A.R., Klaunig, J.E., Pastoor, T., Dellarco, V.L., Dragan, Y.P., 2006. Mode of action in relevance of rodent liver tumors to human cancer risk. *Toxicol. Sci.* 89, 51–56.

¹⁷ Billington R, Lewis R.W, Mehta J.M, Dewhurst I (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.

¹⁸ McClain RM (1990). Mouse liver tumors and microsomal enzyme-inducing drugs: experimental and clinical perspectives with phenobarbital. In: Stevenson DE, Popp JA, Ward JM, McClain RM, Slaga TJ, Pitot HC, editors. *Mouse Liver Carcinogenesis: Mechanisms and Species Comparisons*. Symposium, Nov. 30-Dec. 3, 1988, Austin, Texas. (Progress in Clinical and Biological Research, vol 331). New York (NY): Wiley-Liss, pp. 345-365. Cited In: Carmichael et al., 1997 [Ref. #34].

in 2-year bioassays with various agents are an indirect result of dose-related chronic toxicity and resulting cellular proliferation. In the absence of this chronic toxicity, these tumors are not considered to represent a cancer hazard for humans (Cohen et al., 2004).²⁰

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-190), p-nitrosodiphenylamine caused “positive” findings of liver tumors in male mice and male rats:

“Under the conditions of this bioassay, p-nitrosodiphenylamine was carcinogenic when administered in the diet to male B6C3F1 mice, causing hepatocellular carcinomas. The chemical was also carcinogenic in male Fisher 344 rats, causing liver neoplasms. No evidence was provided for the carcinogenicity of p-nitrosodiphenylamine in female B6C3F1 mice or in female Fisher 344 rats.”²¹

In 1989, NTP identified p-nitrosodiphenylamine as a carcinogen in its Fifth Annual Report on Carcinogens. Subsequently, NTP delisted p-nitrosodiphenylamine for insufficient evidence of carcinogenicity in its Sixth Annual Report on Carcinogens, which was published in 1991. We are currently searching for a copy of the Sixth Annual Report on Carcinogens to further investigate the reason for delisting this substance. But, based on the results of the NTP bioassay, it is clear that the only reason

²⁰ Cohen S.M., Klaunig J., Meek M.E., Hill R.N., Pastoor T., Lehman-McKeeman L., Bucher J., Longfellow D.G., Seed J., Dellarco, V. 2004. Evaluating the human relevance of chemically induced animal tumors. *Toxicol. Sci.* 78: 181–186.

²¹ National Toxicology Program (NTP). 1979. NTP Bioassay of p-nitrosodiphenylamine for possible carcinogenicity. (NTP TR-190). National Toxicology Program, Research Triangle Park, NC.

for initially listing it as a carcinogen was the rodent liver tumors, including the statistically significant increase in hepatocellular carcinoma in male mice. 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.

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open the record until we are able to obtain a copy of the Sixth Annual Report on Carcinogens and evaluate this information.

C. B6C3F1 mouse liver tumors are not a reliable indicator of carcinogenic hazard

Although the NTP Technical Report states there is “clear evidence of carcinogenic activity” in male mice exposed to β -myrcene because of liver tumors, the Report did not address the issue of relevance of these mouse liver tumors to cancer hazard identification for other species. In fact, the high spontaneous incidence of hepatocellular tumors observed in B6C3F1 mice and the relevance of the development of these tumors in mice with regard to human cancer risk has been repeatedly questioned by scientists, including NTP scientists (Maronpot *et al.*, 1987; Velazquez *et al.*, 1996).²² The background incidence of liver tumors has been steadily rising over the past decade in the B6C3F1 mice used by the NTP in its cancer bioassays. Because of their high background rate of and high degree of susceptibility to liver tumors, B6C3F1 mice are not a reliable indicator of carcinogenic hazard for β -myrcene.

The background incidence of liver tumors in the B6C3F1 mice reported in NTP bioassays has historically been high, but in recent years, the background incidence of these tumors has significantly increased over even the historically high background rate. Prior to this recent dramatic change in the background incidence of liver tumors, the historical spontaneous incidence of liver neoplasms (combined hepatocellular adenoma and carcinomas) in control male B6C3F₁ mice in NTP bioassays was 32.4% with a

²² Maronpot RR, Haseman JK, Boorman GA, Eustis SL, Rao GN, Huff JE (1987). Liver lesions in B6C3F1 mice: the National Toxicology Program, experience and position. *Arch Toxicol Suppl* 10:10-26.

range of 20-47%.²³ More recently, rates of combined hepatocellular adenoma and carcinomas in male B6C3F1 control mice exceeding 50% have been reported (e.g., 56% in the isoeugenol study (NTP, 2008), 58% in the pulegone study (NTP, 2011), and an astounding 66% in the β -myrcene study.^{24,25,26} Thus, the incidence of combined hepatocellular adenoma and carcinoma in the control group of male B6C3F1 mice is outside the historical control range published by NTP in 2006, suggesting genetic drift in the mice used in the most recent NTP bioassays, including the bioassay of β -myrcene. The NTP has recognized the limitations of data pertaining to the development of liver tumors in the 2-year mouse bioassays, particularly in susceptible strains of mice (e.g., B6C3F1), with respect to extrapolating the results to other species and has noted that alternative rodent strains are being examined to supplement rat studies.

²³ National Toxicology Program (NTP). 2006. NTP Technical Report on the Toxicology and Carcinogenesis Studies of Benzophenone (CAS NO. 119-61-9) in F344/N Rats and B6C3F1 Mice. NTP TR 533. NIH Publication No. 06-4469. National Toxicology Program, Research Triangle Park, NC. <http://ntp.niehs.nih.gov/>

²⁴ National Toxicology Program (NTP). 2008. Draft Report: NTP Technical Report on the Toxicology and Carcinogenesis Studies of Isoeugenol (CAS NO. 97-54-1) in F344/N Rats and B6C3F1 Mice. NTP TR 551.

²⁵ National Toxicology Program (NTP). 2011. NTP Technical Report on the Toxicology and Carcinogenesis Studies of Pulegone (CASRN 89-82-7) in F344/N Rats and B6C3F1 Mice. NTP TR 563. NIH Publication No. National Toxicology Program, Research Triangle Park, NC.

²⁶ National Toxicology Program (NTP). 2010. NTP Technical Report on the Toxicology and Carcinogenesis Studies of β -Myrcene (CASRN 123-35-3) in F344/N Rats and B6C3F1 Mice. NTP TR 557. NIH Publication No. National Toxicology Program, Research Triangle Park, NC.

IV. β -Myrcene is not genotoxic

β -Myrcene has not shown any evidence of genotoxicity. A battery of genotoxicity studies of β -myrcene was conducted by NTP.²⁷ No mutagenicity was observed in any of several strains of *Salmonella typhimurium* or *E. Coli* in two independent Ames test conducted with and without metabolic activation. In addition, β -myrcene was negative in a micronucleus test in male and female mice administered β -myrcene by gavage for three months.

Interestingly, two publications have reported that β -myrcene protects against known genotoxic substances. Investigators have studied the protective effects of monoterpenes, including β -myrcene, against t-butyl hydroperoxide-induced genotoxicity in reverse mutation assays with two strains of *E. coli* and with the comet assay in human hepatoma and lymphoid cells.²⁸ β -Myrcene had a substantial protective effect against oxidant-induced genotoxicity, which is predominately mediated by its radical scavenging activity. β -Myrcene also inhibited sister chromatid exchanges (SCE) caused by certain mutagens (i.e., cyclophosphamid, benzo[a]pyrene) in a dose-related manner, but it had no effect on SCE produced by aflatoxin B1 and DMBA; β -myrcene also reduced cyclophosphamid-induced SCE frequency in a hepatic tumor cell line.²⁹

²⁷ National Toxicology Program (NTP). 2010. NTP Technical Report on the Toxicology and Carcinogenesis Studies of β -Myrcene (CASRN 123-35-3) in F344/N Rats and B6C3F1 Mice. NTP TR 557. NIH Publication No. National Toxicology Program, Research Triangle Park, NC, p. 9.

²⁸ Miti \ddot{A} , Culafi \ddot{A} D, Zegura B, Nikoli \ddot{A} B, Vukovi \ddot{A} , -Gaci \ddot{A} B, Knezevi \ddot{A} , Vukcevi \ddot{A} J, Filipic M.(2009) Protective effect of linalool, myrcene and eucalyptol against t-butyl hydroperoxide induced genotoxicity in bacteria and cultured human cells. *Food Chem Toxicol* 47(1):260-6. Epub 2008 Nov 18.

²⁹ Rascheisen C, Zamith H, Paumgartten FJ, Speit G (1991) Influence of beta-myrcene on sister-chromatid exchanges induced by mutagens in V79 and HTC cells. *Mutat Res.* 264(1):43-9.

V. β -Myrcene should not be listed because the NTP has not found “sufficient evidence” of carcinogenicity in animals.

OEHHA does not have the authority to list β -myrcene as a carcinogen because the NTP did not “conclude” that β -myrcene “causes cancer” in animals.³⁰ The “primary” Proposition 65 listing mechanism for candidate carcinogens is review by the “state’s qualified experts,” the Carcinogen Identification Committee (“CIC”).³¹ The “authoritative body” listing mechanism is supposed to be a shortcut, allowing listing without CIC review where an authoritative body has already done the work that the CIC would otherwise be required to do.³² As relevant here, that mechanism is triggered only when a chemical has been “formally identified by an authoritative body as causing cancer” in a report which “concludes” that “[s]ufficient evidence of carcinogenicity exists from studies in experimental animals.”³³ To constitute a “sufficient evidence” finding, the authoritative body’s formal “report” must “conclude[]” that “studies in experimental animals indicate that there is an increased incidence of [cancer].”³⁴ OEHHA is not authorized to substantively evaluate the data on β -myrcene and conclude on its own that “sufficient evidence” of carcinogenicity exists. OEHHA’s role is limited by regulation to the “ministerial” task of reviewing the authoritative body’s formal reports and

³⁰ 27 CCR § 25306(a), (d)(1), and (e)(2).

³¹ See Final Statement of Reasons (“FSR”) for 27 CCR § 25306 (then 22 CCR § 12306) at 8.

³² *Id.* at 5, 8.

³³ 27 CCR § 25306(a), (d)(1), (e)(2).

³⁴ 27 CCR § 25306(e)(2).

determining whether the authoritative body has, itself, issued a qualifying sufficient evidence “conclu[sion].”³⁵

NTP has never “conclude[d]” that “sufficient evidence of carcinogenicity exists from studies in experimental animals” within the meaning of section 25306 for β -myrcene. Rather, 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*. Moreover, as noted above, NTP commented that the effect seen was species specific, which further emphasizes the limited nature of the NTP statements and the absence of a “sufficient evidence” finding. NTP stated that “[t]he interpretative conclusions presented in NTP Technical Reports are based only on the results of these NTP studies. Extrapolation of these results to other species requires analyses beyond the intent of these reports.”³⁶ NTP does evaluate chemicals for “sufficient evidence” of carcinogenicity in studies of experimental animals, applying a standard equivalent to section 25306(e)(2), but its current practice is to do so when evaluating chemicals for inclusion in its “Report on Carcinogens.”

The plain language of section 25306 equates “sufficient evidence” with what “studies in experimental animals indicate” generally, and the regulatory history makes clear that this standard was intended to mirror the scientific consensus on sufficient evidence reflected in the language California borrowed directly from the EPA’s 1986 Guidelines for Carcinogen Risk Assessment.³⁷ Those Guidelines require consideration

³⁵ FSR at 8.

³⁶ TR-577 at Foreword.

³⁷ 27 CCR § 25306(e)(2); (FSR at 15 (language drawn from EPA Guidelines).)

of all relevant studies, not just individual studies in isolation. OEMMA’s interpretation would require it to list a chemical on the basis of a single positive study—even if other Technical Reports summarize equally valid, or more valid, data that calls into question the single positive study. NTP almost certainly would not agree in those circumstances that “studies in experimental animals indicate that there is an increased incidence of [cancer],” §25306(e)(2).

To the extent the language leaves any doubt, the regulatory history dispels it. It is undisputed that section 25306(e)’s “causing cancer” definition regarding animal evidence is the well known “sufficient evidence” test taken from the EPA’s 1986 Guidelines for Carcinogen Risk Assessment, with the “same or substantially similar criteria” in use by the NTP, the authoritative body in question.³⁸ The FSR explains the regulation, repeatedly emphasizing that “sufficient evidence” is not a new standard for OEMMA scientists to administer or for industry scientists and observers to understand, but instead a standard already used by authoritative bodies to make their own cancer causing determinations. The FSR states:

Subsection (e) provides that, for purposes of section 12306 [now 25306], the phrase “as causing cancer” means that either of two scientific criteria have been satisfied. Generally, the authoritative body may *rely* on either studies in humans or studies in animals. These criteria are consistent with the criteria the Panel presently uses in evaluating chemicals for listing. The Panel utilizes the EPA’s Classification System for Categorizing Weight of Evidence for Carcinogens From Humans and Animal Studies (51 Fed. Reg. 33999 (Sept. 24, 1986)). The same, or substantially similar criteria have been adopted by many regulatory agencies and scientific organizations involved in hazard identification. The use of these criteria will ensure that *the standards applied by an authoritative body* are the same as or substantially similar to those used by the Panel to evaluate chemicals. (FSR at 15 (emphasis added)).

³⁸ Compare 27 CCR § 25306(e)(2) with 1986 EPA Cancer Guidelines at 33999.

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It is not the intention of the Agency to *substitute its scientific judgment for that of the authoritative body*. The Agency's inquiry will be limited to whether the authoritative body *relied upon scientific data in an amount sufficient to conclude that the chemical causes cancer*. . . . *Because the body is considered authoritative, and the body utilizes the same or substantially the same criteria as set forth in section (e)*, it will be assumed that the data relied upon is scientifically valid. The Agency will look to determine whether the authoritative body relied upon animal or human data in an amount sufficient to satisfy the criteria. If so, the chemical will be proposed for listing.³⁹

These FSR passages make it clear that the California Health and Welfare Agency, which wrote the regulation, expected the sufficient evidence standard would be “applied” by the authoritative body to “conclude that the chemical causes cancer.” These two passages emphasize that the authoritative body is expected to exercise judgment in making the ultimate “causing cancer” conclusion according to substantially the same criteria as set forth in paragraph (e).

The 1986 EPA Guidelines for Carcinogen 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. EPA summarizes its standard as follows: “At various points in the above discussion, EPA has emphasized the need for an overall, balanced judgment of the totality of the available evidence.”⁴⁰ The EPA Guidelines also state that “[r]eplicate negative studies

³⁹ FSR at 18 (emphasis added)).

⁴⁰ 33996 (left column).

that are essentially identical in all other respects to a positive study may indicate that the positive results are spurious.”^{41, 42}

Thus, the EPA cancer risk assessment guidelines, from which section (e)(2) was taken, require that all relevant “studies” be considered as a whole in making a “sufficient evidence” determination, whether based on animal or human data. Section (e)(2) was intended to implement the same standard. The regulation’s copied language and the FSR make this abundantly clear. The NTP has not yet performed that overall analysis for β -myrcene, and thus its Technical Report does not contain a “sufficient evidence” determination required to support an authoritative body listing, or to render the CIC’s consideration of β -myrcene unnecessary.

The NTP did not make a “sufficient evidence” finding with regard to β -myrcene. The Technical Report expresses carcinogenicity conclusions limited to “the conditions of these 2-year feed studies.”⁴³ It does not render an overall conclusion about what “studies in experimental animals indicate, nor does it analyze the implications of the likely species-specific tumors for hazard identification” 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 “analyses beyond the intent” of the report.⁴⁴

⁴¹ 33995 (middle column).

⁴² The EPA Guidelines also state expressly that the classification scheme “is not meant to be applied rigidly or mechanically,” whenever there questionable positive data, but instead provides that “Results and conclusions concerning the agent, derived from different types of information, whether indicating positive or negative responses, are melded together....” *Id.* at 33996 (left column), 33994 (left column).

⁴³ TR-557 at 9.

⁴⁴ TR 577 at Foreword.

The Final Statement of Reasons expressly confirms, twice, that the “sufficient evidence” standard of section 25306(e) is meant to embody the standard that NTP applies when conducting a “reasonably anticipated” analysis for determining whether a chemical should be placed on the Report on Carcinogens:

This [(e)(2)] definition of “sufficient evidence” is also well-established in the scientific community, and several references to this concept are further offered by way of illustration in the bibliography. Under these references, chemicals having sufficient evidence from animal studies have been identified as chemicals ‘reasonably anticipated to be carcinogens’ (NTP) When the evidence from experimental animals concerning the carcinogenicity of a chemical is not sufficient, the NTP list of carcinogens does not include it.⁴⁵

When a chemical is nominated for the Report on Carcinogens, and thus evaluated to see if the evidence of carcinogenicity is “sufficient,” the NTP makes a detailed evaluation, weighing all available information, accepting public comment, and subjecting its conclusions to peer review. First, the NTP “initially evaluates each nomination to determine whether the scientific information available for a nomination justifies its formal review and consideration.” The NTP then announces which nominations are “proposed for review and solicits public comments through announcements in the Federal Register and NTP publications.”⁴⁶ After receiving and responding to public and agency comments on the substances proposed for review, the NTP’s formal evaluation process begins.⁴⁷ As part of that process, NTP scientists prepare additional evaluations, subject those evaluations to multiple rounds of peer

⁴⁵ FSR at 18-19.

⁴⁶ *Id.*

⁴⁷ *Id.*

review (both internal and external), and convene a round of public hearings.⁴⁸ Only then does the NTP reach a preliminary determination about whether a substance satisfies the “sufficient evidence of carcinogenicity” standards required for listing in the Report on Carcinogens.⁴⁹ β -myrcene was not subjected to this comprehensive NTP “sufficient evidence” review process. Importantly for β -myrcene, the Report on Carcinogens is the document in which the NTP analyzes issues such as unique, likely species specific mouse liver tumors and rat kidney tumors

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 animal studies. Instead, it simply could add chemicals to the Report on Carcinogens based on its work in the Technical Report. That is not at all what happens, however.

VI. Conclusion

β -Myrcene should not be listed as a Proposition 65 carcinogen under the authoritative bodies mechanism based solely on the results of a NTP 2-year bioassay in rats and mice. In the rat, clear evidence of carcinogenic activity in the male rat is based on kidney tumors associated with alpha 2u-globulin nephropathy. These tumors are of questionable relevance for cancer hazard identification since they represent a species- and sex-specific response that is unique to certain strains of the male rat. The clear evidence of carcinogenic activity in the male mouse is based on an increased incidence of liver tumors, common to this strain of mice and which are present even in control

⁴⁸ *Id.*

⁴⁹ *Id.*

animals at a high background rate. As in the case of the rat kidney tumors, these male mouse liver tumors are of questionable use in the cancer risk assessment for β -myrcene. Both the mouse and rat data upon which the NTP identified carcinogenic activity under the conditions of NTP's study are of dubious relevance to cancer hazard identification and this study alone does not provide sufficient proof of carcinogenic activity. OEHHA should either elect not to proceed with β -myrcene or a full review of all relevant information available for β -myrcene, including the negative genotoxicity results and protective effects, should be conducted by referring this chemical to the Carcinogen Identification Committee for analysis or special input.