β-Myrcene

May Not be Listed as a Proposition 65 Carcinogen

Pursuant to the Authoritative Bodies Listing Process

Comments of

Flavor and Extract Manufacturers Association of the United States

Consumer Healthcare Products Association

International Fragrance Association, North America

Juice Products Association

Personal Care Products Council

and

Renewable Citrus Products Association

Submitted to the
Office of Environmental Health Hazard Assessment

by

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TABLE OF CONTENTS

Page

I. Introduction 1

II. β–Myrcene should not be listed because the NTP has not found “sufficient evidence” of carcinogenicity in animals. 2

A. OEHHA cannot rely on the Summary section cited in support of the proposed listing 4

1. The Summary section statement that β-myrcene "caused kidney cancers" is patently erroneous 4

2. The Summary section statement that "liver tumors in female rats may have been related to β-myrcene" is plainly wrong 7

3. The Summary was not peer reviewed, nor does it have any of the other indicia of reliability cited in section 25306(d)(2). 8

4. Other Technical Report Summary sections also contain troubling errors 9

5. The Summary section must receive no weight. 11

B. None of the NTP statements OEHHA cites reflect a "sufficient evidence" conclusion by NTP and thus OEHHA is not authorized to list β-myrcene through the authoritative bodies listing process. 11

1. NTP did not formally identify β-myrcene "as causing cancer" because it did not express any opinion about whether "sufficient evidence of carcinogenicity exists" from "studies" (plural) in "experimental animals" (plural). 12

2. NTP's "clear evidence" conclusion regarding rat kidney "neoplasms" may not have depended upon a cancer finding. 17

C. OEHHA's assertions of compliance with section 25306 are unpersuasive. 19

1. Two separate, limited conclusions about male rats and male mice are not a "sufficient evidence" conclusion that can support an authoritative body listing. 19

2. The human relevance of mouse liver tumors is suspect, so these tumors cannot per se be the basis for OEHHA to conclude that clear evidence of mouse liver tumors supports a sufficient evidence of carcinogenicity finding. 25

3. The human relevance of male rat kidney tumors is suspect, so these tumors cannot per se be the basis for OEHHA to conclude that clear evidence of male rat kidney tumors supports a sufficient evidence of carcinogenicity finding. 28

III. Conclusion 30

I. Introduction

OEHHA's Notice of Intent to List β-myrcene as a carcinogen pursuant to the authoritative bodies listing process is not consistent with OEHHA's obligations under Proposition 65 and should be withdrawn.[1] OEHHA claims that the National Toxicology Program (NTP), through the publication of its final report of studies that it sponsored, has found that "sufficient evidence of carcinogenicity exists" in experimental animals for β-myrcene. OEHHA's conclusion is not supported by the facts and the statements it cites.

The NTP Technical Report at issue (NTP TR-557 or "Report") does not make a "sufficient evidence" finding concerning animal carcinogenicity, and OEHHA cannot analyze the data to make its own "sufficient evidence" finding in support of a listing. Accordingly, the Flavor and Extract Manufacturers Association of the United States, the Consumer Healthcare Products Association, the International Fragrance Association, North America, the Juice Products Association, the Personal Care Products Council, and the Renewable Citrus Products Association (the Associations) oppose the listing of β-myrcene and respectfully request that OEHHA withdraw its February 7, 2014, Notice of Intent to List β-myrcene ("Notice") through the authoritative bodies process. OEHHA's intent to list β-myrcene based on the cited Technical Report is unauthorized, illegal, and does not represent a sound scientific approach.

[1]All references to "OEHHA" are to the California Office of Environmental Health Hazard Assessment. All references to "NTP" are to the United States National Toxicology Program. All references to "Proposition 65" are to California Health and Safety Code §§ 25249.5 - 25249.13. OEHHA refers to β-myrcene as "beta-Myrcene," CAS No. 123-35-3. All references to "CCR" are to the California Code of Regulations.

II. β–Myrcene should not be listed because the NTP has not found "sufficient evidence" of carcinogenicity in animals.

OEHHA lacks authority to list β-myrcene as a carcinogen because the NTP did not "conclude" that β-myrcene "causes cancer" in animals.[2] The "primary" Proposition 65 listing mechanism for candidate carcinogens is review by the "state's qualified experts," the Carcinogen Identification Committee ("CIC").[3] The "authoritative body" listing mechanism is intended to be an abbreviated listing process, allowing listing without CIC review where an authoritative body has already done the work that the CIC would otherwise be required to do[4] and definitely concluded that "sufficient evidence of carcinogenicity exists" in experimental animals.[5]

The authoritative bodies listing mechanism may be employed by OEHHA 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."[6] 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" malignant or combined tumors under certain circumstances.[7] 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

[2] 27 CCR § 25306(a), (d)(1), and (e)(2).
[3] See Final Statement of Reasons ("FSR") for 27 CCR § 25306 (then 22 CCR § 12306) at 8.
[4] *Id*. at 5, 8.
[5] 27 CCR § 25306(a), (d)(1), (e)(2).
[6] *Id.*
[7] 27 CCR § 25306(e)(2).

authoritative body's formal reports and determining whether the authoritative body has, itself, issued a qualifying sufficient evidence "conclu[sion]."[8]

The record on β-myrcene demonstrates that NTP has never "conclude[d]" that "sufficient evidence of carcinogenicity exists from studies in experimental animals" within the meaning of section 25306. OEHHA has identified three NTP statements that OEHHA claims represent a "sufficient evidence" conclusion within the meaning of section 25306. None of these three statements, however, satisfy the section 25306 requirement that NTP, rather than OEHHA, articulate a "sufficient evidence" conclusion in order to support an authoritative body conclusion.

First, OEHHA noted that the Summary section of the Report states:

> "We conclude that β-myrcene caused kidney cancers in male rats and liver cancer in male mice . . . ."[9]

Second, OEHHA noted that NTP states the following in the Conclusion section of the Report's Abstract :

> "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."[10]

Third, OEHHA noted that the body of the Report 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."[11]

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[8]FSR at 8.
[9]NTP TR-557 at 5.
[10]Notice, quoting TR-557 at 9 (emphasis in original) (mistakenly described by OEHHA as page 8).
[11]*Id*., quoting TR-557 at 63 (emphasis in original).

These statements do not demonstrate that NTP “concluded” β-myrcene “causes” cancer in animals for purposes of the authoritative bodies listing process because: (1) the clause in the Summary is inaccurate and not scientifically reliable, (2) none of the three passages cited by OEHHA reflect the necessary overall evaluation of animal carcinogenicity that is the bare minimum for a legally adequate "sufficient evidence" conclusion, and (3) the overall context of the NTP Report, reporting on discrete studies it sponsored rather than assessing the larger picture of what “studies in experimental animals indicate” does not support a listing.

A. OEHHA cannot rely on the Summary section cited in support of the proposed listing

The sentence in the Summary section cited by OEHHA contains significant factual errors that render it wholly unreliable. Furthermore, other NTP Technical Report Summary sections also contain troubling errors, thereby confirming the unreliability of the Summary section. Interestingly, OEHHA's February 10, 2012 "Request for Relevant Information" on β-myrcene did not rely on the statement in the Summary section.[12] OEHHA may not do so now.

1. The Summary section statement that β-myrcene "caused kidney cancers" is patently erroneous

The Summary section incorrectly states that β-myrcene “caused kidney cancers in male rats." β-Myrcene caused a statistically significant increase in *benign* kidney tumors, not malignant kidney tumors (i.e., kidney cancer) in male F344/N rats. OEHHA does not have the authority to list a chemical through the authoritative bodies process

[12]OEHHA, "Request for Relevant Information on Chemicals Being Considered for Listing by the Authoritative Bodies Mechanism: Isopyrazam, Beta-myrcene, Pulegone, and 3,3',4,4'-Tetrachloroazobenzene," February 10, 2012.

based on benign tumors. Recognizing this, OEHHA normally does not list a chemical as a carcinogen unless it has sufficient evidence of increases in malignant tumors.

Contrary to the Summary section cited by OEHHA, the relevant sentences in the Abstract and Conclusions sections (also cited by OEHHA) both state: "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***."[13] The difference between the Summary statement and the other two statements is significant because "kidney cancers" and "renal tubule neoplasms" are very different. The term "kidney cancers" describes malignant tumors of the kidney. The term "renal tubule neoplasms" includes both benign tumors and malignant tumors (cancer) of the kidney.

Describing the β-myrcene data as reflecting that the chemical *causes* kidney *cancers* in male rats is clearly erroneous. Among the male rats exposed to β-myrcene, the incidences of benign kidney tumors were statistically significantly increased. In contrast, the incidences of malignant kidney tumors (i.e., kidney cancers) were very low, did not reflect any dose response, and certainly were not statistically significantly increased at any dose level. The incidence of combined benign and malignant kidney tumors was statistically significantly increased, but this increase is due to the increase in benign, not malignant, kidney tumors. The incidence of renal tubule carcinoma among male rats was 0/50, 3/50, 1/50, and 0/50 at 0, 250, 500 and 1000 mg/kg of β-myrcene,

[13]NTP TR-557 at 9, 63 (bold emphasis added).

respectively.[14] Thus, the Summary is patently inaccurate and does not reflect either the data or the conclusions in the Report.

Not only did the Summary section incorrectly describe benign tumors as cancer, the Summary also asserted that β-myrcene caused more than one type of cancer -- "cancers." The use of the plural in this sentence also was clearly mistaken.[15] As noted earlier, β-myrcene did not cause a statistically significant increase in any type of malignant kidney tumor. The Report described three different types of malignant tumors appearing in the kidney of the male rats: "renal tubule carcinoma," "kidney, mesenchymal tumor malignant", and "kidney, transitional epithelium, carcinoma."[16] The incidence of "kidney, mesenchymal tumor malignant" among male rats was 0/50, 1/50, 0/50 and 0/50 at 0, 250, 500 and 1000 mg/kg of β-myrcene, respectively.[17] The incidence of "kidney, transitional epithelium, carcinoma" among male rats was 1/50, 0/50, 0/50 and 0/50 at 0, 250, 500 and 1000 mg/kg of β-myrcene, respectively.[18] The incidence of renal tubule carcinoma is described above. These data clearly refute the Summary section statement that "β-myrcene caused kidney cancers in male rats."[19] Not surprisingly, therefore, NTP did not conclude that "β-myrcene caused kidney

[14] *Id.* at Table A-1. NTP did not include (appropriately) the results at 1000 mg/kg in its statistical evaluation of the tumor data because the Maximum Tolerated Dose (MTD) was clearly exceeded. However, the results at all four dose levels are presented in this paragraph and throughout this submission for the sake of completeness, as well as to illustrate the difficulty in interpreting the tumor dose-response relationship when the MTD has been exceeded.

[15] The Summary also mistakenly uses the singular for liver cancers in male mice when two types of liver tumors were significantly increased in male mice exposed to β-myrcene. Again, this series of mistakes calls into question the competence of the author of the Summary section and his or her familiarity with the results of the study.

[16] NTP TR-557, Table A-1.

[17] *Id.*

[18] *Id.*

[19] *Id.* at 5.

cancers in male rats" in the relevant conclusion sections, nor was any such conclusion discussed in any public peer review process.

*2. **The Summary section statement that "liver tumors in female rats may have been related to β-myrcene" is plainly wrong***

The full sentence in the Summary section from which OEHHA drew the clause discussed above contains yet another error. The complete sentence states:

> "We conclude that β-myrcene caused kidney cancers in male rats and liver cancer in male mice, and the occurrence of kidney tumors in female rats and liver tumors in female ***rats*** may have been related to β-myrcene administration."[20]

This sentence wrongly states that "liver tumors in female **rats** may have been related to β-myrcene administration."[21] In contrast, the substantive conclusion regarding female rats expressed in the conclusion sections of the Abstract and the overall Report states:

> "There was *equivocal evidence of carcinogenic activity* of β-myrcene in female F344/N rats based on increased incidences of renal tubule adenoma."[22]

Thus, the conclusion in the Abstract and Conclusions sections does not mention an increase in liver tumors in female rats. Further, the actual study results do not support a conclusion that liver tumors in female rats may have been related to β-myrcene administration. The incidences of hepatocellular carcinoma among female rats were 0/50, 1/50, 0/50, and 0/50 at 0, 250, 500 and 1000 mg/kg, respectively. Similarly, the incidences of hepatocellular adenoma among female rats were 0/50, 0/50, 0/50, and 1/50 at 0, 250, 500 and 1000 mg/kg, respectively.

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[20] *Id.* at 5 (bold emphasis added).
[21] *Id.*
[22] *Id*. at 9, 63 (emphasis in original).

In contrast to the erroneous sentence in the Summary, in the Abstract and Conclusions sections of the report, NTP states:

> "There was *equivocal evidence of carcinogenic activity* of β-myrcene in female B6C3F1 **mice** on marginally increased incidences of hepatocellular adenoma and carcinoma."[23]

Discussing only **rats**, the Summary makes no mention of an increase in liver tumors among female **mice**. The incidences of hepatocellular carcinoma among female mice were 1/50, 7/50, 2/50, and 1/50, at 0, 250, 500 and 1000 mg/kg, respectively. Similarly, the incidences of hepatocellular adenoma among female mice were 6/50, 13/50, 6/50, and 7/50 at 0, 250, 500 and 1000 mg/kg, respectively.

The sentence in the Summary section falsely reported that "liver tumors in female rats may have been related to β-myrcene" when, in fact, the marginally increased incidences of liver tumors was considered "equivocal evidence of carcinogenic activity" in female ***mice,*** not female ***rats***. An error of this magnitude discredits all of the statements in the Summary not corroborated elsewhere in the Report. It appears that the person who wrote the Summary was not qualified to do so.

3. ***The Summary was not peer reviewed, nor does it have any of the other indicia of reliability cited in section 25306(d)(2).***

The Summary section of the Report, including the clause OEHHA cites in its Notice, did not receive *any* external peer-review, whether by the NTP Board of Scientific Counselors (BSC) or otherwise. Moreover, it does not appear that the Summary section received any internal peer review either. "We conclude that β-myrcene caused kidney cancers in male rats and liver cancer in male mice …" does not appear

[23] *Id.* at 9 and 63 (bold emphasis added).

anywhere in the draft Technical Report reviewed by the public and the BSC Technical Reports Review Subcommittee. The BSC peer reviewed the draft Technical Report at its meeting on February 25, 2009. We attach a copy of the draft Technical Report reviewed by the public and the BSC at its February 25, 2009, meeting. Indeed, the draft Technical Report did not contain a Summary section at all. The language of the Summary section that differs from the conclusions in the Abstract and Conclusions sections does not reflect the recommendations of the BSC Subcommittee or the public.[24] Moreover, the Summary section is not published in a publication such as the Federal Register, signed, set forth in an official document used for regulatory purposes, or adopted as a final rule. Thus, none of the indicia of reliability discussed in section 25306(d)(2) apply to the Summary section.

*4. **Other Technical Report Summary sections also contain troubling errors***

The β-myrcene Technical Report is not the only example of a significant inconsistency between the conclusions in the Summary and those in the main body of a NTP Technical Report. Other recent NTP Technical Reports contain similar inconsistencies between the conclusions in the Summary section and the conclusions in the Abstract and Conclusions sections. For example, the Summary section of the Technical Report (TR-538) for methyl isobutyl ketone (MIBK) contains a sentence similar to the sentence that appears in the Summary section of the Technical Report on β-myrcene:

[24] *See* TR-557 at 14-15.

> "We conclude that methyl isobutyl ketone caused cancer of the kidney in male rats and of the liver in male and female mice."[25]

Yet, there was no "clear evidence of carcinogenic activity" in male rats, female rats, male mice or female mice in the conclusions in the Abstract and Conclusions sections of the Technical Report:

> "Under the conditions of these 2-year studies, there was *some evidence of carcinogenic activity** of methyl isobutyl ketone in male F344/N rats based on increased incidences of renal tubule neoplasms. Increased incidences of mononuclear cell leukemia in 1,800 ppm male F344/N rats may have been related to methyl isobutyl ketone exposure. There was *equivocal evidence of carcinogenic activity* of methyl isobutyl ketone in female F344/N rats based on the occurrence of renal mesenchymal tumors in the 1,800 ppm group. There was *some evidence of carcinogenic activity* of methyl isobutyl ketone in male and female $B6C3F_1$ mice based on increased incidences of liver neoplasms."[26]

Thus, the sentence in the Summary section of the Technical Report concludes that MIBK "caused cancer" in animals based on no more than "some evidence of carcinogenic activity." If MIBK had been listed based upon the sentence in the Summary section of the NTP Technical Report, MIBK would have been listed with no "clear evidence of carcinogenic activity." Historically, for a NTP Technical Report, OEHHA has taken the position that "clear evidence of carcinogenic activity" is necessary to list a chemical under the authoritative bodies regulations. This is further evidence that OEHHA should not rely on a conflicting and contrary sentence in the Summary section of an NTP Technical Report for purposes of an authoritative bodies listing.

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[25] NTP TR-538, "Toxicology and Carcinogenesis Studies of Methyl Isobutyl Ketone, (February 2007) at 5.
[26] *Id.* at 8, 46 (emphasis in original).

*5. **The Summary section must receive no weight.***

In determining whether the section 25306 criteria have been met, OEHHA may not “cherry pick” an inaccurate clause from the Summary section of the β-myrcene Report, especially when the clause appears in a sentence riddled with errors and not characterized by any indicia of reliability.

OEHHA therefore should place no weight on the statement in the Summary section of the Report. Alternatively, OEHHA should pause the authoritative body listing process for β-myrcene in order to confer with the Proposition 65 Carcinogen Identification Committee (CIC) concerning whether the Summary section of a NTP Technical Report should be considered “authoritative.”

OEHHA notes that, in 1998, the Carcinogen Identification Committee (CIC) considered and affirmatively retained NTP Technical Reports as “authoritative”. However, it is difficult to imagine that the CIC would be comfortable with a situation where an inaccurate sentence in the Summary section of the Technical Report, which is in conflict with the main conclusions in the Abstract and Conclusions sections of the Technical Report, and which was not the subject of scientific peer review, is used as part or all of the basis for an authoritative bodies listing.

B. None of the NTP statements OEHHA cites reflect a "sufficient evidence" conclusion by NTP and thus OEHHA is not authorized to list β-myrcene through the authoritative bodies listing process.

NTP never "conclude[d]" that "sufficient evidence of carcinogenicity exists from studies in experimental animals" within the meaning of section 25306 for β-myrcene. The inaccurate and unreliable Summary section is not a sufficient evidence conclusion. Similarly, the two separate and limited conclusions OEHHA cites about carcinogenic

activity in one strain of mice and one strain of rats *under the conditions of NTP's experiment* are not “sufficient evidence” conclusions either.

These two sentences fail to support listing under the authoritative bodies mechanism for two independent reasons. First, NTP made no overall conclusion that "sufficient evidence of carcinogenicity exists" from "studies" in "experimental animals." Second, the sentence concerning the rat data is ambiguous because one cannot determine how much or how little the carcinomas, as opposed to the adenomas, contributed to the NTP's "clear evidence" conclusion.

1. ***NTP did not formally identify β-myrcene "as causing cancer" because it did not express any opinion about whether "sufficient evidence of carcinogenicity exists" from "studies" (plural) in "experimental animals" (plural).***

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.[27] Those Guidelines require consideration of *all* relevant studies, not just individual studies in isolation. OEHHA’s interpretation would require it to list a chemical based on a single positive study—even if other Technical Reports summarize equally valid, or more valid, data that undermines 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],” 27 CCR § 25306(e)(2).

[27] 27 CCR § 25306(e)(2); Final Statement of Reasons (FSR) for Section 12306, February 1990 at 15 (language drawn from EPA Guidelines) (Section 12306 of title 22 was moved without substantive change to 27 CCR § 25306).

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" used by the NTP, the authoritative body in question.[28] The FSR explains the regulation, repeatedly emphasizing that "sufficient evidence" is not a novel standard for OEHHA scientists to administer or for industry scientists and observers to understand, but instead an existing standard authoritative bodies already employed 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. 33992-34003 (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)).
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> * * * *
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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

[28] *Compare* 27 CCR § 25306(e)(2) *with* 1986 EPA Cancer Guidelines, 51 Fed. Reg. 33992-34003 (Sept. 24, 1986) at 33999.

> 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.[29]

These FSR passages confirm 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 (not OEHHA) is expected to exercise judgment in making the ultimate "causing cancer" conclusion using 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."[30] The EPA Guidelines also state that "[r]eplicate negative studies that are essentially identical in all other respects to a positive study may indicate that the positive results are spurious."[31, 32]

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

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[29] FSR at 18 (emphasis added).
[30] 51 Fed. Reg. 33992-34003 (September 24,1986).
[31] *Id.* at 33995.
[32] 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).

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."[33] 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.[34] Moreover, NTP made clear in the Report 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." NTP does evaluate chemicals for "sufficient evidence" of carcinogenicity in experimental animals in the Technical Reports, 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."

[33] TR-557 at 9.
[34] 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.[35]

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."[36] After receiving and responding to public and agency comments on the substances proposed for review, the NTP's formal evaluation process begins.[37] As part of that process, NTP scientists prepare additional evaluations, subject those evaluations to multiple rounds of peer

[35] FSR at 18-19.
[36] *Id.*
[37] *Id*.

review (both internal and external), and convene a round of public hearings.[38] 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.[39] 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” were 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 as part of its Report on Carcinogens process. 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.

2. ***NTP's "clear evidence" conclusion regarding rat kidney "neoplasms" may not have depended upon a cancer finding.***

Proposition 65 requires that a chemical be demonstrated to "cause cancer," not just benign tumors, before listing. The data indicates that the male rat "clear evidence" statement by NTP may not have been materially influenced by the very few malignant tumors observed in the study. These tumors were not statistically significant at any dose or by trend, did not exhibit dose-response, and were not noted by NTP as being biologically important. Indeed, NTP's male rat "clear evidence" finding might have been the same even if no malignant tumors were present. One can reasonably infer from the NTP conclusion, which does not mention malignancy at all (the “clear evidence of

[38] *Id.*
[39] *Id.*

carcinogenic activity" in male rats was "based on increased incidences of renal tubule neoplasms"), that the "clear evidence" determination may have been the same if there were no malignant tumors present.[40] NTP's criteria specifically permit this:

> "Clear evidence of carcinogenic activity is demonstrated by studies that are interpreted as showing a dose-related . . . (iii) marked increase of benign neoplasms if there is an *indication* from this or other studies of the ability of such tumors to progress to malignancy."[41]

Confirming this, "clear evidence" conclusions in NTP Technical Reports have been based on increased incidences of renal tubule adenomas alone. For example, in its evaluation of tris(2-chloroethyl)phosphate in TR-391, NTP stated: "Under the conditions of these 2-year gavage studies, there was *clear evidence of carcinogenic activity* for male and female F344/N rats receiving tris(2-chloroethyl)phosphate as shown by increased incidences of renal tubule adenomas."[42]

Since NTP's conclusion may well have been the same even if no malignancies were observed, NTP's "clear evidence" statement on male rat liver tumors cannot contribute to a Proposition 65 "authoritative bodies" listing.

We recognize that NTP conducted statistical analyses on the incidences of benign (only), malignant (only), and combined malignant and benign renal tubule tumors among both male and female rats. A statistically significant increase in benign renal tubule tumors (but not malignant renal tubule tumors) was observed in male rats exposed to β-myrcene. The incidence of combined malignant and benign renal tubule tumors was also increased among male rats, but this was accounted for by the

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[40] *See*, e.g., NTP TR-391.
[41] NTP TR-557 at 12.
[42] NTP TR-391 at 6, 54. Of note, unlike some other NTP Technical Reports, NTP TR-391 did not contain a "Summary" section.

statistically significant increase in benign tumors. If there had been no carcinomas among the male rats, there would have still been a marked, statistically significant increase in combined malignant and benign renal tubule tumors.

C. OEHHA's assertions of compliance with section 25306 are unpersuasive.

Our prior comments, submitted in April 2012, expressed essentially the same substantive concerns presented above in section II.B.1.[43] OEHHA's response to those comments is unpersuasive for the reasons set forth below.

*1. **Two separate, limited conclusions about male rats and male mice are not a "sufficient evidence" conclusion that can support an authoritative body listing.***

Our April 2012 comments note that section 25306 requires that the authoritative body, the NTP, make the "sufficient evidence" finding, not OEHHA. OEHHA asserts that the Technical Report "concludes that the chemical causes cancer," but OEHHA's basis for this assertion is inadequate. Section 25306(a) states, in relevant part, that:

> (a) Pursuant to section 25249.8(b) of the Act, a chemical is known to the state to cause cancer . . . if the lead agency determines that an authoritative body has formally identified the chemical *as causing cancer* . . . as specified in this section. (*emphasis* added)

Section 25306(e)(2) defines what "as causing cancer" means for this section. In relevant part:

> "as causing cancer" means that . . . 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

[43]"β-Myrcene Should Not be Listed as a Proposition 65 Carcinogen Pursuant to the Authoritative Bodies Listing Process," Flavor and Extract Manufacturers Association and others, April 2012. These comments (hereafter, Associations' April 2012 Comments) are fully incorporated herein by reference.

> increased incidence of malignant tumors or combined malignant and benign tumors in multiple species or strains, in multiple experiments . . . , or, to an unusual degree, in a single experiment with regard to high incidence, site or type of tumor, or age at onset.

NTP must make the overall assessment of what "studies in experimental animals indicate," and NTP has not done this.

OEHHA does not address this point head on. Instead, it simply repeats the inadequate statement that "clear evidence" was found when certain rat data was examined in isolation and when other mouse data was examined in isolation.[44] OEHHA admits "that the NTP Technical Report on β-myrcene expressed separate conclusions about four individual experiments"; thus recognizing that NTP did not make an overall assessment of the sufficiency of the evidence. Rather than responding to the legal deficiency of this situation, OEHHA instead notes that the Carcinogen Identification Committee (CIC) in 1998 declined a request to limit the authoritative status of NTP to what it says in the Report on Carcinogens. The CIC in 1998, however, did not directly confront the issue of whether NTP makes an overall "sufficient evidence" finding which would authorize OEHHA to conclude, pursuant to section 25306(a), that the NTP has identified β-myrcene "as causing cancer." The CIC has not made any finding that the Technical Reports make a "sufficient evidence" conclusion as is required.

In response to our earlier comments, OEHHA interpreted the cautionary statement in the Forward about not extrapolating the NTP Technical Report findings to

[44]OEHHA, "Response to Comments Pertaining to the Request for Relevant Information on β-myrcene as Causing Cancer under Proposition 65," February 2014, pp. 2-3 (hereafter "OEHHA Response to Comments").

other species and another sentence in the Explanation of Levels of Evidence of Carcinogenic Activity on page 12 of the Technical Report as follows:

> 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. 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.[45]

We respectfully disagree with OEHHA's interpretation of NTP's statement in the Forward to the Technical Report. First, OEHHA has cited sentences from two sections of the NTP Technical Report out of context. The full paragraph in the Forward states:

> The Technical Report series began in 1976 with carcinogenesis studies conducted by the National Cancer Institute. In 1981, this bioassay program was transferred to the NTP. 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). Substances selected for NTP toxicity and *carcinogenicity* studies are chosen primarily on the basis of human exposure, level of production, and chemical structure. The interpretive conclusions presented in NTP Technical Reports are based only on the results of these NTP studies. Extrapolation of these results to other species, including characterization of hazards and risks to humans, requires analyses beyond the intent of these reports. Selection *per se* is not an indicator of a substance's carcinogenic potential.[46]

[45]OEHHA Response to Comments at 5.
[46]NTP TR-557 at Foreword.

Thus, NTP is explaining that the conclusions in these reports are based only on the results of the NTP studies and that extrapolation of these results to human hazard identification and risk assessment is beyond the intent of the Technical reports. It is noteworthy that NTP does not limit its statement to *risk assessment*. It states "***hazards*** and risks to humans." [emphasis added] NTP clearly communicates that both human hazard identification and human risk assessment are beyond the scope of these reports. Nothing in the levels of evidence passage noted by OEHHA is inconsistent with NTP's clear statement that the assessment of "hazards to humans" is beyond the scope of the Technical Report. Saying that certain data "indicate that exposure to the chemical has the potential for hazard to humans" is consistent with saying that when one undertakes a hazard identification assessment, these data will be relevant but not necessarily sufficient. "Potential for hazard" does not a "known carcinogen" make. Proposition 65 cases have consistently said that chemicals placed on the list must be known carcinogens, not suspected ones. "Although we concluded in *Deukmejian* that both human and animal carcinogens must be included on the Proposition 65 list, we cautioned that the standard remains *known* carcinogens." *Styrene Information and Research Center v. OEHHA*, 210 Cal.App.4th 1082, 1094 (emphasis in original) (2012) (referring to *AFL-CIO v. Deukmejian*, 212 Cal.App.3d 425 (1989)).

Moreover, OEHHA's reliance on the "potential for hazard to humans" is not consistent with OEHHA's actions or common sense. The "potential for hazard to humans" statement applies equally to "clear evidence" and "some evidence" chemicals, and not even OEHHA has suggested, ever, that a "some evidence" call by NTP is a proper basis for an authoritative body listing.

Proposition 65 is designed to protect humans, not rats and mice, from cancer hazards. It is appropriate that animal studies are allowed to form the basis for certain listings under Proposition 65 because certain animal studies (and increases in certain tumors) have the ability to predict a cancer hazard to humans. However, NTP (and the scientific community) recognize that not every chemical that causes cancer or tumors in mice or rats poses a "sufficient" cancer hazard to humans to constitute a "known carcinogen" for purposes of Proposition 65. It is well understood that certain chemicals cause cancer in laboratory animals by mechanisms not considered relevant to humans. NTP indicates that it is not considering all the available information, including existing data on the relevance of specific animal tumors to human hazard identification, in its Technical Reports. NTP is simply reporting the results of its animal study "under the conditions of the study," nothing more. To evaluate whether a chemical is a "known carcinogen," one must consider what is known about the human relevance of specific tumors increased in animals, as well as other factors.

Distinguishing between chemicals that pose a carcinogenic hazard to humans and those that don't is beyond the scope of the NTP Technical Reports.[47]

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[47] It is illustrative to consider what would happen if NTP had conducted a 2-year carcinogenicity study of the artificial sweetener, saccharin. Numerous studies in rats have shown an increased incidence of urinary bladder cancer at high doses of saccharin, particularly in male rats. If NTP had conducted such a study, the most likely outcome would be "clear evidence of carcinogenic activity" in male and female rats due to increased incidences of urinary bladder cancer. In identifying the level of "carcinogenic activity" in its Technical Report of saccharin, NTP would not have considered the questionable relevance of these tumors because "characterization of hazards and risks to humans, requires analyses beyond the intent of these reports." However, according to the National Cancer Institute, mechanistic studies have shown that these results with saccharin apply only to rats. (National Cancer Institute. Fact Sheet. Artificial Sweeteners and Cancer. http://www.cancer.gov/cancertopics/factsheet/Risk/artificial-sweeteners). Human epidemiology studies have shown no consistent evidence that saccharin is associated with bladder cancer incidence in humans. Because the bladder tumors seen in rats are due to a mechanism not relevant to humans and because there is no clear evidence that saccharin causes cancer in humans, saccharin was removed in 2000 from the U.S. National Toxicology Program's *Report on Carcinogens*, where it had been listed since 1981 as a substance "reasonably anticipated to be a human carcinogen." Yet, by OEHHA's interpretation of statements in the NTP Technical Report, if saccharin had been the

OEHHA has dismissed the difference between NTP Technical Reports and the NTP Report on Carcinogens (RoC) as "as separate and distinct" activities. This is an over-simplification that does not recognize the differences between the two activities. The RoC considers the weight of the scientific evidence, including studies not conducted by NTP, and the human relevance of findings in animal studies, whereas the Technical Report does not.

Although it clearly has the authority to do so, and has done so with respect to many other chemicals, NTP has not even nominated β-myrcene for evaluation as a substance "reasonably anticipated to be a human carcinogen" in its RoC. Yet, Congress mandates that NTP list in its RoC "all substances (1) which either are known to be human carcinogens or may reasonably be anticipated to be human carcinogens and (2) to which a significant number of persons residing in the United States are exposed." Clearly, a significant number of persons residing in the United States are exposed to β-myrcene. Presumably, β-myrcene was not nominated for inclusion in the RoC because NTP neither assumes nor concludes that the "clear evidence of carcinogenic activity" in its Technical Report on β-myrcene indicates a cancer hazard to humans. In other words, NTP does not consider every substance found to cause cancer in laboratory animals in the NTP Technical Reports as "reasonably anticipated to be a human carcinogen." While the RoC may be a "separate and distinct" activity, it sheds light on NTP's evaluation of β-myrcene. If NTP had *actually* identified β-myrcene as causing cancer in the Technical Report, it would have been obligated to nominate if

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subject of a NTP bioassay, the Technical Report would have triggered an authoritative bodies listing of saccharin because the study would have provided "clear evidence of carcinogenic activity" in rats, even though it poses no cancer hazard to humans.

for and include it in the RoC. The absence of β-myrcene in the RoC indicates that NTP did not identify β-myrcene "as causing cancer."

OEHHA further argues at page 6 of its 2014 Response to Comments that it is sufficient for OEHHA to stitch together the separate and limited findings that NTP made in its Technical Report and call that a "sufficient evidence" finding that satisfies section 25306. This assertion defies standard rules of statutory construction, does not address the interpretive points made above, and fails to explain the Final Statement of Reasons statements that NTP must make the sufficient evidence finding. This constitutes OEHHA asserting it is authorized to make the ultimate "sufficient evidence" determination supporting an authoritative body listing. OEHHA does not have that authority.

2. ***The human relevance of mouse liver tumors is suspect, so these tumors cannot per se be the basis for OEHHA to conclude that clear evidence of mouse liver tumors supports a sufficient evidence of carcinogenicity finding.***

Our 2012 comments on β-myrcene spent six pages explaining that the human relevance of mouse liver tumors has been seriously questioned, particularly when the chemical is not genotoxic (as is the case for β-myrcene).[48] We also noted that such tumors, if relevant at all, require additional expert analysis before any "sufficient evidence" finding or statement about hazard identification can be made. Among other things, we noted that a significant body of scientific literature has criticized the relevance of mouse liver tumors for cancer hazard identification, and the European Food Safety

[48]Associations' April 2012 Comments at 8-13.

Authority and Commonwealth of Australia have taken a consistent approach to considering such liver tumors not relevant to humans.[49]

OEHHA's response concerning mouse liver tumors can be summarized as follows:

> 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. . . . [¶] 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 . . . under Proposition 65. . . . [¶] . . . Neither the European Food Safety Authority nor the National Industrial Chemical *Notification* Assessment Scheme of Australia are considered "authoritative" under Proposition 65.[50]

OEHHA's response is not adequate to establish that clear evidence of mouse liver tumors for a non-genotoxic chemical is *per se* adequate to establish one positive experiment or one positive strain for purposes of a "sufficient evidence of carcinogenicity" determination *in the view of NTP*. First, OEHHA cites no support for its statement that the NTP considers mouse liver tumors relevant for cancer hazard identification without any “discounting.” Indeed, OEHHA does not address the published statements by NTP scientists (e.g., Maronpot et al., 1987; Velazquez et al., 1996) questioning the relevance to humans of mouse liver tumors. Moreover, neither of the two references cited elsewhere in OEHHA's discussion of mouse liver tumors support this proposition.

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[49]*Id*. at 8-9.
[50]OEHHA Response to Comments at 13-14.

Second, “relevance” alone is not necessarily adequate for a sufficient evidence finding. Indeed, the phrase "sufficient evidence" would not be meaningful unless some evidence is not sufficient; OEHHA has offered no basis to conclude that NTP considers all mouse liver tumors adequate to support one half of a "sufficient evidence" conclusion. OEHHA neither states the proposition nor supports the proposition that NTP considers all mouse liver tumor results adequate to form half of a "sufficient evidence" conclusion.

The Associations have put forward significant, credible evidence that not all mouse liver tumors add sufficient weight to comprise half of a "sufficient evidence" conclusion. OEHHA has not directly addressed most of that evidence.

Absent a clear basis for OEHHA to conclude that *NTP* considers *all* mouse liver tumors adequate to support one-half of a sufficient evidence conclusion, and absent NTP affirming that view as it relates to β-myrcene, the listing of β-myrcene cannot proceed pursuant to the authoritative bodies listing process. The Associations do not dispute that the NTP is an authoritative body and that it still uses B6C3F1 mice for its cancer bioassays. Those facts, however, are wholly inadequate to support the conclusion that NTP considers mouse liver tumors for non-genotoxic chemicals adequate to support a sufficient evidence conclusion. Likewise, it is not the Associations' burden to establish that mouse liver tumors are irrelevant to hazard identification. Given the significant literature and regulatory concern that mouse liver tumors are of less value to cancer hazard identification than other tumors, OEHHA must identify where in the Technical Report NTP concludes that the β-myrcene liver tumors are enough to support half of a "sufficient evidence" conclusion. A finding of "clear

evidence" of liver tumors is not sufficient for a variety of reasons, including that it does not describe what weight NTP assigns those tumors in cancer hazard identification.

OEHHA cites to *AFL-CIO v Deukmejian* (1989) as evidence that causing cancer in animals is sufficient to list. However, what constitutes sufficient evidence was not at issue in that case. The case clearly does not stand for the proposition that all animal evidence, however its human relevance has been questioned, is "sufficient" evidence.

3. ***The human relevance of male rat kidney tumors is suspect, so these tumors cannot per se be the basis for OEHHA to conclude that clear evidence of male rat kidney tumors supports a sufficient evidence of carcinogenicity finding.***

As noted in our earlier comments, male rat kidney tumors are of questionable relevance for cancer hazard identification, since they appear to be a species- and sex-specific response. While renal tubule tumors are uncommon spontaneous tumors in rats, there is an increasingly large number of substances (e.g., d-limonene, 1,4-dichlorobenzene, isophorone, pinene) that have been reported to produce increases in renal tubule neoplasms in rats, particularly male rats. Some of these substances act through α2u-globulin, a mode of action which is specific to the male rat. NTP noted that there is evidence that this mechanism plays a role in the increased incidence of renal tubule neoplasms observed in male rats exposed to β-myrcene. Others act through exacerbation of chronic progressive nephropathy (CPN), to which male rats are particularly susceptible.

OEHHA responded to this point by noting that NTP concluded “several lines of evidence suggest that β-myrcene might cause nephrotoxicity by a mechanism other than, or in addition to, α2u-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 the α2u-globulin mechanism." However, even if α2u-globulin is not the sole mechanism of action, the other most likely mechanism of action involves chronic progressive nephropathy (CPN).

NTP discussed the likelihood that CPN may play a role in the induction of rat kidney tumors. Of note, male rats are more susceptible to CPN than are female rats. And the kidney tumor data in rats in the NTP bioassay of β-myrcene are consistent with CPN playing a significant role in kidney tumor induction. But, CPN, like α2u-globulin, is a mechanism of action considered by many not to be relevant to humans.[51]

OEHHA responded that "NTP took this information [its discussion of the mechanism(s) of action] into account when concluding that β-myrcene showed 'clear evidence of carcinogenic activity' in male rats." However, OEHHA provided no support for this statement. To the contrary, in its Technical Report, NTP identified the level of carcinogenic activity without regard to the relevance of the tumors for purposes of human hazard identification. The NTP's own explanation of the levels of carcinogenic activity fully rebuts OEHHA's unsupported assertion.[52] The levels of carcinogenic activity are clearly limited to an analysis of the strength of the neoplastic response, not the processes in the rat not present in humans that likely led to the neoplastic response.[53] Regardless of whether NTP considered the renal tubule tumors to be relevant to human hazard identification, the level of carcinogenic activity would be the

[51] *Id*. Hard, GC, *et al*., "Consideration of rat chronic progressive nephropathy in regulatory evaluations for carcinogenicity. *Toxicol. Sci*., Apr. 2013, pp. 268-75.
[52] NTP TR-557 at 12.
[53] *Id*.

same. NTP does not consider the relevance of tumors to human hazard identification when determining the level of carcinogenic activity in the NTP Technical Report.

III. Conclusion

For the reasons stated above, the Associations respectfully submit that OEHHA does not have the authority to list β-myrcene through the authoritative bodies listing process based on the current record and request that OEHHA withdraw the Notice of Intent to List.

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NTP TECHNICAL REPORT

ON THE

TOXICOLOGY AND CARCINOGENESIS

STUDIES OF β-MYRCENE

(CAS NO. 123-35-3)

IN F344/N RATS AND B6C3F1 MICE

(GAVAGE STUDIES)

Scheduled Peer Review Date: February 25, 2009

NOTICE

This DRAFT Technical Report is distributed solely for the purpose of predissemination peer review under the applicable information quality guidelines. It has not been formally disseminated by the NTP. It does not represent and should not be construed to represent NTP determination or policy.

NTP TR 557

NIH Publication No. 09-5898

National Toxicology Program

National Institutes of Health
Public Health Service
U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES

FOREWORD

The National Toxicology Program (NTP) is an interagency program within the Public Health Service (PHS) of the Department of Health and Human Services (HHS) and is headquartered at the National Institute of Environmental Health Sciences of the National Institutes of Health (NIEHS/NIH). Three agencies contribute resources to the program: NIEHS/NIH, the National Institute for Occupational Safety and Health of the Centers for Disease Control and Prevention (NIOSH/CDC), and the National Center for Toxicological Research of the Food and Drug Administration (NCTR/FDA). Established in 1978, the NTP is charged with coordinating toxicological testing activities, strengthening the science base in toxicology, developing and validating improved testing methods, and providing information about potentially toxic substances to health regulatory and research agencies, scientific and medical communities, and the public.

The Technical Report series began in 1976 with carcinogenesis studies conducted by the National Cancer Institute. In 1981, this bioassay program was transferred to the NTP. 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). Substances selected for NTP toxicity and carcinogenicity studies are chosen primarily on the basis of human exposure, level of production, and chemical structure. The interpretive conclusions presented in NTP Technical Reports are based only on the results of these NTP studies. Extrapolation of these results to other species, including characterization of hazards and risks to humans, requires analyses beyond the intent of these reports. Selection *per se* is not an indicator of a substance's carcinogenic potential.

The NTP conducts its studies in compliance with its laboratory health and safety guidelines and FDA Good Laboratory Practice Regulations and must meet or exceed all applicable federal, state, and local health and safety regulations. Animal care and use are in accordance with the Public Health Service Policy on Humane Care and Use of Animals. Studies are subjected to retrospective quality assurance audits before being presented for public review.

NTP Technical Reports are indexed in the NIH/NLM PubMed database and are available free of charge electronically on the NTP website (*http://ntp.niehs.nih.gov*) or in hardcopy upon request from the NTP Central Data Management group at *cdm@niehs.nih.gov* or (919) 541-3419.

NTP TECHNICAL REPORT

ON THE

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National Toxicology Program

National Institutes of Health
Public Health Service
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CONTENTS

ABSTRACT 5

EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY 12

TECHNICAL REPORTS REVIEW SUBCOMMITTEE 13

SUMMARY OF TECHNICAL REPORTS REVIEW SUBCOMMITTEE COMMENTS 14

INTRODUCTION 15

MATERIALS AND METHODS 25

RESULTS 41

DISCUSSION AND CONCLUSIONS 79

REFERENCES 85

APPENDIX A Summary of Lesions in Male Rats in the 2-Year Gavage Study of β-Myrcene A-1

APPENDIX B Summary of Lesions in Female Rats in the 2-Year Gavage Study of β-Myrcene B-1

APPENDIX C Summary of Lesions in Male Mice in the 2-Year Gavage Study of β-Myrcene C-1

APPENDIX D Summary of Lesions in Female Mice in the 2-Year Gavage Study of β-Myrcene D-1

APPENDIX E Genetic Toxicology E-1

APPENDIX F Clinical Pathology Results F-1

APPENDIX G Organ Weights and Organ-Weight-to-Body-Weight Ratios G-1

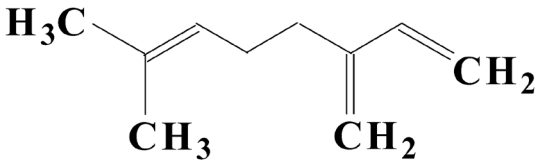
APPENDIX H Reproductive Tissue Evaluations and Estrous Cycle Characterization H-1

APPENDIX I Chemical Characterization and Dose Formulation Studies I-1

APPENDIX J Ingredients, Nutrient Composition, and Contaminant Levels in NTP-2000 Rat and Mouse Ration J-1

APPENDIX K Sentinel Animal Program K-1

ABSTRACT



β-MYRCENE

CAS No. 123-35-3

Chemical Formula: $C_{10}H_{16}$ Molecular Weight: 136.24

Synonyms: 2-Methyl-6-methylene-2,7-octadiene; 7-methyl-3-methylene-1,6-octadiene; myrcene

β-Myrcene, an acyclic unsubstituted monoterpene, and the essential oils which contain it are used as intermediates in the production of terpene alcohols (geraniol, nerol, and linalool), which, in turn, serve as intermediates in the production of aroma and flavor chemicals; thus, β-myrcene is used widely in cosmetics, soaps, and detergents and as a flavoring additive in food and beverages. β-Myrcene is also the major constituent of hop and bay oils, which are used in the manufacture of alcoholic beverages. β-Myrcene was nominated for study by the National Institute of Environmental Health Sciences based on its high production volume, high level of human exposure, and structural relationship to *d*-limonene, which induced neoplasms in the kidneys of male rats in association with hyaline droplet nephropathy (NTP, 1990). Male and female F344/N rats and B6C3F1 mice were administered β-myrcene (greater than 90% pure) by gavage for 3 months or 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium*, *Escherichia coli*, and mouse peripheral blood erythrocytes.

3-Month Study in Rats

Groups of 10 male and 10 female rats were administered 0, 0.25, 0.5, 1, 2, or 4 g β-myrcene/kg body weight in corn oil by gavage, 5 days per week for 14 weeks. Additional groups of 10 male and 10 female special study rats were administered the same doses for 22 days. All core study rats in the 4 g/kg groups died during the first week of the study except one male that died on day 11. One to three rats in the 1 and 2 g/kg groups and one 0.5 g/kg male died by week 9 of the study. One 2 g/kg female died during the last week of the study. Except for lesion incidence data in groups administered 2 g/kg or less, data from rats that died early were excluded from the analysis and summary tables. Mean body weights were significantly decreased in the 0.5, 1, and 2 g/kg groups. Special study rats in the 4 g/kg groups died by the end of the first week. Dose-related clinical findings in animals that died early included thinness, lethargy, abnormal breathing, and ruffled fur. Right kidney and liver weights of dosed males and females were generally significantly greater than those of the vehicle controls.

In special study rats evaluated on day 23, the incidences and severities of chronic progressive nephropathy (CPN) and renal tubule necrosis were increased in 2 g/kg males. At the end of the 3-month study, the incidences of renal tubule necrosis were significantly increased in all dosed groups of males and females.

At 3 months, the incidences of olfactory epithelium degeneration in 2 g/kg males and females were significantly increased, and the severities were increased. The incidences of chronic inflammation in 1 and 2 g/kg males and females were significantly increased. All 2 g/kg males and females had splenic atrophy. In the mesenteric lymph node, significantly increased incidences of atrophy occurred in 2 g/kg males and 1 and 2 g/kg females. Acute inflammation of the forestomach occurred in four 2 g/kg females. The incidences of porphyrin pigmentation in the Harderian gland of males administered 0.5 g/kg or greater were significantly increased.

3-Month Study in Mice

Groups of 10 male and 10 female mice were administered 0, 0.25, 0.5, 1, 2, or 4 g β-myrcene/kg body weight in corn oil by gavage, 5 days per week for 14 weeks. All 4 g/kg male and female mice died during week 1; nine 2 g/kg males and eight 2 g/kg females died by week 4. The mean body weights of 1 g/kg males and 0.5 and 2 g/kg females were significantly less than those of the vehicle controls. Clinical findings in animals that did not survive to the end of the study included thinness, lethargy, and abnormal breathing. The right kidney weights of 1 g/kg females and the liver weights of females administered 0.5 or 1 g/kg were significantly increased. No histopathology changes were observed in mice administered 1 g/kg or less. The 2 and 4 g/kg mice were not evaluated due to early deaths.

2-Year Study in Rats

Groups of 50 male and 50 female rats were administered 0, 0.25, 0.5, or 1 g β-myrcene/kg body weight in corn oil by gavage, 5 days per week for 105 weeks. All 1 g/kg male rats died before the end of the study due to renal toxicity. Compared to vehicle controls, the mean body weights of 0.25 and 0.5 g/kg males were slightly greater, and mean body weights of 1 g/kg females were slightly less at the end of the study.

In the standard evaluation of the kidney, the incidence of renal tubule adenoma was significantly increased in 0.5 g/kg male rats, and the combined incidences of renal tubule adenoma or carcinoma were significantly increased in 0.25 and 0.5 g/kg males. In both the extended evaluation and the combined standard and extended evaluations, the incidences of renal tubule adenoma and the combined incidences of renal tubule adenoma or carcinoma were significantly increased in the 0.25 and 0.5 g/kg groups of males. The incidences of renal tubule nephrosis (nephrosis) were markedly increased in all dosed groups of both sexes except in 0.25 g/kg females. The incidences of papillary mineralization in 0.25 and 0.5 g/kg males were significantly increased. Significantly increased incidences of nephropathy occurred in dosed females, and the severity was increased in the 0.5 and 1 g/kg males and females. The incidences of hyperplasia of the transitional epithelium lining the pelvis and overlying the renal

papilla were significantly increased in all dosed groups of males and females. In male rats, the incidences of focal suppurative inflammation were significantly increased in the 0.25 and 0.5 g/kg groups.

A significantly increased incidence of chronic active inflammation of the nose occurred in 0.5 g/kg males. Also in 0.5 g/kg males, the incidence of chronic active inflammation of the forestomach was increased.

2-Year Study in Mice

Groups of 50 male and 50 female mice were administered 0, 0.25, 0.5, or 1 g β-myrcene/kg body weight in corn oil by gavage, 5 days per week for 104 or 105 weeks. Survival of 1 g/kg mice was significantly less than that of the vehicle controls; the cause of the deaths was uncertain. Mean body weights of 1 g/kg males were 8% less than those of the vehicle controls after week 8. Mean body weights of 0.5 g/kg females were 7% less than those of the vehicle controls after week 17, and those of 1 g/kg females were 8% less after week 11.

The incidences of liver neoplasms were significantly increased in 0.25 and/or 0.5 g/kg males and 0.25 g/kg females. Liver neoplasms included hepatocellular adenoma and hepatocellular carcinoma in males and females and hepatoblastoma in males. The incidences of hepatocellular hypertrophy were significantly increased in 0.5 g/kg males and females, as was the incidence of mixed cell focus in 0.5 g/kg females.

The incidences of bone marrow atrophy and lymph node follicle atrophy in the spleen were significantly increased in 0.5 g/kg females. In the forestomach, there were significantly increased incidences of inflammation and epithelial hyperplasia in 0.5 g/kg females.

Genetic Toxicology

β-Myrcene did not show evidence of genotoxicity in assays conducted by the NTP. No mutagenicity was observed in any of several strains of *Salmonella typhimurium* or *Escherichia coli* in two independent Ames assays conducted with and without exogenous metabolic activation. In addition, no significant increase in frequency of micronucleated normochromatic erythrocytes, biomarkers of chromosomal damage, was observed in male or female mice administered β-myrcene for 3 months by gavage.

Conclusions

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 *equivocal evidence of carcinogenic activity* of β-myrcene in female F344/N rats based on increased incidences of renal tubule adenoma. There was *clear evidence of carcinogenic activity* of β-myrcene in male B6C3F1 mice based on increased incidences of liver neoplasms. There was *equivocal evidence of carcinogenic activity* of β-myrcene in female B6C3F1 mice based on marginally increased incidences of hepatocellular neoplasms.

Administration of β-myrcene induced nonneoplastic lesions in the kidney of male and female rats, nose of male rats, and liver of male and female mice.

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* Explanation of Levels of Evidence of Carcinogenic Activity is on page 12.

Summary of the 2-Year Carcinogenesis and Genetic Toxicology Studies of β-Myrcene[a]

	Male F344/N Rats	Female F344/N Rats	Male B6C3F1 Mice	Female B6C3F1 Mice
Doses in corn oil by gavage	0, 0.25, 0.5, 1 g/kg	0, 0.25, 0.5, 1 g/kg	0, 0.25, 0.5, 1 g/kg	0, 0.25, 0.5, 1 g/kg
Body weights	0.25 and 0.5 g/kg groups greater than vehicle control group after week 81	1 g/kg group less than vehicle control group after week 13	1 g/kg group less than vehicle control group after week 8	1 g/kg group less than vehicle control group after week 11; 0.5 g/kg group less than vehicle control group after week 17
Survival rates	29/50, 36/50, 28/50, 0/50	31/50, 33/50, 28/50, 33/50	35/50, 35/50, 31/50, 21/50	39/50, 34/50, 35/50, 17/50
Nonneoplastic effects	Kidney: renal tubule, nephrosis (0/50, 42/50, 46/50); papilla, mineralization (1/50, 48/50, 40/50); severity of nephropathy (1.2, 2.0, 2.6); transitional epithelium, hyperplasia (0/50, 21/50, 19/50); inflammation, suppurative, focal (1/50, 22/50, 22/50) Nose: inflammation, chronic active (14/50, 19/50, 27/50)	Kidney: renal tubule, nephrosis (0/50, 2/50, 27/50, 45/50); nephropathy (26/50, 43/50, 41/50, 44/50); severity of nephropathy (1.0, 1.0, 1.3, 1.7); transitional epithelium, hyperplasia (1/50, 12/50, 15/50, 19/50)	Liver: hepatocyte, hypertrophy (1/50, 2/50, 16/50)	Liver: hepatocyte, hypertrophy (0/50, 0/50, 6/50); mixed cell focus (1/50, 4/50, 6/50)
Neoplastic effects	Kidney: renal tubule adenoma (standard evaluation - 0/50, 4/50, 8/50; extended evaluation - 0/50, 8/50, 7/50; standard and extended evaluations combined - 0/50, 12/50, 13/50); renal tubule adenoma or carcinoma (standard evaluation - 0/50, 7/50, 9/50; extended evaluation - 0/50, 10/50, 7/50; standard and extended evaluations combined - 0/50, 14/50, 13/50)	None	Liver: hepatocellular adenoma (26/50, 41/50, 43/50); hepatocellular carcinoma (14/50, 20/50, 28/50); hepatocellular adenoma or carcinoma (33/50, 44/50, 48/50); hepatoblastoma (4/50, 6/50, 11/50); hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma (34/50, 45/50, 48/50)	None
Equivocal findings	None	Kidney: renal tubule adenoma (standard evaluation - 0/50, 1/50, 0/50, 2/50; extended evaluation - 0/50, 1/50, 1/50, 2/50; standard and extended evaluation - 0/50, 2/50, 1/50, 3/50)	None	Liver: hepatocellular adenoma (6/50, 13/50, 6/50); hepatocellular carcinoma (1/50, 7/50, 2/50); hepatocellular adenoma or carcinoma (7/50, 18/50, 8/50)

Summary of the 2-Year Carcinogenesis and Genetic Toxicology Studies of β-Myrcene

	Male F344/N Rats	Female F344/N Rats	Male B6C3F1 Mice	Female B6C3F1 Mice
Level of evidence of carcinogenic activity	Clear evidence	Equivocal evidence	Clear evidence	Equivocal evidence
Genetic toxicology				
Salmonella typhimurium gene mutations:		Negative in strains TA97, TA98, TA100, and TA1535 with and without S9; negative in *Escherichia coli* strain WP2 *uvrA*/pKM101 with and without S9		
Micronucleated erythrocytes				
Mouse peripheral blood *in vivo:*		Negative		

[a] Neoplasm and nonneoplastic lesion incidences are not presented for 1 g/kg male rats or 1 g/kg male or female mice due to high mortality.

EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY

The National Toxicology Program describes the results of individual experiments on a chemical agent and notes the strength of the evidence for conclusions regarding each study. Negative results, in which the study animals do not have a greater incidence of neoplasia than control animals, do not necessarily mean that a chemical is not a carcinogen, inasmuch as the experiments are conducted under a limited set of conditions. Positive results demonstrate that a chemical is carcinogenic for laboratory animals under the conditions of the study and indicate that exposure to the chemical has the potential for hazard to humans. Other organizations, such as the International Agency for Research on Cancer, assign a strength of evidence for conclusions based on an examination of all available evidence, including animal studies such as those conducted by the NTP, epidemiologic studies, and estimates of exposure. Thus, 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.

Five categories of evidence of carcinogenic activity are used in the Technical Report series to summarize the strength of the evidence observed in each experiment: 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. For each separate experiment (male rats, female rats, male mice, female mice), one of the following five categories is selected to describe the findings. These categories refer to the strength of the experimental evidence and not to potency or mechanism.

- **Clear evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a dose-related (i) increase of malignant neoplasms, (ii) increase of a combination of malignant and benign neoplasms, or (iii) marked increase of benign neoplasms if there is an indication from this or other studies of the ability of such tumors to progress to malignancy.
- **Some evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a chemical-related increased incidence of neoplasms (malignant, benign, or combined) in which the strength of the response is less than that required for clear evidence.
- **Equivocal evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a marginal increase of neoplasms that may be chemical related.
- **No evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing no chemical-related increases in malignant or benign neoplasms.
- **Inadequate study** of carcinogenic activity is demonstrated by studies that, because of major qualitative or quantitative limitations, cannot be interpreted as valid for showing either the presence or absence of carcinogenic activity.

For studies showing multiple chemical-related neoplastic effects that if considered individually would be assigned to different levels of evidence categories, the following convention has been adopted to convey completely the study results. In a study with clear evidence of carcinogenic activity at some tissue sites, other responses that alone might be deemed some evidence are indicated as “were also related” to chemical exposure. In studies with clear or some evidence of carcinogenic activity, other responses that alone might be termed equivocal evidence are indicated as “may have been” related to chemical exposure.

When a conclusion statement for a particular experiment is selected, consideration must be given to key factors that would extend the actual boundary of an individual category of evidence. Such consideration should allow for incorporation of scientific experience and current understanding of long-term carcinogenesis studies in laboratory animals, especially for those evaluations that may be on the borderline between two adjacent levels. These considerations should include:

- adequacy of the experimental design and conduct;
- occurrence of common versus uncommon neoplasia;
- progression (or lack thereof) from benign to malignant neoplasia as well as from preneoplastic to neoplastic lesions;
- some benign neoplasms have the capacity to regress but others (of the same morphologic type) progress. At present, it is impossible to identify the difference. Therefore, where progression is known to be a possibility, the most prudent course is to assume that benign neoplasms of those types have the potential to become malignant;
- combining benign and malignant tumor incidence known or thought to represent stages of progression in the same organ or tissue;
- latency in tumor induction;
- multiplicity in site-specific neoplasia;
- metastases;
- supporting information from proliferative lesions (hyperplasia) in the same site of neoplasia or in other experiments (same lesion in another sex or species);
- presence or absence of dose relationships;
- statistical significance of the observed tumor increase;
- concurrent control tumor incidence as well as the historical control rate and variability for a specific neoplasm;
- survival-adjusted analyses and false positive or false negative concerns;
- structure-activity correlations; and
- in some cases, genetic toxicology.

NATIONAL TOXICOLOGY PROGRAM BOARD OF SCIENTIFIC COUNSELORS TECHNICAL REPORTS REVIEW SUBCOMMITTEE

The members of the Technical Reports Review Subcommittee who evaluated the draft NTP Technical Report on β-myrcene on February 25, 2009, are listed below. Subcommittee members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, subcommittee members have five major responsibilities in reviewing the NTP studies:

- to ascertain that all relevant literature data have been adequately cited and interpreted,
- to determine if the design and conditions of the NTP studies were appropriate,
- to ensure that the Technical Report presents the experimental results and conclusions fully and clearly,
- to judge the significance of the experimental results by scientific criteria, and
- to assess the evaluation of the evidence of carcinogenic activity and other observed toxic responses.

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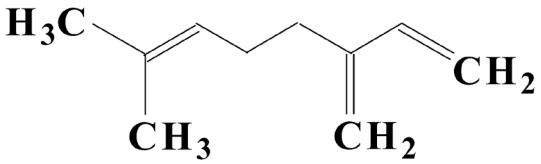
SUMMARY OF TECHNICAL REPORTS REVIEW SUBCOMMITTEE COMMENTS

NOTE: A summary of the Technical Reports Review Subcommittee's remarks will appear in a future draft of this report.

INTRODUCTION



β-MYRCENE

CAS No. 123-35-3

Chemical Formula: $C_{10}H_{16}$ Molecular Weight: 136.24

Synonyms: 2-Methyl-6-methylene-2,7-octadiene; 7-methyl-3-methylene-1,6-octadiene; myrcene

Chemical and Physical Properties

β-Myrcene is an acyclic unsubstituted monoterpene (colorless oil) with a boiling point of 167° C (*CRC Handbook*, 1981; *Hawley's*, 1993) and a density of 0.794 at 20° C (*Merck*, 1996). β-Myrcene has a peppery, spicy, or balsam odor. β-Myrcene is insoluble in water but soluble in the organic solvents alcohol, chloroform, ether, and glacial acetic acid (*Merck*, 1996).

Production, Use, and Human Exposure

Commercially available β-myrcene is produced by the thermal rearrangement of β-pinene at 450° to 600° C (Stolle *et al.*, 2008) or pyrolysis at temperatures above 713° C (Kolicheski *et al.*, 2007). Under either of these conditions, the product contains up to 30% side reaction and consecutive reaction by-products (R. Gorman, 1996, SCM GLIDCO Organics, personal communication; Kolicheski, *et al.*, 2007). Pyrolysis of β-pinene at 550° to 600° C yields 75% to 90% β-myrcene. Impurities include limonene, *psi*-limonene, *dl*-limonene, terpenes, and isomers

and dimers of β-myrcene (R. Gorman, 1996, SCM GLIDCO Organics, personal communication; Kolicheski *et al.*, 2007; Stolle *et al.*, 2008). A polymerization inhibitor such as butylhydroxytoluene or tenox propyl gallate is normally added to crude or high purity myrcene during shipment or extended storage.

Production volume of β-myrcene is large, but reliable production figures are unavailable (SRI International, 1996). SCM GLIDCO Organics (Jacksonville, FL) was listed as the only producer of β-myrcene in the United States in 1996 (SRI International, 1996).

β-Myrcene is found in verbena oil, galbanum oil, and lemongrass oil (Guenther *et al.*, 1994). In commercial production, β-myrcene and the essential oils that contain it are used as intermediates in the production of terpene alcohols (geraniol, nerol, and linalool), which, in turn, serve as intermediates in the production of aroma and flavor chemicals (Kuney, 1994); thus, β-myrcene is used widely in cosmetics, soaps, and detergents and as a flavoring additive in food and beverages (Lorente *et al.*, 1989; Delgado *et al.*, 1993a). Furthermore, β-myrcene is the major constituent of hop and bay oils, which are used in the manufacture of alcoholic beverages (Madyastha and Srivatsan, 1987).

β-Myrcene is the chief ingredient in lemongrass tea used in Brazilian folk medicine to treat gastrointestinal disturbances and as a sedative and antipyretic (Delgado *et al.*, 1993b). It acts as a peripheral analgesic (Lorenzetti *et al.*, 1991; Delgado *et al.*, 1993b).

Consumers are exposed to β-myrcene via inhalation, oral routes, and dermal contact. Occupational exposure is via inhalation and dermal contact.

β-Myrcene has been identified in over 200 plants, in emission samples from plywood veneer dryers (up to 38.6 mg/m^3; Cronn *et al.*, 1983), in emissions by many tree species (up to 3 μg/g per hour; Guenther *et al.*, 1994),

in household waste (Wilt *et al.*, 1988; Wilkins, 1994; Wilkins and Larsen, 1995), and in indoor air (Wilkins, 1994; Kostiainen, 1995).

β-Myrcene is generally recognized as a safe substance in food both in its naturally occurring form in essential oils (21 CFR § 172.510) and as a synthetic substance (21 CFR § 172.515). β-Myrcene in effluents is not regulated by the United States Environmental Protection Agency (40 CFR § 414.101).

Absorption, Distribution, Metabolism, and Excretion

Experimental Animals

β-Myrcene was well absorbed through the skin of rats (Opdyke, 1976). In female rats administered 1 g/kg body weight orally, the blood level of β-myrcene at 60 minutes was 14.1 ± 3.0 μg/mL, and the elimination half-life was 285 minutes (Delgado *et al.*, 1993b). In that study, the parent chemical was detected in adipose tissue, brain, liver, kidney, and testis. β-Myrcene was primarily metabolized to 10-hydroxylinalool (II) by rat liver microsomes as shown in Figure 1 (Madyastha and Srivatsan, 1987). The rate of metabolism was significantly greater in microsomal preparations from phenobarbital (PB)-treated rats than in 3-methylcholanthrene-treated rats, indicating that β-myrcene was preferentially metabolized by CYP2B. Induction of CYP2B isozymes by β-myrcene was indicated after repeated oral treatment (daily for 14 days) of 1 g/kg of β-myrcene to rats resulted in a marked decrease in pentobarbital sleeping time (Freitas *et al.*, 1993). Further, increased levels of CYP2B1/2 and pentoxy-resorufin-O-dealkylation were observed in liver microsomes of female Wistar rats receiving three daily oral doses of the same concentration (De-Oliveira *et al.*, 1997). In contrast, Madyastha and Srivatsan (1987) and Austin *et al.* (1988) detected no changes in the amount of PB-inducible CYP450 in rat liver microsomes of rats administered β-myrcene. Male rats receiving 800 mg/kg per day for 20 days metabolized and excreted β-myrcene in urine as 10-hydroxylinalool (II), 7-methyl-3-methylele-oct-6-ene-1,2-diol (IV), 1-hydroxymethyl-4-isopropenyl cyclohexanol (VI), 10-carboxylinalool (III), and 2-hydroxy-7-methyl-3-methylene-oct-6-enoic acid (V) as shown in Figure 1. 10-Hydroxylinalool [II and also myrcene-3(10)-glycol], arising through apparent formation of a

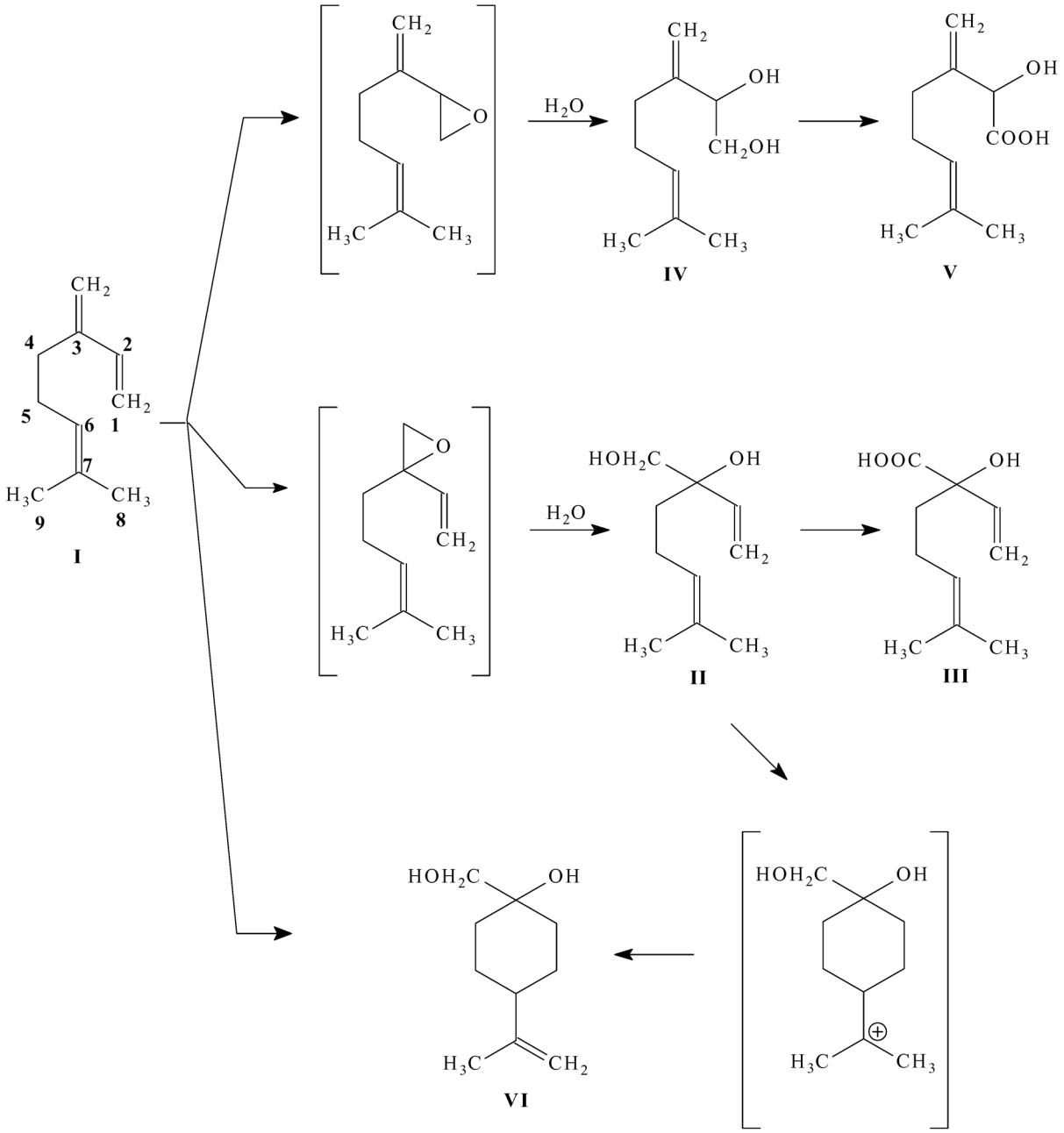


Figure 1
Metabolism of β-Myrcene by Rats (Madyastha and Srivatsan, 1987)
I. β-myrcene; II. 10-hydroxylinalool; III. 10-carboxylinalool; IV. 7-methyl-3-methylene-oct-6-ene-1,2-diol; V. 2-hydroxy-7-methyl-3-methylene-oct-6-enoic acid; VI. 1-hydroxymethyl-4-isopropenyl cyclohexanol

3,10-epoxide, was the major metabolite detected in the urine of rabbits receiving β-myrcene by gavage (Ishida *et al.*, 1981). β-Myrcene was preferentially metabolized to 10-hydroxylinalool by larvae of the common cutworm *Spodoptera litura* (Miyazawa and Murata, 2000). The acidic metabolite, 10-carboxylinalool (III) arising from II in rats was detected in rabbits, but not cutworms (Ishida *et al.*, 1981; Madyastha and Srivatsan, 1987). Oxidation of the 1,2-double bond led to formation, through a 1,2-epoxide intermediate, of 7-methyl-3-methylele-oct-6-ene-1,2-diol (IV; myrcene-1,2-glycol) in all three species, and formation of the acidic metabolite V in rats and rabbits (Ishida *et al.*, 1981; Madyastha and Srivatsan, 1987; Miyazawa and Murata, 2000). Preferential oxidation occurred at the 3,10-double bond in these studies. Bond formation between C1 and C6 of β-myrcene resulted in ring closure and excretion of uroterpenol in rabbit (not shown) and metabolite VI in rat urine (Figure 1).

β-Myrcene shares similarities in epoxide formation with *d*-limonene, a rat kidney carcinogen (NTP, 1990). A major component in some essential oils of citrus fruits, *d*-limonene was metabolized by CYPs in rat liver microsomes to the glycols, *d*-limonene-8,9-diol (major metabolite) and *d*-limonene-1,2-diol, through the respective 8,9- and 1,2-epoxide intermediates (Watabe *et al.*, 1980). Neither epoxide was mutagenic to *Salmonella typhimurium* strain TA100. However, interaction between cis-*d*-limonene-1,2-oxide and α2u-globulin is the apparent causal factor in nephrotoxicity and nephrocarcinogenicity specific to male rats exposed to *d*-limonene (Lehman-McKeeman *et al.*, 1989). 8,9-Diol metabolites have been detected in rat, guinea pig, hamster, dog, and humans exposed to *d*-limonene (Kodama *et al.*, 1976). Uroterpenol, identified as a metabolite of β-myrcene in rabbits is an 8,9-diol metabolite of *d*-limonene (Kodama *et al.*, 1976; Ishida *et al.*, 1981). The partial structure of β-myrcene containing C1, C2, C3, and C10 is similar to 1,3-butadiene, a carcinogen in rodents and humans (Melnick and Sills, 2001) and is a site for potential formation of a diepoxide. 1,3-Butadiene is metabolized to two monoepoxides (1,2-epoxy-3-butene and 3,4-epoxy-1,2-butanediol) and also forms diepoxybutane (NTP, 1993). Diepoxybutane and 1,2-epoxy-3-butene have been shown to be mutagenic to *S. typhimurium* strains TA98 and/or TA100 (Gervasi *et al.*, 1985). Further, DNA adduct studies suggest that 3,4-epoxy-1,2-butanediol is the major epoxide involved in the mutagenesis and carcinogenesis of 1,3-butadiene (Melnick, 2002). The partial structure of β-myrcene containing C1, C2, C3, C4, and C10 is similar to isoprene, also known to form monoexpoxides and

a diepoxide (Watson *et al.*, 2001). Mutagenic activity was observed for the diepoxide, but not the monoepoxides, of isoprene in *S. typhimurium* strains TA98 and TA100 (Gervasi *et al.*, 1985). Isoprene was not mutagenic to various strains of *S. typhimurium* in studies conducted by the NTP (1999), but inhalation exposure to the chemical did result in evidence of carcinogenicity in both male and female F344/N rats. Epoxide formation is suspected as a causal factor in toxicity related to isoprene exposure, and it is suggested that sex- and species-dependent differences in the stereochemistry of these metabolites influence their reactivity (Watson *et al.*, 2001). Further, steric hindrance of the additional methyl group of isoprene may result in less potential cross-linking of the isoprene diepoxide than for the 1,3-butadiene diepoxide. Formation of a β-myrcene-derived diepoxide is unproven, and the potential for reactivity of the β-myrcene-derived monoepoxides with either α2u-globulin or DNA is uncertain.

Humans

No studies on absorption, distribution, metabolism, or excretion of β-Myrcene in humans were found in the literature.

TOXICITY

Experimental Animals

The oral approximate lethal doses in mice and rats were 5.06 g/kg and 11.39 g/kg, respectively; an oral dose of 10.5 g/kg was not lethal to rats. Intraperitoneal approximate lethal doses in mice and rats were 2.25 g/kg and 5.06 g/kg, respectively (Paumgartten *et al.*, 1990).

Humans

β-Myrcene is an irritant; in humans, β-myrcene exposure induces dermatitis, conjunctivitis, somnolence, and asthma-like symptoms (Newmark, 1978).

Reproductive and Developmental Toxicity

Experimental Animals

β-Myrcene administered orally to pregnant Wistar rats induced reduction in maternal weight gain at 1.2 g/kg per day (Delgado *et al.*, 1993b) and mortality (33%) at 1.5 g/kg per day (Delgado *et al.*, 1993a). Embryo toxicity was also seen at the maternal toxic dose of 1.2 g/kg. The no-observed-adverse-effect level (NOAEL) was 0.5 g/kg per day for embryo-fetotoxicity (Delgado *et al.*, 1993b) and 0.25 g/kg per day for peri- and postnatal development (Delgado *et al.*, 1993a).

Humans

No reproductive or developmental toxicity data for humans exposed to β-Myrcene were found in the literature.

Other Biological Effects

β-Myrcene is analgesic in mice; the ED_{50} is 16 mg/kg orally (Lorenzetti *et al.*, 1991). Mice treated with β-myrcene exhibited an increased antinociceptive activity (hot plate test) (Rao *et al.*, 1990). At 1 g/kg, β-myrcene had no effects on exploratory or emotional behavior, anxiolytic activity in a plus maze, nor inhibition of conditioned avoidance (da-Silva *et al.*, 1991). This same study found that β-myrcene did not protect against pentylenetetrazol-induced seizures in mice.

β-Myrcene (0.8 g/kg per day *per os* for 4 days or 40 mg/kg per day intraperitoneally for 3 days) did not elevate liver drug metabolizing enzyme activity in rats (Madyastha and Srivatsan, 1987; Austin *et al.*, 1988). However, it was shown that β-myrcene at 1.0 g/kg *per os* interferes with pentobarbital metabolism by increasing sleeping time in rats (Freitas *et al.*, 1993).

β-Myrcene at 1 mM and 3 mM inhibits 20% and 18%, respectively, 21 to 26 kDa protein, a family of isoprenylated proteins consisting of the *ras* superfamily of small G proteins, in HT–29 colon carcinoma cells

(Crowell *et al.*, 1994). Its ability to inhibit post-translational isoprenylation of small G proteins is more potent than that of limonene. β-Myrcene at 1% in the diet had no effect on 7,12-dimethylbenzanthracene (DMBA) induction of mammary tumors in Sprague-Dawley rats (Russin *et al.*, 1989).

CARCINOGENICITY

No carcinogenicity studies of β-myrcene in experimental animals or epidemiology studies in humans were found in a review of the literature.

GENETIC TOXICITY

No mutagenic activity has been observed with β-myrcene in bacterial or mammalian test systems. No increase in mutant colonies was observed in *Salmonella typhimurium* strains TA100, TA1535, TA98, or TA97a treated with up to 1,500 μg/plate β-myrcene, with or without induced rat liver S9 activation enzymes; α-terpinene and (-)-α-pinene, two other monoterpenes, were also negative in this *Salmonella* mutagenicity assay (Gomes-Carneiro *et al.*, 2005). No increases in the frequency of *hprt* mutations were reported in hamster V79 cells incubated with up to 1,000 μg/mL β-myrcene, with and without S9, and no increase in chromosomal aberrations or sister chromatid exchanges were seen in V79 cells exposed to β-myrcene (Kauderer *et al.*, 1991). No increases in chromosomal aberrations were reported in bone marrow cells of male or female Wistar rats sampled 24 or 48 hours after administration of β-myrcene (up to 1 g/kg) by corn oil gavage (Zamith *et al.*, 1993).

No mutagenic activity has been observed with *d*-limonene, which is structurally related to β-myrcene, in *S. typhimurium* (Haworth *et al.*, 1983), cultured mouse lymphoma cells (Myhr *et al.*, 1990; NTP, 1990), or Big Blue™ mice (Turner *et al.*, 2001). In addition, *d*-limonene did not increase the frequency of chromosomal aberrations or sister chromatid exchanges in cultured Chinese hamster ovary cells (Anderson *et al.*, 1990; NTP, 1990).

Study Rationale

β-Myrcene was nominated by NIEHS for carcinogenicity studies. β-Myrcene was nominated for study based on its high production volume, high level of human exposure, and structural relationship to *d*-limonene, which induced tumors in the kidneys of male rats in association with hyaline droplet nephropathy (NTP, 1990).

MATERIALS AND METHODS

Procurement and Characterization of β-Myrcene

β-Myrcene was obtained from Millennium Specialty Chemicals (Jacksonville, FL) in two lots (0LB410 for 3-month studies and 1WB503 for 2-year studies). Identity and purity studies were performed by the analytical chemistry laboratory at Battelle Columbus Operations (Chemistry Support Services, Columbus, OH), Galbraith Laboratories, Inc. (Knoxville, TN), and the study laboratory at Battelle Columbus Operations (Columbus, OH); Karl Fischer titration was performed by Galbraith Laboratories, Inc. (Appendix I). Reports on analyses performed in support of the β-myrcene studies are on file at the National Institute of Environmental Health Sciences.

Both lots of the chemical, a clear to slightly yellow liquid, were identified as β-myrcene by infrared, proton, and carbon-13 nuclear magnetic resonance (NMR) spectroscopy and by boiling point determinations. The water content of each lot was determined by Karl Fischer titration. The purity of each lot was determined by elemental analyses, gas chromatography with flame ionization detection (GC-FID), and high-performance liquid chromatography (HPLC).

For lot 0LB410, Karl Fischer titration indicated a water content of less than 0.016%, and elemental analyses of carbon and hydrogen were generally consistent with the theoretical values. HPLC revealed a major peak and one impurity with a peak area of 0.36%. GC-FID indicated one major peak, nine impurity peaks with areas of 0.1% or greater, and a total peak area for all impurities of 9.55%. The largest peak (4.4% of total peak area) was identified as *psi*-limonene. The second largest impurity (1.4% of total peak area) was tentatively identified as *dl*-limonene. The other seven impurity peaks, small by comparison, were chiefly terpene hydrocarbons and were tentatively identified as isomers and dimers of β-myrcene. The overall purity of lot 0LB410 was determined to be greater than 90%.

For lot 1WB503, Karl Fischer titration indicated a water content of less than 0.06%, and elemental analyses of carbon and hydrogen were generally consistent with the theoretical values. HPLC revealed a major peak and one impurity with a peak area of 0.4%. GC-FID indicated one major peak, 12 impurities with areas of 0.1% or greater, a total peak area for all impurities of 6.5%. The largest impurity in β-myrcene was identified as *psi*-limonene (approximately 5%). The other 11 impurity peaks, small by comparison, were chiefly the same as in the earlier lot, tentatively identified as isomers and dimers of β-myrcene. The overall purity of lot 1WB503 was determined to be greater than 93%.

To ensure stability, the bulk chemical was stored in amber glass bottles sealed with Teflon®-lined lids at less than or equal to –20° C. Stability was monitored during the 3-month and 2-year studies using GC-FID; no degradation of the bulk chemical was detected.

Preparation and Analysis of Dose Formulations

The dose formulations were prepared by mixing β-myrcene (lot 0LB410 for 3-month studies and lot 1WB503 for 2-year studies) with corn oil (Table I1). Stability studies of a 50 mg/mL β-myrcene (lot 09116TQ obtained from Aldrich Chemical Company, Milwaukee, WI) dose formulation were performed by the analytical chemistry laboratory using GC-FID. Stability was confirmed for at least 37 days for dose formulations stored in amber glass bottles sealed with Teflon®-lined lids at temperatures up to room temperature, and for up to 3 hours under simulated animal room conditions.

Periodic analyses of the dose formulations of β-myrcene were conducted by the study laboratory using GC-FID. During the 3-month studies, the dose formulations were analyzed at the beginning, midpoint, and end of the studies; all 13 dose formulations analyzed for rats and all 15 for mice were within 10% of the target concentrations (Table I2). Animal room samples of these dose formulations were also analyzed; all 13 animal room samples

for rats and mice were within 10% of the target concentrations. During the 2-year studies, the dose formulations were analyzed approximately every 3 months; animal room samples were also analyzed (Table I3). All 27 dose formulations for rats and mice were within 10% of the target concentrations; all nine animal room samples for rats and mice were within 10% of the target concentrations. Periodic analyses of the corn oil vehicle by the study laboratory demonstrated peroxide concentrations below the acceptable limit of 3 mEq/kg.

3-Month Studies

The 3-month studies were conducted to evaluate the cumulative toxic effects of repeated exposure to β-myrcene and to determine the appropriate doses to be used in the 2-year studies.

Male and female F344/N rats and B6C3F1 mice were obtained from Taconic Farms, Inc. (Germantown, NY). On receipt, the mice were approximately 3 to 4 weeks old. Animals were quarantined for 11 to 14 days and were approximately 5 to 6 weeks old on the first day of the study. Before the studies began, five male and five female mice were randomly selected for parasite evaluation and gross observation for evidence of disease. At 1 month and at the end of the studies, serologic analyses were performed on five male and five female sentinel rats and mice using the protocols of the NTP Sentinel Animal Program (Appendix K).

Groups of 10 male and 10 female rats and mice were administered β-myrcene in corn oil by gavage at doses of 0.25, 0.5, 1, 2, or 4 g/kg body weight 5 days per week for 14 weeks. Groups of 10 male and 10 female rats and mice received the corn oil vehicle alone and served as controls. The dose levels were selected based on published acute toxicity data. Additional groups of 10 male and 10 female special study rats were administered the same doses for 22 days. Feed and water were available *ad libitum*. All rats and female mice were housed five per cage. Male mice were housed individually. Clinical findings were recorded and core study animals were weighed initially, weekly, and at the end of the studies. Details of the study design and animal maintenance are summarized in Table 1.

Blood was collected from the retroorbital sinus of special study rats on day 23 and core study rats and mice at the end of the studies for hematology (rats and mice) and clinical chemistry (rats) analyses. Animals were anesthetized with a mixture of CO_2/O_2. Blood samples for hematology were put into microcollection tubes containing EDTA as the anticoagulant. Erythrocyte, platelet, and leukocyte counts; hematocrit value; hemoglobin concentration; and mean cell volume, hemoglobin, and hemoglobin concentration were measured using a Cell-Dyn 3500 hematology analyzer and reagents from Abbott Diagnostics (Abbott Park, IL). Samples for clinical chemistry analyses were collected into microcollection separator tubes and allowed to clot at room temperature; serum was obtained by centrifugation. Samples were analyzed using a Hitachi 911® chemistry analyzer (Boehringer Mannheim Diagnostics, Indianapolis, IN) with reagents supplied by the manufacturer. The parameters measured are listed in Table 1.

At the end of the 3-month studies, samples were collected for sperm morphology and vaginal cytology evaluations on core study rats and mice administered 0, 0.25, 0.5, or 1 g/kg. The parameters evaluated are listed in Table 1. For 12 consecutive days prior to scheduled terminal sacrifice, the vaginal vaults of the females were moistened with saline, if necessary, and samples of vaginal fluid and cells were stained. Relative numbers of leukocytes, nucleated epithelial cells, and large squamous epithelial cells were determined and used to ascertain estrous cycle stage (i.e., diestrus, proestrus, estrus, and metestrus). Male animals were evaluated for sperm count and motility. The left testis and left epididymis were isolated and weighed. The tail of the epididymis (cauda epididymis) was then removed from the epididymal body (corpus epididymis) and weighed. Test yolk (rats) or modified Tyrode's buffer (mice) was applied to slides, and a small incision was made at the distal border of the cauda epididymis. The sperm effluxing from the incision were dispersed in the buffer on the slides, and the numbers of motile and nonmotile spermatozoa were counted for five fields per slide by two observers. Following completion of sperm motility estimates, each left cauda epididymis was placed in buffered saline solution. Caudae were finely minced, and the tissue was incubated in the saline solution and then heat fixed at 65° C. Sperm density was then determined microscopically with the aid of a hemacytometer. Four sperm morphology slides were prepared for each animal evaluated. An aliquot of killed sperm suspension was stained in a test tube, spread on a microscope

slide under a coverslip, and examined for sperm count and motility. To quantify spermatogenesis, the testicular spermatid head count was determined by removing the tunica albuginea and homogenizing the left testis in phosphate-buffered saline containing 10% dimethyl sulfoxide. Homogenization-resistant spermatid nuclei were counted with a hemacytometer.

Necropsies were performed on all core study animals. The heart, right kidney, liver, lung, right testis, and thymus were weighed. Tissues for microscopic examination were fixed and preserved in 10% neutral buffered formalin (except eyes were fixed in Davidson's solution), processed and trimmed, embedded in paraffin, sectioned to a thickness of 4 to 6 µm, and stained with hematoxylin and eosin. Complete histopathologic examinations were performed on all core study vehicle control, 2, and 4 g/kg rats; vehicle control, 1, 2, and 4 g/kg mice; and all early death animals. Tissues were examined in the remaining core study groups to a no-effect level. Right kidneys of special study and core study rats were also examined for hyaline droplets using the Mallory Heidenhain technique. Tissues examined microscopically are listed in Table 1.

2-Year Studies

Study Design

Groups of 50 male and 50 female rats and mice received β-myrcene in corn oil by gavage at doses of 0.25, 0.5, and 1 g/kg body weight 5 days per week for 104 (female mice) or 105 weeks (rats and male mice). Groups of 50 male and 50 female vehicle control rats and mice received the corn oil vehicle alone.

Source and Specification of Animals

Male and female F344/N rats and B6C3F1 mice were obtained from Taconic Farms, Inc. (Germantown, NY), for use in the 2-year studies. Rats were quarantined for 11 (males) or 12 (females) days, and mice were quarantined for 13 (females) or 14 (males) days before the beginning of the studies. Five male and five female rats and mice were randomly selected for parasite evaluation and gross observation of disease. Rats were 5 to 6 weeks old, and

mice were 6 to 7 weeks old at the beginning of the studies. The health of the animals was monitored during the studies according to the protocols of the NTP Sentinel Animal Program (Appendix K).

Animal Maintenance

Male rats were housed three per cage; female rats and mice were housed five per cage, and male mice were housed individually. Feed and water were available *ad libitum*. Cages and racks were rotated every 2 weeks. Further details of animal maintenance are given in Table 1. Information on feed composition and contaminants is provided in Appendix J.

Clinical Examinations and Pathology

All animals were observed twice daily. Body weights were recorded on study day 1, weekly for the first 13 weeks, at 4-week intervals thereafter, and at study termination. Clinical findings were recorded at study week 5, at 4-week intervals thereafter, and at study termination.

Complete necropsies and microscopic examinations were performed on all rats and mice. At necropsy, all organs and tissues were examined for grossly visible lesions, and all major tissues were fixed and preserved in 10% neutral buffered formalin (except eyes, which were fixed in Davidson's solution before being transferred to 10% neutral buffered formalin), processed and trimmed, embedded in paraffin, sectioned to a thickness of 4 to 6 µm, and stained with hematoxylin and eosin for microscopic examination. For all paired organs (e.g., adrenal gland, kidney, ovary), samples from each organ were examined. For extended evaluation of renal proliferative lesions, kidneys were step sectioned at approximately 1 mm intervals and three to four additional sections were obtained from each kidney. Tissues examined microscopically are listed in Table 1.

Microscopic evaluations were completed by the study laboratory pathologist, and the pathology data were entered into the Toxicology Data Management System. The slides, paraffin blocks, and residual wet tissues were sent to the NTP Archives for inventory, slide/block match, and wet tissue audit. The slides, individual animal data records, and pathology tables were evaluated by an independent quality assessment laboratory. The individual

animal records and tables were compared for accuracy; the slide and tissue counts were verified, and the histotechnique was evaluated. For the 2-year studies, a quality assessment pathologist evaluated slides from all tumors and all potential target organs, which included the forestomach, kidney, liver, and spleen of rats and mice; nose of rats and female mice; eye of male rats; thyroid gland and uterus of female rats; bone marrow, heart, lung, and mesenteric lymph node of male and female mice; adrenal cortex, esophagus, mandibular lymph node, and testis of male mice; and thymus of female mice.

The quality assessment report and the reviewed slides were submitted to the NTP Pathology Working Group (PWG) coordinator, who reviewed the selected tissues and addressed any inconsistencies in the diagnoses made by the laboratory and quality assessment pathologists. Representative histopathology slides containing examples of lesions related to chemical administration, examples of disagreements in diagnoses between the laboratory and quality assessment pathologists, or lesions of general interest were presented by the coordinator to the PWG for review. The PWG consisted of the quality assessment pathologist and other pathologists experienced in rodent toxicologic pathology. This group examined the tissues without any knowledge of dose groups or previously rendered diagnoses. When the PWG consensus differed from the opinion of the laboratory pathologist, the diagnosis was changed. Final diagnoses for reviewed lesions represent a consensus between the laboratory pathologist, reviewing pathologist(s), and the PWG. Details of these review procedures have been described, in part, by Maronpot and Boorman (1982) and Boorman *et al.* (1985). For subsequent analyses of the pathology data, the decision of whether to evaluate the diagnosed lesions for each tissue type separately or combined was generally based on the guidelines of McConnell *et al.* (1986).

Table 1
Experimental Design and Materials and Methods in the Gavage Studies of β-Myrcene

3-Month Studies	2-Year Studies
Study Laboratory	
Battelle Columbus Operations (Columbus, OH)	Battelle Columbus Operations (Columbus, OH)
Strain and Species	
F344/N rats B6C3F1 mice	F344/N rats B6C3F1 mice
Animal Source	
Taconic Farms, Inc. (Germantown, NY)	Taconic Farms, Inc. (Germantown, NY)
Time Held Before Studies	
Rats: 11 (males) or 12 (females) days Mice: 13 (females) or 14 (males) days	Rats: 11 (males) or 12 (females) days Mice: 13 (females) or 14 (males) days
Average Age When Studies Began	
Rats: 5 weeks Mice: 5-6 weeks	Rats: 5-6 weeks Mice: 6-7 weeks
Date of First Dose	
Rats: January 29 (males) or 30 (females), 2001 Mice: January 31 (females) or February 1 (males), 2001	Rats: March 25 (males) or 26 (females), 2002 Mice: April 10 (females) or 11 (males), 2002
Duration of Dosing	
Rats: core study, 5 days/week for 14 weeks; special study, 5 days/week for 22 days Mice: 5 days/week for 14 weeks	Rats and male mice: 5 days/week for 105 weeks Female mice: 5 days/week for 104 weeks
Date of Last Dose	
Rats: April 30 (males) or May 1 (females), 2001 Mice: May 2 (females) or 3 (males), 2001	Rats: March 23 (males) or 25 (females), 2004 Mice: April 6 (females) or 8 (males), 2004
Necropsy Dates	
Rats: May 1 (males) or 2 (females), 2001 Mice: May 3 (females) or 4 (males), 2001	Rats: March 22-24 (males) or 24-26 (females), 2004 Mice: April 5-7 (females) or 7-9 (males), 2004
Average Age at Necropsy	
Rats: 18 weeks (core study) Mice: 18-19 weeks	110 weeks
Size of Study Groups	
10 males and 10 females	50 males and 50 females
Method of Distribution	
Animals were distributed randomly into groups of approximately equal initial mean body weights.	Same as 3-month studies
Animals per Cage	
Rats: 5 Mice: 1 (males) or 5 (females)	Rats: 3 (males) or 5 (females) Mice: 1 (males) or 5 (females)

TABLE 1
Experimental Design and Materials and Methods in the Gavage Studies of β-Myrcene

3-Month Studies	2-Year Studies
Method of Animal Identification	
Tail tattoo	Tail tattoo
Diet	
Irradiated NTP-2000 wafer feed (Zeigler Brothers, Inc., Gardners, PA), available *ad libitum,* changed at least weekly	Same as 3-month studies
Water	
Tap water (City of Columbus municipal supply) via automatic watering system (Edstrom Industries, Waterford, WI), available *ad libitum*	Same as 3-month studies
Cages	
Polycarbonate cages (Lab Products, Inc., Seaford, DE), changed at least twice weekly (rats) or at least weekly (mice)	Polycarbonate cages (Lab Products, Inc., Seaford, DE), changed at least twice weekly (rats and female mice) or at least weekly (male mice)
Bedding	
Irradiated Sani-Chips® (P.J. Murphy Forest Products Corp., Montville, NJ), changed at least twice weekly (rats) or at least weekly (mice)	Irradiated Sani-Chips® (P.J. Murphy Forest Products Corp., Montville, NJ), changed at least twice weekly (rats and female mice) or at least weekly (male mice)
Cage Filters	
Spun-bonded polyester filter sheets (Snow Filtration Co., Cincinnati, OH), changed every 2 weeks	Same as 3-month studies
Racks	
Stainless steel (Lab Products, Inc., Seaford, DE), changed and rotated every 2 weeks	Same as 3-month studies
Animal Room Environment	
Temperature: 72° ± 3° F Relative humidity: 50% ± 15% Room fluorescent light: 12 hours/day Room air changes: 10/hour	Temperature: 72° ± 3° F Relative humidity: 50% ± 15% Room fluorescent light: 12 hours/day Room air changes: 10/hour
Doses	
0, 0.25, 0.5, 1, 2, and 4 g/kg	0, 0.25, 0.5, and 1 g/kg
Type and Frequency of Observation	
Observed twice daily; core study animals were weighed and clinical findings were recorded initially, weekly, and at the end of the studies.	Observed twice daily; animals were weighed initially, weekly for 13 weeks, monthly thereafter, and at the end of the studies. Clinical findings were recorded monthly beginning at week 5 and at the end of the studies.
Method of Sacrifice	
Carbon dioxide asphyxiation	Same as 3-month studies
Necropsy	
Necropsies were performed on all core study animals. Organs weighed were heart, right kidney, liver, lung, right testis, and thymus.	Necropsies were performed on all animals.

Table 1
Experimental Design and Materials and Methods in the Gavage Studies of β-Myrcene

3-Month Studies	2-Year Studies
Clinical Pathology	
Blood was collected from the retroorbital sinus of special study rats on day 23 and core study rats and mice surviving to the end of the studies for hematology (rats and mice) and clinical chemistry (rats). ***Hematology:*** hematocrit; hemoglobin concentration; erythrocyte, reticulocyte, and platelet counts; mean cell volume; mean cell hemoglobin; mean cell hemoglobin concentration; and leukocyte count and differentials ***Clinical chemistry:*** urea nitrogen, creatinine, total protein, albumin, alanine aminotransferase, alkaline phosphatase, creatine kinase, sorbital dehydrogenase, and total bile acids	None
Histopathology	
Complete histopathologic examinations were performed on all core study 0, 2, and 4 g/kg rats; 0, 1, 2, and 4 g/kg mice;.and all animals that died early. In addition to gross lesions and tissue masses, the following tissues were examined to the no-effect level: adrenal gland, bone with marrow, brain, clitoral gland, esophagus, eye, gallbladder (mice only), Harderian gland, heart, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), liver, lung, lymph nodes (mandibular and mesenteric), mammary gland, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, skin, spleen, stomach (forestomach and glandular), right testis with epididymis and seminal vesicle, thymus, thyroid gland, trachea, urinary bladder, and uterus. The right kidney was examined in all special study and core study rats and mice. The bone marrow, lymph nodes (mandibular and mesenteric), nose, pancreas, spleen, and thymus were examined in all dosed groups of mice remaining.	Complete histopathologic examinations were performed on all rats and mice. In addition to gross lesions and tissue masses, the following tissues were examined: adrenal gland, bone with marrow, brain, clitoral gland, esophagus, eye, gallbladder (mice only), Harderian gland, heart, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, liver, lung, lymph nodes (mandibular and mesenteric), mammary gland, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, skin, spleen, stomach (forestomach and glandular), testis with epididymis and seminal vesicle, thymus, thyroid gland, trachea, urinary bladder, and uterus.
Sperm Motility and Vaginal Cytology	
At the end of the studies, sperm samples were collected from core study male animals in the 0, 0.25, 0.5, and 1 g/kg groups for sperm motility evaluations. The following parameters were evaluated: spermatid heads per testis and per gram testis, spermatid counts, and epididymal spermatozoal motility and concentration. The left cauda, left epididymis, and left testis were weighed. Vaginal samples were collected for up to 12 consecutive days prior to the end of the studies from core study females dosed with 0, 0.25, 0.5, and 1 g/kg for vaginal cytology evaluations. The percentage of time spent in the various estrous cycle stages and estrous cycle length were evaluated.	None

Statistical Methods

Survival Analyses

The probability of survival was estimated by the product-limit procedure of Kaplan and Meier (1958) and is presented in the form of graphs. Animals found dead of other than natural causes were censored from the survival analyses; animals dying from natural causes were not censored. Statistical analyses for possible dose-related effects on survival used Cox's (1972) method for testing two groups for equality and Tarone's (1975) life table test to identify dose-related trends. All reported P values for the survival analyses are two sided.

Calculation of Incidence

The incidences of neoplasms or nonneoplastic lesions are presented in Tables A1, A4, B1, B4, C1, C4, D1, and D4 as the numbers of animals bearing such lesions at a specific anatomic site and the numbers of animals with that site examined microscopically. For calculation of statistical significance, the incidences of most neoplasms (Tables A2, B2, C2, and D2) and all nonneoplastic lesions are given as the numbers of animals affected at each site examined microscopically. However, when macroscopic examination was required to detect neoplasms in certain tissues (e.g., Harderian gland, intestine, mammary gland, and skin) before microscopic evaluation, or when neoplasms had multiple potential sites of occurrence (e.g., leukemia or lymphoma), the denominators consist of the number of animals on which a necropsy was performed. Tables A2, B2, C2, and D2 also give the survival-adjusted neoplasm rate for each group and each site-specific neoplasm; the 1 g/kg male rats and male and female mice were excluded due to early mortality. This survival-adjusted rate (based on the Poly-3 method described below) accounts for differential mortality by assigning a reduced risk of neoplasm, proportional to the third power of the fraction of time on study, only to site-specific, lesion-free animals that do not reach terminal sacrifice.

Analysis of Neoplasm and Nonneoplastic Lesion Incidences

The Poly-k test (Bailer and Portier, 1988; Portier and Bailer, 1989; Piegorsch and Bailer, 1997) was used to assess neoplasm and nonneoplastic lesion prevalence. This test is a survival-adjusted quantal-response procedure that modifies the Cochran-Armitage linear trend test to take survival differences into account. More specifically, this

method modifies the denominator in the quantal estimate of lesion incidence to approximate more closely the total number of animal years at risk. For analysis of a given site, each animal is assigned a risk weight. This value is one if the animal had a lesion at that site or if it survived until terminal sacrifice; if the animal died prior to terminal sacrifice and did not have a lesion at that site, its risk weight is the fraction of the entire study time that it survived, raised to the kth power.

This method yields a lesion prevalence rate that depends only upon the choice of a shape parameter for a Weibull hazard function describing cumulative lesion incidence over time (Bailer and Portier, 1988). Unless otherwise specified, a value of k=3 was used in the analysis of site-specific lesions. This value was recommended by Bailer and Portier (1988) following an evaluation of neoplasm onset time distributions for a variety of site-specific neoplasms in control F344 rats and B6C3F1 mice (Portier *et al.,* 1986). Bailer and Portier (1988) showed that the Poly-3 test gave valid results if the true value of k was anywhere in the range from 1 to 5. A further advantage of the Poly-3 method is that it does not require lesion lethality assumptions. Variation introduced by the use of risk weights, which reflect differential mortality, was accommodated by adjusting the variance of the Poly-3 statistic as recommended by Bieler and Williams (1993).

Tests of significance included pairwise comparisons of each dosed group with controls and a test for an overall dose-related trend. Continuity-corrected Poly-3 tests were used in the analysis of lesion incidence, and reported P values are one sided. The significance of lower incidences or decreasing trends in lesions is represented as 1–P with the letter N added (e.g., P=0.99 is presented as P=0.01N).

Analysis of Continuous Variables

Two approaches were employed to assess the significance of pairwise comparisons between dosed and control groups in the analysis of continuous variables. Organ and body weight data, which historically have approximately normal distributions, were analyzed with the parametric multiple comparison procedures of Dunnett (1955) and Williams (1971, 1972). Hematology, clinical chemistry, spermatid, and epididymal spermatozoal data, which have

typically skewed distributions, were analyzed using the nonparametric multiple comparison methods of Shirley (1977) (as modified by Williams, 1986) and Dunn (1964). Jonckheere's test (Jonckheere, 1954) was used to assess the significance of the dose-related trends and to determine whether a trend-sensitive test (Williams' or Shirley's test) was more appropriate for pairwise comparisons than a test that does not assume a monotonic dose-related trend (Dunnett's or Dunn's test). Prior to statistical analysis, extreme values identified by the outlier test of Dixon and Massey (1957) were examined by NTP personnel, and implausible values were eliminated from the analysis. Because vaginal cytology data are proportions (the proportion of the observation period that an animal was in a given estrous stage), an arcsine transformation was used to bring the data into closer conformance with a normality assumption. Treatment effects were investigated by applying a multivariate analysis of variance (Morrison, 1976) to the transformed data to test for simultaneous equality of measurements across doses. Proportions of regular cycling females in each dosed group were compared to the vehicle control group using the Fisher exact test (Gart *et al.*, 1979). Tests for extended periods of estrus and diestrus were constructed based on a Markov chain model proposed by Girard and Sager (1987). For each dose group, a transition probability matrix was estimated for transitions among the proestrus, estrus, metestrus, and diestrus stages, with provision for extended stays within estrus and diestrus. Equality of transition matrices among dose groups and between the control group and each dosed group was tested using chi-square statistics.

Historical Control Data

The concurrent control group represents the most valid comparison to the treated groups and is the only control group analyzed statistically in NTP bioassays. However, historical control data are often helpful in interpreting potential treatment-related effects, particularly for uncommon or rare neoplasm types. For meaningful comparisons, the conditions for studies in the historical database must be generally similar. One significant factor affecting the background incidence of neoplasms at a variety of sites is diet. In 1995, the NTP incorporated a new diet (NTP-2000) that contains less protein and more fiber and fat than the NIH-07 diet previously used in toxicity and carcinogenicity studies (Rao, 1996, 1997). The current NTP historical database contains all studies that use the NTP-2000 diet with histopathology findings completed within the most recent 5-year period. A second

potential source of variability is route of administration. In general, the historical database for a given study will include studies using the same route of administration, and the overall incidences of neoplasms for all routes of administration are included for comparison, including the present study.

Quality Assurance Methods

The 3-month and 2-year studies were conducted in compliance with Food and Drug Administration Good Laboratory Practice Regulations (21 CFR, Part 58). In addition, as records from the 2-year studies were submitted to the NTP Archives, these studies were audited retrospectively by an independent quality assurance contractor. Separate audits covered completeness and accuracy of the pathology data, pathology specimens, final pathology tables, and a draft of this NTP Technical Report. Audit procedures and findings are presented in the reports and are on file at NIEHS. The audit findings were reviewed and assessed by NTP staff, and all comments were resolved or otherwise addressed during the preparation of this Technical Report.

Genetic Toxicology

The genetic toxicity of β-myrcene was assessed by testing the ability of the chemical to induce mutations in various strains of *Salmonella typhimurium* and *Escherichia coli* and increases in the frequency of micronucleated erythrocytes in mouse peripheral blood. Micronuclei (literally "small nuclei" or Howell-Jolly bodies) are biomarkers of induced structural or numerical chromosomal alterations and are formed when acentric fragments or whole chromosomes fail to incorporate into either of two daughter nuclei during cell division (Schmid, 1975; Heddle *et al.*, 1983). The protocols for these studies and the results are given in Appendix E.

The genetic toxicity studies have evolved from an earlier effort by the NTP to develop a comprehensive database permitting a critical anticipation of a chemical's carcinogenicity in experimental animals based on numerous considerations, including the molecular structure of the chemical and its observed effects in short-term *in vitro* and *in vivo* genetic toxicity tests (structure-activity relationships). The short-term tests were originally developed to

clarify proposed mechanisms of chemical-induced DNA damage based on the relationship between electrophilicity and mutagenicity (Miller and Miller, 1977) and the somatic mutation theory of cancer (Straus, 1981; Crawford, 1985). However, it should be noted that not all cancers arise through genotoxic mechanisms.

DNA reactivity combined with *Salmonella* mutagenicity is highly correlated with induction of carcinogenicity in multiple species/sexes of rodents and at multiple tissue sites (Ashby and Tennant, 1991). A positive response in the *Salmonella* test was shown to be the most predictive *in vitro* indicator for rodent carcinogenicity (89% of the *Salmonella* mutagens are rodent carcinogens) (Tennant *et al.,* 1987; Zeiger *et al.,* 1990). Additionally, no battery of tests that included the *Salmonella* test improved the predictivity of the *Salmonella* test alone. However, these other tests can provide useful information on the types of DNA and chromosomal damage induced by the chemical under investigation.

The predictivity for carcinogenicity of a positive response in acute *in vivo* bone marrow chromosome aberration or micronucleus tests appears to be less than that in the *Salmonella* test (Shelby *et al.,* 1993; Shelby and Witt, 1995). However, clearly positive results in long-term peripheral blood micronucleus tests have high predictivity for rodent carcinogenicity (Witt *et al.,* 2000); negative results in this assay do not correlate well with either negative or positive results in rodent carcinogenicity studies. Because of the theoretical and observed associations between induced genetic damage and adverse effects in somatic and germ cells, the determination of *in vivo* genetic effects is important to the overall understanding of the risks associated with exposure to a particular chemical. Most organic chemicals that are identified by the International Agency for Research on Cancer as human carcinogens, other than hormones, are genotoxic. The vast majority of these are detected by both the *Salmonella* assay and rodent bone marrow cytogenetics tests (Shelby, 1988; Shelby and Zeiger, 1990).

RESULTS

RATS

3-MONTH STUDY

Male and female rats administered 0.25 g/kg and females administered 0.5 g/kg or less survived until the end of the study (Table 2). All core study female rats in the 4 g/kg group died within the first week of the study; all 4 g/kg male rats died within 4 days of dosing except for one male that died on study day 11. One (0.5 and 1 g/kg) and two (2 g/kg) males died before the end of the study, and one (1 g/kg) and four (2 g/kg) females died before the end of the study. Vehicle control rats survived to the end of the study. Final mean body weights and mean body weight gains of males and females administered 0.5 g/kg or greater were significantly less than those of the vehicle controls. Except for lesion incidence data in groups administered 2 g/kg or less, data from rats that died early were excluded from the analysis and summary tables. Similarly, survival of special study rats in the 0.25, 0.5, and 1 g/kg groups of both sexes was similar to that of the vehicle controls. Survival of special study rats in the 2 g/kg group was significantly decreased during the third week of the study. Special study male and female rats in the 4 g/kg groups died by the end of the first week; their kidneys were not evaluated. No dose-related clinical findings were noted in animals surviving to the end of study. The dose-related clinical findings that occurred in animals that died early (some by moribund sacrifice) included thinness, lethargy, abnormal breathing, and ruffled fur. The cause of death of animals that died early was not determined. No clinical findings were seen in either sex receiving 1 g/kg or less.

In special study rats on day 23, a decrease (approximately 25%) in leukocyte counts, characterized by a decrease in lymphocytes, occurred in 2 g/kg males and females and was consistent with a transient, physiological (corticosteroid-induced-type) response (Tables 3 and F1). Such a response is consistent with the decreases in

TABLE 2
Survival and Body Weights of Rats in the 3-Month Gavage Study of β-Myrcene

Dose (g/kg)	Survival[a]	Mean Body Weight[b] (g) Initial	Final	Change	Final Weight Relative to Controls (%)
Male					
0	10/10	89 ± 6	330 ± 7	241 ± 7	
0.25	10/10	88 ± 5	323 ± 6	235 ± 8	98
0.5[c]	9/10[d]	88 ± 6	289 ± 10**	199 ± 9**	88
1[c]	9/10[e]	90 ± 6	293 ± 8**	201 ± 6**	89
2	8/10[f]	87 ± 6	256 ± 7**	169 ± 7**	78
4	0/10[g]	88 ± 5	——	—	
Female					
0	10/10	87 ± 4	195 ± 3	108 ± 4	
0.25	10/10	89 ± 4	195 ± 3	105 ± 4	100
0.5	10/10	88 ± 4	184 ± 3*	96 ± 2*	94
1[c]	9/10[h]	88 ± 4	184 ± 4*	96 ± 4*	94
2	6/10[i]	87 ± 4	176 ± 5**	86 ± 3**	90
4[c]	0/10[j]	89 ± 4	——	—	

* Significantly different (P≤0.05) from the vehicle control group by Williams' test
** P≤0.01
[a] Number of animals surviving at 3 months/number initially in group.
[b] Weights and weight changes are given as mean ± standard error. Subsequent calculations are based on animals surviving to the end of the study.
[c] One death in this group was a dosing accident.
[d] Week of death: 5
[e] Week of death: 4
[f] Weeks of death: 1, 3
[g] Weeks of death: 1, 1, 1, 1, 1, 1, 1, 1, 1, 2
[h] Week of death: 9
[i] Weeks of death: 1, 1, 1, 14
[j] Week of deaths: 1

final body weights in the 2 g/kg males and females. In core study rats at week 14, dose-related decreases (up to 30%) in creatinine concentration occurred in males and females and would be consistent with the decreased body weights. The leukon and creatinine effects were likely secondary biological effects. Other changes in hematology and clinical chemistry parameters seem inconsistent between sexes and in comparison to similar or complimentary markers.

TABLE 3
Selected Clinical Pathology Data for Rats in the 3-Month Gavage Study of β-Myrcene[a]

	Vehicle Control	0.25 g/kg	0.5 g/kg	1 g/kg	2 g/kg
Male					
Hematology					
n					
Day 23	10	10	10	9	5
Week 14	9	10	9	9	8
Leukocytes ($10^3/\mu L$)					
Day 23	10.54 ± 0.55	10.24 ± 0.40	9.65 ± 0.37	10.09 ± 0.64	7.70 ± 0.46**
Week 14	7.32 ± 0.28	8.49 ± 0.50	7.86 ± 0.55	7.52 ± 0.61	7.33 ± 0.39
Lymphocytes ($10^3/\mu L$)					
Day 23	9.22 ± 0.53	8.78 ± 0.42	8.43 ± 0.33	8.65 ± 0.54	6.00 ± 0.41**
Week 14	6.15 ± 0.29	7.31 ± 0.50	6.70 ± 0.56	6.45 ± 0.59	5.66 ± 0.19
Clinical Chemistry					
n					
Day 23	10	10	10	9	6
Week 14	10	10	9	9	8
Creatinine (mg/dL)					
Day 23	0.44 ± 0.02	0.49 ± 0.01	0.47 ± 0.02	0.49 ± 0.01*	0.52 ± 0.02**
Week 14	0.56 ± 0.02	0.58 ± 0.01	0.50 ± 0.00*	0.47 ± 0.02**	0.40 ± 0.00**
Female					
Hematology					
n					
Day 23	9	7	8	8	5
Week 14	10	9	10	9	6
Leukocytes ($10^3/\mu L$)					
Day 23	11.08 ± 0.37	12.14 ± 0.67	10.45 ± 0.36	9.60 ± 0.58	8.36 ± 0.48**
Week 14	7.83 ± 0.66	7.53 ± 0.29	8.45 ± 0.62	8.92 ± 0.66	7.72 ± 0.52
Lymphocytes ($10^3/\mu L$)					
Day 23	9.74 ± 0.34	10.76 ± 0.63	9.26 ± 0.34	8.46 ± 0.51	7.27 ± 0.63**
Week 14	6.76 ± 0.52	6.23 ± 0.24	7.14 ± 0.47	7.83 ± 0.58	6.42 ± 0.43
Clinical Chemistry					
n	10	10	10	9	6
Creatinine (mg/dL)					
Day 23	0.49 ± 0.01	0.48 ± 0.01	0.48 ± 0.01	0.48 ± 0.02	0.48 ± 0.02
Week 14	0.57 ± 0.02	0.50 ± 0.02**	0.48 ± 0.01**	0.49 ± 0.01**	0.43 ± 0.02**

* Significantly different ($P \leq 0.05$) from the vehicle control group by Dunn's or Shirley's test
** $P \leq 0.01$
[a] Mean ± standard error. Statistical tests were performed on unrounded data. No data available for 4 g/kg males or females due to 100% mortality.

The absolute and relative right kidney and liver weights of both sexes in all dosed groups were significantly greater than those of the vehicle controls with the exception of the absolute liver weight of the 2 g/kg males (Tables 4 and G1). Decreased absolute and relative thymus weights occurred in the 2 g/kg males (Tables 4 and G1). There were no significant changes seen in the weights of the reproductive organs nor in the sperm parameters or estrous cyclicity of the male or female rats at any dose level (Tables H1 and H2).

TABLE 4
Selected Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 3-Month Gavage Study of β-Myrcene[a]

	Vehicle Control	0.25 g/kg	0.5 g/kg	1 g/kg	2 g/kg
Male					
n	10	10	9	9	8
Necropsy body wt	341 ± 7	335 ± 7	318 ± 5*	300 ± 8**	255 ± 8**
R. Kidney					
Absolute	0.964 ± 0.020	1.186 ± 0.021**	1.306 ± 0.028**	1.524 ± 0.033**	1.792 ± 0.064**
Relative	2.826 ± 0.049	3.545 ± 0.033**	4.109 ± 0.045**	5.099 ± 0.092**	7.014 ± 0.121**
Liver					
Absolute	11.47 ± 0.21	12.76 ± 0.35*	12.78 ± 0.29*	13.44 ± 0.32**	12.55 ± 0.43
Relative	33.688 ± 0.742	38.084 ± 0.398**	40.205 ± 0.563**	44.930 ± 0.653**	49.121 ± 0.647**
Thymus					
Absolute	0.350 ± 0.016	0.340 ± 0.018	0.285 ± 0.009**	0.272 ± 0.015**	0.205 ± 0.017**
Relative	1.024 ± 0.040	1.013 ± 0.048	0.899 ± 0.038	0.913 ± 0.052	0.795 ± 0.052**
Female					
n	10	10	10	9	6
Necropsy body wt	196 ± 3	196 ± 3	187 ± 3	188 ± 3	185 ± 5
R. Kidney					
Absolute	0.633 ± 0.012	0.799 ± 0.012**	0.828 ± 0.019**	0.953 ± 0.027**	1.197 ± 0.043**
Relative	3.229 ± 0.056	4.091 ± 0.062**	4.418 ± 0.056**	5.055 ± 0.085**	6.483 ± 0.143**
Liver					
Absolute	5.990 ± 0.162	6.717 ± 0.109**	7.022 ± 0.164**	7.819 ± 0.219**	9.421 ± 0.326**
Relative	30.533 ± 0.641	34.407 ± 0.622**	37.463 ± 0.463**	41.499 ± 0.831**	51.003 ± 0.867**
Thymus					
Absolute	0.265 ± 0.009	0.256 ± 0.009	0.248 ± 0.012	0.266 ± 0.009	0.224 ± 0.008*
Relative	1.353 ± 0.046	1.313 ± 0.050	1.321 ± 0.057	1.410 ± 0.036	1.213 ± 0.042

* Significantly different ($P \leq 0.05$) from the vehicle control group by Williams' or Dunnett's test
** $P \leq 0.01$
[a] Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error). No data available for 4 g/kg males or females due to 100% mortality.

In special study rats evaluated on day 23, the incidences and severities of chronic progressive nephropathy (CPN) and renal tubule necrosis were increased in 2 g/kg males, and the incidence and severity of hyaline droplet accumulation in the renal tubule epithelium was decreased in this group (Table 5). All 4 g/kg rats of both sexes, four males and four females from the 2 g/kg groups, and one male and one female from the 1 g/kg groups died before day 23, and their kidneys were not evaluated. Hyaline droplets were not noted in any of the females. CPN is a spontaneous disease of F344/N rats that may be exacerbated by exposure to some toxins. In this study, the lesions were multifocally distributed in the renal cortex and were characterized by tubule basophilia, chronic interstitial inflammation, and protein casts within the renal cortex and occasionally in the medulla. Renal tubule necrosis was characterized by tubule epithelial cell vacuolation and necrosis with flattening and elongation of the remaining epithelial cells, tubule dilation, formation of cellular and protein casts, and interstitial edema and was located in the outer stripe of the outer medulla. Hyaline droplets were characterized by the presence of small, round, eosinophilic granules in the cytoplasm of tubule epithelial cells in the renal cortex. They were present in all male groups, including vehicle controls. There was a slight dose-related increase in the size and/or number of

TABLE 5
Incidences of Selected Nonneoplastic Lesions of the Kidney in Rats in the 23-Day Gavage Special Study of β-Myrcene[a]

	Vehicle Control	0.25 g/kg	0.5 g/kg	1 g/kg	2 g/kg
Male					
Number Examined Microscopically	10	10	10	9	6
Renal Tubule, Necrosis[b]	0	0	0	0	6** (3.0)[c]
Nephropathy	0	0	0	1 (1.0)	6** (1.7)
Renal Tubule, Accumulation, Hyaline Droplet	10 (1.8)	10 (2.4)	10 (2.5)	9 (2.0)	2 (1.0)
Female					
Number Examined Microscopically	10	10	10	9	6
Renal Tubule, Necrosis	0	0	2 (1.0)	9** (2.1)	6** (2.8)
Nephropathy	0	2 (1.0)	0	0	6** (1.2)

** Significantly different (P≤0.01) from the vehicle control group by the Fisher exact test
[a] No data presented for 4 g/kg males or females due to early mortality.
[b] Number of animals with lesion
[c] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

hyaline droplets in the 0.25, 0.5, and 1 g/kg groups relative to the vehicle controls. However, in 2 g/kg males, the incidence of hyaline droplet accumulation was decreased and the droplets were typically fewer, smaller, and more uniformly round than those in vehicle controls; in vehicle controls and 0.25, 0.5, and 1 g/kg groups, the droplets were often elliptical or brick shaped.

The renal response to β-myrcene administration is complex, having multiple manifestations. At the end of the 3-month study, the only treatment-related gross lesion was a granular appearance of the kidney in the core study 2 g/kg males. Microscopically, the incidences of renal tubule necrosis were significantly increased in all dosed groups of males and females, but this lesion did not occur in the vehicle controls (Table 6). Slight increases in the incidence of CPN in 1 g/kg females and the severity of CPN in 2 g/kg males were found as well as significant increases in the incidences of nephrosis in 1 and 2 g/kg males and females. Additionally, there was an increase in the severity of nephrosis in the 2 g/kg males. Renal tubule necrosis was characterized by tubule epithelial cell vacuolation and necrosis (evidenced by occasional karyorrhectic and karyolytic tubule epithelium) with flattening and elongation of the remaining epithelial cells, tubule dilation, and interstitial edema. Nephrosis was originally diagnosed in the 2-year study of β-myrcene in rats as a constellation of related lesions (see description in the results section for the 2-year study in rats). An early nephrosis lesion, characterized by nuclear enlargement of the renal tubule cells and, in the more severe cases, minimal dilation of the tubules, was subsequently identified in the subchronic study during a reexamination of the slides. The nephrosis was restricted to the outer stripe of the outer medulla. CPN was characterized as described for the special study animals on day 23.

Treatment-related increases in the incidences and severities of hyaline droplet accumulation were found in 0.25, 0.5, and 1 g/kg males; hyaline droplet accumulation was not observed in the 2 g/kg males. No evidence of hyaline droplet accumulation was found in any female rats. In males, the severity of the hyaline droplet accumulation was greatest in the 0.5 g/kg group, but the severity of nephrosis was greatest at 2 g/kg. In the core study, the term *hyaline droplet accumulation* reflected not only an increase in the number of cells containing hyaline droplets but also a change in the normal pattern of the hyaline droplets in the proximal renal tubule epithelial cells and the

TABLE 6
Incidences of Selected Nonneoplastic Lesions in Rats in the 3-Month Gavage Study of β-Myrcene[a]

	Vehicle Control	0.25 g/kg	0.5 g/kg	1 g/kg	2 g/kg
Male					
Kidney[b]	7	10	9	10	10
Renal Tubule, Necrosis[c]	0	10** (1.0)[d]	9** (1.1)	10** (1.8)	10** (2.9)
Nephropathy	7 (1.0)	10 (1.0)	9 (1.3)	8 (1.0)	9 (1.9)
Nephrosis	0	0	1 (1.0)	10** (1.0)	9** (2.7)
Renal Tubule, Accumulation, Hyaline Droplet	0	10** (2.0)	9** (2.4)	10** (2.1)	0
Nose	10	10	10	10	10
Olfactory Epithelium, Degeneration	0	0	0	2 (1.0)	8** (2.6)
Inflammation, Suppurative	0	0	0	1 (1.0)	3 (1.0)
Inflammation, Chronic	0	0	1 (1.0)	6** (1.0)	8** (1.1)
Spleen	10	10	10	10	10
Atrophy	0	0	1 (2.0)	0	10** (1.8)
Mesenteric Lymph Node	10	0	1	1	10
Atrophy	0		0	1 (1.0)	6** (1.7)
Harderian Gland	10	10	10	10	9
Pigmentation, Porphyrin	1 (1.0)	3 (1.0)	7** (1.0)	8** (1.1)	9** (1.3)
Female					
Kidney	10	10	10	10	10
Renal Tubule, Necrosis	0	10** (1.0)	10** (1.0)	9** (2.2)	9** (2.4)
Nephropathy	1 (1.0)	2 (1.0)	3 (1.0)	4 (1.0)	1 (1.0)
Nephrosis	0	0	0	10** (1.0)	7** (1.1)
Nose	10	0	10	10	10
Olfactory Epithelium, Degeneration	0		0	2 (1.5)	7** (2.9)
Inflammation, Suppurative	0		0	1 (1.0)	6** (1.7)
Inflammation, Chronic	0		0	9** (1.0)	4* (1.5)
Spleen	10	0	10	10	10
Atrophy	0		0	1 (1.0)	10** (1.8)
Mesenteric Lymph Node	10	0	0	3	10
Atrophy	0			2** (1.0)	4* (1.8)
Forestomach	10	0	0	10	10
Inflammation, Acute	0			0	4* (1.8)

* Significantly different (P≤0.05) from the vehicle control group by the Fisher exact test
** P≤0.01
[a] No data presented for 4 g/kg males or females due to early mortality.
[b] Number of animals with tissue examined microscopically
[c] Number of animals with lesion
[d] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

presence of granular casts or developing granular casts in the outer medulla. These lesions are consistent with α2u-globulin nephropathy (Hard *et al.*, 1993). The change in the hyaline droplet pattern consisted of decreased numbers of cells with apical accumulations of hyaline droplets and increased numbers of large, angular, crystalloid droplets. In addition to hematoxylin and eosin, kidney sections were stained using the Mallory-Heidenhain method in order to better visualize and assess hyaline droplet accumulation.

The chemical-related lesions of the nose were degeneration of the olfactory epithelium, necrosis of the respiratory epithelium, suppurative inflammation, and chronic inflammation (Table 6). The incidences and severities of olfactory epithelial degeneration were greater in the 2 g/kg males and females than in the vehicle controls. The incidence of respiratory epithelial necrosis was slightly increased in 2 g/kg females. The incidences of suppurative inflammation in the nose were greater in the 2 g/kg males and females than in the vehicle controls, and the increase was significant in 2 g/kg females. The incidences of chronic inflammation in 1 and 2 g/kg males and females were significantly increased compared to the vehicle controls. Olfactory epithelial degeneration was characterized by decreased cellularity, disorganization of epithelial cells, and nuclear pyknosis. Respiratory epithelial necrosis was characterized by decreased cellularity, disorganization of epithelial cells, nuclear pyknosis, erosions and ulcers, and focal accumulation of inflammatory cells. A decrease in the size of the glands associated with the respiratory epithelium and a change from columnar cells to cuboidal or flattened cells within the glands were also noted. Both lesions primarily involved the dorsal to middle septum and turbinates.

The incidences of splenic atrophy were significantly increased in the 2 g/kg groups of both sexes (Table 6). One male and three females in the 2 g/kg groups had thymic necrosis. In the mesenteric lymph node, the incidences of atrophy were increased in 2 g/kg males and 1 and 2 g/kg females. Splenic atrophy was distinguished by an overall decrease in splenic size due to lymphoid depletion with focal areas of lymphocytic necrosis and loss of red pulp. In the thymus, necrosis was characterized by multifocal pyknosis of cortical lymphocytes. In the mesenteric lymph node, atrophy was characterized by lymphoid depletion. The lymphoid changes in these organs are considered secondary to morbidity rather than a direct toxic effect of β-myrcene.

The incidence of acute inflammation of the forestomach, characterized by neutrophilic infiltration of the forestomach epithelium, was significantly increased in 2 g/kg females (Table 6).

The incidences of porphyrin pigment in the Harderian gland of males from the 0.5 g/kg or greater groups were significantly increased in comparison to the vehicle control incidences. While porphyrin pigment is normally found in the Harderian gland, the increased amounts of pigment were distinguished by the intracytoplasmic accumulation of brownish-gold, granular material (concretions) beyond that seen in vehicle controls.

Dose Selection Rationale: Based on decreased survival and body weight gains of the 2 and 4 g/kg groups, the highest dose selected for the 2-year gavage study in rats was 1 g/kg. The severity of the renal tubule necrosis seen in the 1 g/kg rats was not considered sufficient to adversely affect survival in a 2-year study. Lack of clinical chemistry abnormalities at 1 g/kg also suggested that the morphologic changes were not impairing renal function.

2-Year Study

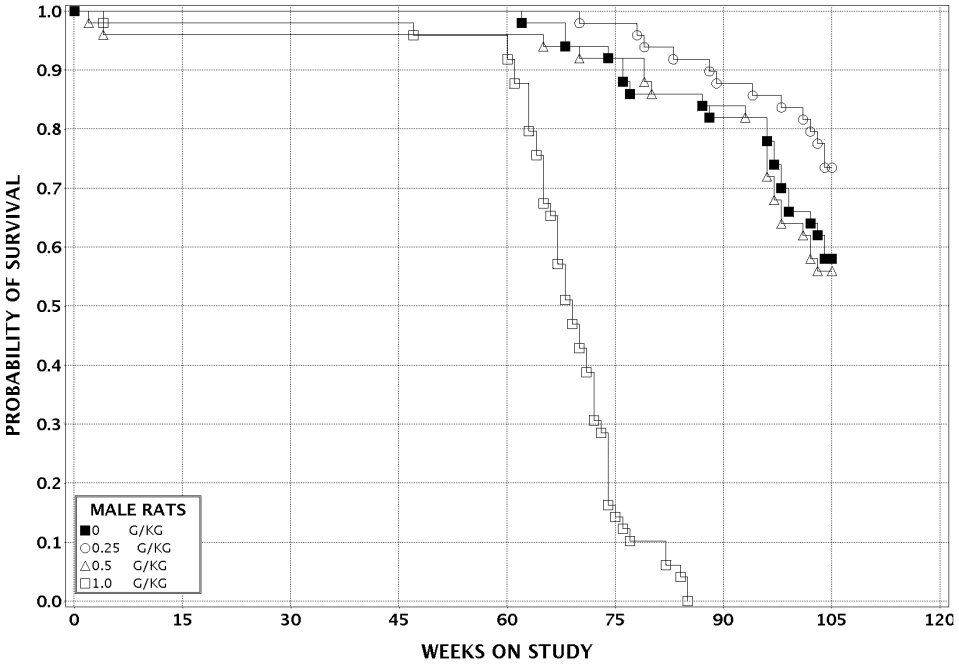
Survival

Estimates of 2-year survival probabilities for male and female rats are shown in Table 7 and in the Kaplan-Meier survival curves (Figure 2). Three males administered 1 g/kg died before week 60, and the remainder died by week 89. Death in male rats was attributed to renal toxicity. Survival of 0.25 and 0.5 g/kg males and all dosed groups of females was similar to that of the vehicle controls.

Table 7
Survival of Rats in the 2-Year Gavage Study of β-Myrcene

	Vehicle Control	0.25 g/kg	0.5 g/kg	1 g/kg
Male				
Animals initially in study	50	50	50	50
Accidental deaths[a]	0	1	0	1
Moribund	18	8	13	24
Natural deaths	3	5	9	25
Animals surviving to study termination	29	36	28	0
Percent probability of survival at end of study[b]	58	74	56	0
Mean survival (days)[c]	683	696	664	467
Survival analysis[d]	P<0.001	P=0.127N	P=0.895	P<0.001
Female				
Animals initially in study	50	50	50	50
Accidental deaths	0	2	0	1
Moribund	11	9	12	7
Natural deaths	8	6	10	9
Animals surviving to study termination	31	33	28	33
Percent probability of survival at end of study	62	69	56	67
Mean survival (days)	668	664	651	653
Survival analysis	P=0.815N	P=0.566N	P=0.625	P=0.657N

[a] Censored from survival analyses
[b] Kaplan-Meier determinations
[c] Mean of all deaths (uncensored, censored, and terminal sacrifice).
[d] The result of the life table trend test (Tarone, 1975) is in the vehicle control column, and the results of the life table pairwise comparisons (Cox, 1972) with the vehicle controls are in the dosed group columns. A negative trend or lower mortality in a dose group is indicated by **N**.



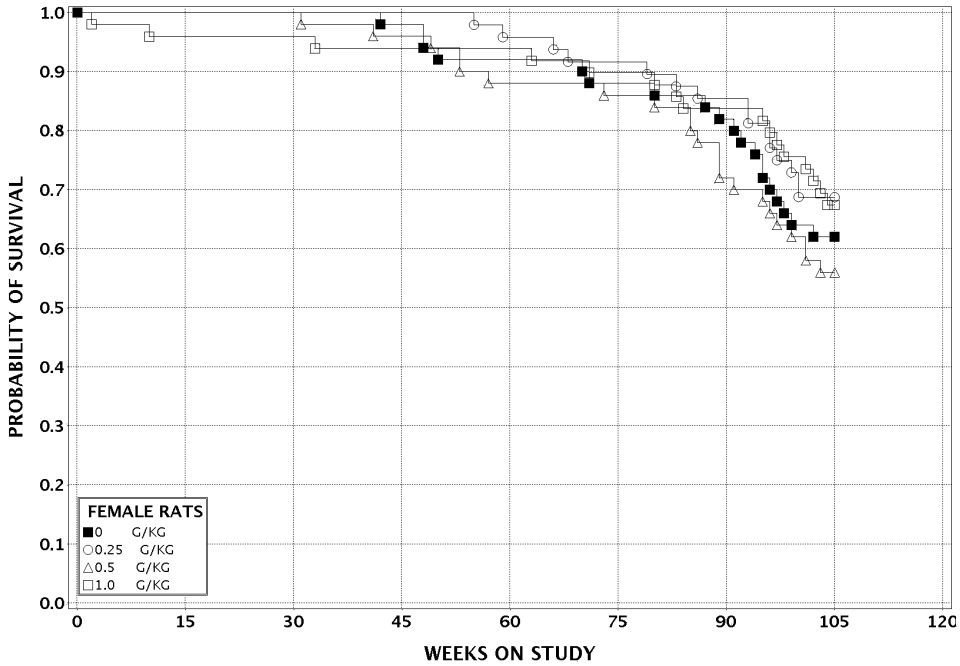
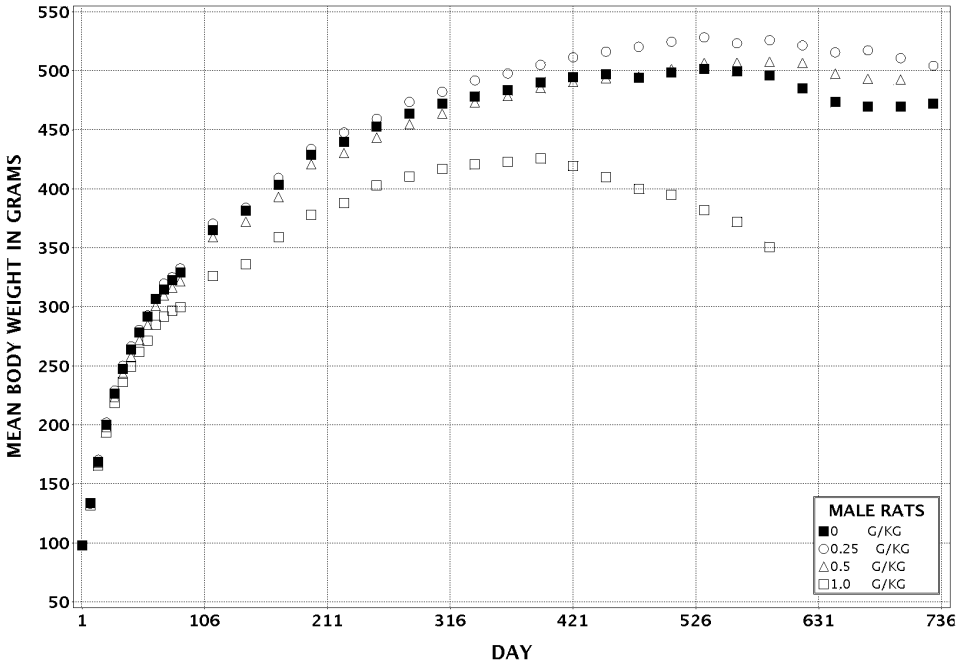


FIGURE 2
Kaplan-Meier Survival Curves for Male and Female Rats Administered β-Myrcene by Gavage for 2 Years

Body Weights and Clinical Findings

Mean body weights of 0.25 g/kg females were similar to those of the vehicle controls (Figure 3; Tables 8 and 9). The mean body weights of 1 g/kg males and females were less than those of the vehicle controls after weeks 7 and 13, respectively. The mean body weights of 0.25 and 0.5 g/kg males were slightly greater than those of the vehicle controls late in the study. The mean body weights of 0.5 g/kg females were less than those of the vehicle controls during much of the study but were similar by the end of the study. Approximately 22% of the 1 g/kg males were thin after approximately week 60 of the study. The most frequent toxicologically relevant clinical observations were thinness, lethargy, ruffled fur, and ocular and nasal discharge.



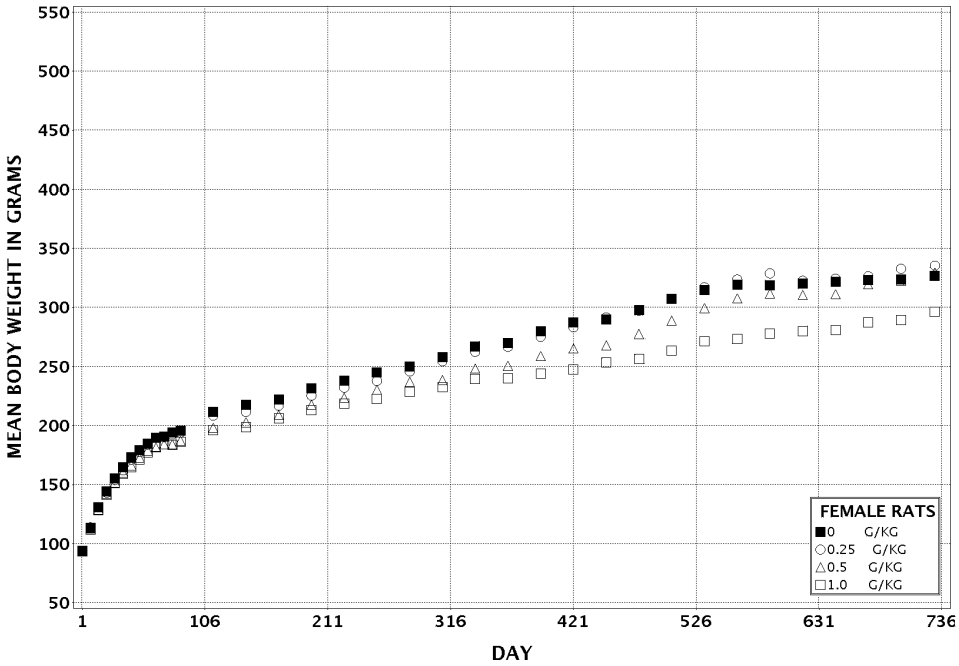


FIGURE 3
Growth Curves for Male and Female Rats Administered β-Myrcene by Gavage for 2 Years

TABLE 8
Mean Body Weights and Survival of Male Rats in the 2-Year Gavage Study of β-Myrcene

Days on Study	Vehicle Control		0.25 g/kg			0.5 g/kg			1 g/kg		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	98	50	98	100	50	98	100	50	98	100	50
8	134	50	133	100	50	134	100	50	132	99	50
15	169	50	171	101	50	168	99	49	166	98	50
22	200	50	202	101	50	198	99	49	194	97	50
29	226	50	229	101	50	224	99	48	218	97	49
36	248	50	250	101	50	244	98	48	237	96	49
43	264	50	267	101	50	258	98	48	249	95	49
50	278	50	281	101	50	272	98	48	262	94	49
57	292	50	293	100	50	285	98	48	271	93	49
64	307	50	307	100	50	301	98	48	285	93	49
71	315	50	320	102	50	310	98	48	292	93	48
78	323	50	325	101	50	316	98	48	297	92	48
85	329	50	333	101	50	322	98	48	300	91	48
113	365	50	371	102	50	359	98	48	326	89	48
141	382	50	384	101	50	372	98	48	336	88	48
169	404	50	409	101	50	393	97	48	359	89	48
197	429	50	434	101	50	421	98	48	378	88	48
225	440	50	448	102	50	431	98	48	388	88	48
253	453	50	460	102	50	444	98	48	403	89	48
281	464	50	474	102	49	455	98	48	411	89	48
309	472	50	482	102	49	464	98	48	417	88	48
337	478	50	492	103	49	473	99	48	421	88	47
365	484	50	498	103	49	479	99	48	423	87	47
393	490	50	505	103	49	486	99	48	426	87	47
421	495	50	512	103	49	491	99	48	419	85	45
449	497	49	516	104	49	494	99	47	410	83	37
477	494	47	520	105	49	495	100	47	400	81	25
505	499	47	525	105	48	502	101	46	395	79	15
533	502	44	528	105	48	507	101	46	382	76	6
561	500	43	523	105	46	507	102	43	372	75	5
589	496	43	526	106	45	508	102	43	351	71	2
617	485	41	522	108	44	507	105	42			
645	474	41	516	109	43	498	105	42			
673	470	39	517	110	42	493	105	35			
701	470	33	511	109	41	493	105	32			
Mean for weeks											
1-13	245		247	101		241	98		231	95	
14-52	432		439	102		423	98		382	89	
53-101	489		517	106		497	102		398	80	

TABLE 9
Mean Body Weights and Survival of Female Rats in the 2-Year Gavage Study of β-Myrcene

Days on Study	Vehicle Control		0.25 g/kg			0.5 g/kg			1 g/kg		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	94	50	94	101	50	94	100	50	94	100	50
8	113	50	114	101	50	113	100	50	112	99	50
15	131	50	131	100	50	129	98	50	128	98	49
22	145	50	143	99	50	142	99	50	141	98	49
29	156	50	154	99	50	152	98	50	151	97	49
36	165	50	162	98	50	160	97	50	159	97	49
43	174	50	171	99	50	167	96	50	165	95	49
50	179	50	178	99	50	173	96	50	171	95	48
57	185	50	184	100	50	179	97	50	178	96	48
64	190	50	190	100	50	182	96	50	182	96	47
71	191	50	190	100	49	184	97	50	184	97	47
78	194	50	193	99	49	184	95	50	184	95	47
85	196	50	194	99	49	187	96	50	186	95	47
113	212	50	209	99	49	198	94	50	196	93	47
141	218	50	212	97	49	203	93	50	199	91	47
169	222	50	217	98	49	209	94	50	206	93	47
197	231	50	225	97	48	218	94	50	213	92	47
225	238	50	232	97	48	224	94	49	219	92	46
253	245	50	238	97	48	230	94	49	223	91	46
281	250	50	246	98	48	237	95	49	229	92	46
309	258	49	255	99	48	239	92	48	233	90	46
337	267	47	263	98	48	248	93	48	240	90	46
365	270	46	267	99	48	251	93	47	240	89	46
393	280	46	275	98	47	259	92	45	244	87	46
421	288	46	283	99	46	265	92	44	248	86	46
449	290	46	292	101	46	268	93	44	253	87	45
477	298	46	297	100	44	278	93	44	257	86	45
505	307	44	307	100	44	289	94	43	264	86	44
533	315	44	317	101	44	299	95	43	272	86	44
561	319	43	324	101	43	308	96	42	274	86	43
589	319	43	329	103	42	312	98	42	278	87	41
617	320	42	323	101	41	311	97	39	280	87	41
645	322	39	324	101	41	311	97	35	281	87	41
673	323	35	326	101	36	320	99	32	287	89	39
701	324	32	333	103	33	323	100	30	289	89	37
Mean for weeks											
1-13	162		161	99		157	97		157	97	
14-52	238		233	98		223	94		217	91	
53-101	306		307	100		292	95		267	87	

Pathology and Statistical Analyses

This section describes the statistically significant or biologically noteworthy changes in the incidences of neoplasms and/or nonneoplastic lesions of the kidney, liver, lung, nose, forestomach, thyroid gland, and uterus. Due to the early mortality in 1 g/kg male rats, data from this group are not presented in this section. Summaries of the incidences of neoplasms and nonneoplastic lesions, statistical analyses of primary neoplasms that occurred with an incidence of at least 5% in at least one animal group, and historical incidences for the neoplasms mentioned in this section are presented in Appendix A for male rats and Appendix B for female rats.

Kidney: In the original kidney sections, renal tubule adenoma occurred with a positive trend in males, and the incidence was significantly increased in 0.5 g/kg males (Tables 10, A1, and A2). Though not significantly different from the vehicle controls, the incidences of renal tubule adenoma and renal tubule carcinoma in 0.25 g/kg males exceeded the historical control means from all routes of administration. When compared to vehicle control males, the incidences of renal tubule adenoma or carcinoma (combined) were significantly increased in 0.25 and 0.5 g/kg males; and the combined incidences occurred with a positive trend. Two renal tubule adenomas occurred in 1 g/kg females, and this incidence was higher than the historical control mean from all routes of study (Tables 10, B1, and B3). Renal tubule hyperplasia occurred in only two 0.5 g/kg males and one 1 g/kg female (Tables 10, A4, and B4).

In the kidney, renal tubule hyperplasia, adenoma, and carcinoma are thought to represent a continuum of renal tubule proliferative lesions. The renal tubule adenomas were well-circumscribed, variably sized masses composed of large, polygonal cells with well defined borders, abundant, well developed, basophilic cytoplasm, and variably sized nuclei with large nucleoli (Plates 1 and 2). The adenomas often exhibited growth of blood vessels and/or fibroblasts into the tumors. Mitotic figures, small areas of central necrosis, and pleomorphism of the neoplastic cells occasionally characterized the adenomas, particularly the larger ones. Small adenomas were differentiated from renal tubule hyperplasia by extension beyond the confines of a single renal tubule. Generally, lesions with greater than seven small lobules, too many to be consistent with the convolutions of a single tubule, were

TABLE 10
Incidences of Neoplasms and Nonneoplastic Lesions of the Kidney in Rats in the 2-Year Gavage Study of β-Myrcene[a]

	Vehicle Control	0.25 g/kg	0.5 g/kg
Male			
Single Sections (Standard Evaluation)			
Number Examined Microscopically	50	50	50
Renal Tubule, Hyperplasia[b]	0	0	2 (1.0)[c]
Renal Tubule, Nephrosis	0	42** (1.8)	46** (2.7)
Papilla, Mineralization	1 (1.0)	48** (2.1)	40** (1.9)
Nephropathy	45 (1.2)	48 (2.0)	48 (2.6)
Transitional Epithelium, Hyperplasia	0	21** (1.4)	19** (1.4)
Inflammation, Suppurative, Focal	1 (1.0)	22** (1.0)	22** (1.0)
Vein, Thrombosis	0	0	3 (2.3)
Renal Tubule, Adenoma, Multiple	0	2	1
Renal Tubule, Adenoma (includes multiple)[d]			
Overall rate[e]	0/50 (0%)	4/50 (8%)	8/50 (16%)
Adjusted rate[f]	0.0%	8.8%	18.7%
Terminal rate[g]	0/29 (0%)	3/36 (8%)	5/28 (18%)
First incidence (days)	—[i]	717	551
Poly-3 test[h]	P=0.002	P=0.068	P=0.003
Renal Tubule, Carcinoma[j]	0	3	1
Renal Tubule, Adenoma or Carcinoma[k]			
Overall rate	0/50 (0%)	7/50 (14%)	9/50 (18%)
Adjusted rate	0.0%	15.4%	21.0%
Terminal rate	0/29 (0%)	5/36 (14%)	6/28 (21%)
First incidence (days)	—	652	551
Poly-3 test	P=0.002	P=0.010	P=0.002
Single Sections and Step Sections (Combined)			
Number Examined Microscopically	50	50	50
Renal Tubule, Hyperplasia	1 (1.0)	5 (1.6)	3 (1.0)
Renal Tubule, Adenoma			
Overall rate	0/50 (0%)	12/50 (24%)	13/50 (26%)
Adjusted rate	0.0%	26.5%	30.2%
Terminal rate	0/29 (0%)	11/36 (31%)	9/28 (32%)
First incidence (days)	—	717	551
Poly-3 test	P<0.001	P<0.001	P<0.001
Renal Tubule, Carcinoma	0	3	1
Renal Tubule, Adenoma or Carcinoma			
Overall rate	0/50 (0%)	14/50 (28%)	13/50 (26%)
Adjusted rate	0.0%	30.8%	30.2%
Terminal rate	0/29 (0%)	12/36 (33%)	9/28 (32%)
First incidence (days)	—	652	551
Poly-3 test	P<0.001	P<0.001	P<0.001

TABLE 10
Incidences of Neoplasms and Nonneoplastic Lesions of the Kidney in Rats in the 2-Year Gavage Study of β-Myrcene

	Vehicle Control	0.25 g/kg	0.5 g/kg	1 g/kg
Female				
Single Sections (Standard Evaluation)				
Number Examined Microscopically	50	50	50	50
Renal Tubule, Hyperplasia	0	0	0	1 (1.0)
Renal Tubule, Nephrosis	0	2 (1.0)	27** (1.0)	45** (1.2)
Papilla, Mineralization	5 (1.0)	3 (1.0)	1 (1.0)	0*
Nephropathy	26 (1.0)	43** (1.0)	41** (1.3)	44** (1.7)
Transitional Epithelium, Hyperplasia	1 (1.0)	12** (1.3)	15** (1.3)	19** (1.2)
Inflammation, Suppurative, Focal	0	1 (1.0)	0	1 (1.0)
Renal Tubule, Adenoma[l]	0	1	0	2
Single Sections and Step Sections (Combined)				
Number Examined Microscopically	50	50	50	50
Renal Tubule, Hyperplasia	1 (1.0)	5 (1.4)	1 (1.0)	2 (1.0)
Renal Tubule, Adenoma				
Overall rate	0/50 (0%)	2/50 (4%)	1/50 (2%)	3/50 (6%)
Adjusted rate	0.0%	4.8%	2.5%	7.2%
Terminal rate	0/31 (0%)	1/33 (3%)	0/28 (0%)	3/33 (9%)
First incidence (days)	—	689	701	730 (T)
Poly-3 test	P=0.105	P=0.239	P=0.491	P=0.121

* Significantly different (P≤0.05) from the vehicle control group by the Poly-3 test
** P≤0.01
(T) Terminal sacrifice
[a] Data for the 1 g/kg male group are not presented due to early mortality; the incidences are included in Tables A1 and A4.
[b] Number of animals with lesion
[c] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked
[d] Historical incidence for 2-year gavage studies with corn oil vehicle control groups (mean ± standard deviation): 1/150 (0.7% ± 1.2%), range 0%-2%; all routes: 8/1,394 (0.6% ± 0.9%), range 0%-2%
[e] Number of animals with neoplasm per number of animals with kidney examined microscopically
[f] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[g] Observed incidence at terminal kill
[h] Beneath the vehicle control incidence is the P value associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal sacrifice.
[i] Not applicable; no neoplasms in animal group
[j] Historical incidence for corn oil gavage studies: 0/150; all routes: 2/1,394 (0.1% ± 0.5%), range 0%-2%
[k] Historical incidence for corn oil gavage studies: 1/150 (0.7% ± 1.2%), range 0%-2%; all routes: 10/1,394 (0.7% ± 1.2%), range 0%-4%
[l] Historical incidence for corn oil gavage studies: 0/150; all routes, 1/1,340 (0.1% ± 0.4%), range 0%-2%

considered adenomas (Hard and Seely, 2005). Renal tubule carcinomas had cellular features similar to adenomas but with more marked pleomorphism. They also had more mitotic figures, were larger, had much more extensive central necrosis, and occasionally had invaded adjacent tissue.

In contrast, renal tubule hyperplasia (not associated with nephropathy or nephrosis), a putative preneoplastic lesion, had cellular features similar to adenomas but with fewer mitotic figures and rare cellular pleomorphism (Plates 3 and 4). They also lacked ingrowth of fibroblasts and blood vessels and did not extend beyond the confines of a single renal tubule. Two important features of this type of renal tubule hyperplasia that distinguished it from the hyperplasia associated with nephrosis and nephropathy were a rim of fibroblasts in intimate contact with the rim of the expansile lesion and a glassy, basophilic sheen that was characteristic of the hyperplastic cells (Hard and Seely, 2005) (Plate 3).

Due to the dose-related increase in renal tubule proliferative lesions found in the standard evaluations, extended evaluations of 3 to 4 additional step sections of kidney from each animal were performed. The extended evaluation substantiated the results of the standard evaluation. In both the extended evaluation and the combined standard and extended evaluations, the incidences of renal tubule adenoma and of renal tubule adenoma or carcinoma (combined) were significantly increased in all dosed groups of males (Tables 10 and A2). The incidence of renal tubule hyperplasia was slightly increased in 0.25 g/kg males and females in the extended evaluation.

The incidences of renal tubule nephrosis (nephrosis) were markedly increased compared to vehicle controls in all dosed groups of both sexes except the 0.25 g/kg females (Tables 10, A4, and B4). Additionally, a dose-related increase in the severity of nephrosis was observed in males. A spectrum of related histologic lesions constitutes nephrosis: renal tubule dilatation, renal tubule karyomegaly (of tubule epithelial cells), renal tubule hyperplasia, collecting duct hyperplasia, interstitial fibrosis, and renal tubule atrophy (Plates 5 to 9). Tubule dilatation and karyomegaly were the predominant histologic changes and began in the outer stripe of the outer

medulla (Plates 5, 6, and 9). In more severe cases, the tubular dilation also affected some cortical tubules. Renal tubule hyperplasia, which differed from the preneoplastic renal tubule hyperplasia previously described, was widespread in more severely affected animals and was evident in both cortical tubules and collecting ducts. The cells of the hyperplastic tubules were generally enlarged; had granular to slightly vacuolated cytoplasm that lacked the basophilic sheen of the preneoplastic hyperplasias; and were generally surrounded by eosinophilic, fibrillar material (fibrosis). The fibrotic regions, which often contained scattered fibroblasts and mononuclear cells, often surrounded and separated dilated and hyperplastic tubules. Interstitial fibrosis and tubule atrophy were prominent in some rats but were multifocal in others (Plates 5 and 7). In mid- and low-dose male rats, dose-related changes were noted in all of the parameters used to assess nephrosis. In female rats, tubule dilatation and karyomegaly, predominantly affecting the outer stripe of the outer medulla, were the most remarkable changes and exhibited a dose-related increase in severity, though the severity was reduced compared to the males. The other components of nephrosis were present sporadically and to a lesser degree in the female rats.

In the males, the incidences and severities of papillary mineralization were significantly increased in the 0.25 and 0.5 g/kg groups (Tables 10 and A4). There were no increases in the incidence of papillary mineralization in the dosed female rats compared to vehicle controls (Tables 10 and B4). The papillary mineralization was identified by linear accumulations of angular to stippled basophilic material within the loops of Henle in the renal papilla. Epithelial changes ranged from complete loss of the epithelial lining to flattening and elongation of the epithelial cells. Occasionally, necrotic cells could be seen within the tubules. This type of renal mineralization is considered a chronic manifestation of α2u-globulin nephropathy (Hard *et al.*, 1993).

The incidences of CPN were significantly increased in dosed females, and the severity was increased in the 0.5 and 1 g/kg groups (Tables 10 and B4). CPN was observed in virtually all males, and the severity was increased in the dosed groups (Tables 10 and A4). CPN is an age-related disease process characterized by a spectrum of tubule, glomerular, and interstitial lesions. The tubule lesions include varying degrees of tubule dilation; proteinaceous tubule casts; thickening of tubule basement membrane; and atrophy, degeneration, regeneration, and hypertrophy

of the tubule epithelium (Plate 10). Glomerular lesions include thickening of glomerular basement membrane; glomerular mesangial proliferation; adhesions between the glomerular tuft and the wall of Bowman's capsule; glomerular parietal and visceral epithelial hyperplasia and hypertrophy; and glomerulosclerosis. Interstitial lesions include fibrosis and infiltration by varying numbers and aggregates of mononuclear inflammatory cells. Minimal CPN was characterized by a few scattered foci of tubule regeneration. These regenerative tubules had increased numbers of more intensely stained basophilic cells. Basement membranes, both in glomeruli and around tubules, were slightly thickened. As CPN became more severe, tubule dilatation, proteinaceous casts, and interstitial fibrosis were evident along with glomerular changes including periglomerular fibrosis and thickening of glomerular basement membranes, hyperplasia of parietal and visceral epithelial cells, and adhesions of the glomerular tufts to Bowman's capsule. Many of these lesions are listed above as components of nephrosis; and indeed, it was often difficult to differentiate between the two lesions, particularly in the high dose males. The main differentiating factors were the presence of proteinaceous tubule casts and thickened basement membranes around hyperplastic, dilated, and atrophic tubules which are both characteristic of CPN; and the character and cellular features of the hyperplastic and dilated tubules, which, when associated with CPN, tended to be more basophilic and have more crowded nuclei.

The incidences of hyperplasia of the transitional epithelium lining the pelvis and overlying the renal papilla in all dosed groups of both sexes were significantly increased relative to the vehicle controls (Tables 10, A4, and B4). This lesion frequently accompanies severe nephropathy, and the increased incidences may reflect the enhanced nephropathy (Plates 8 and 10).

In the male rats, the incidences of focal suppurative inflammation, which was typically within renal tubules, was significantly increased in the 0.25 and 0.5 g/kg groups compared to the vehicle controls (Tables 10 and A4). Three male rats in the 0.5 g/kg group had venous thrombosis compared to none in the vehicle controls (Tables 10 and A4).

Liver: The incidences of all forms of hepatic foci in male rats were decreased, with statistically significant decreases in basophilic focus and mixed cell focus at 0.25 and 0.5 g/kg (Table A4). In the females, the incidence of basophilic focus was decreased in the 1 g/kg group compared to vehicle controls, but the incidences of eosinophilic focus were increased in the 0.5 and 1 g/kg groups (Table B4). The incidence of chronic inflammation was decreased in the 0.5 g/kg male group, and negative trends for incidences of chronic inflammation were found in both sexes. A negative trend in the incidences of bile duct hyperplasia in the liver was found in the male rats. Bile duct hyperplasia is a common lesion in aged rats.

Other Findings: In the lung, the incidence of alveolar/bronchiolar adenoma in the 0.25 g/kg males (Table A2) exceeded the control means from NTP 2-year studies using corn oil gavage [6/150 (4%)] and all routes [34/1,399 (2%)]. In the nose, the incidence of chronic active inflammation of the nose was significantly increased in the 0.5 g/kg males (Table A4). This irritation is likely a secondary effect on the nasal epithelium due to regurgitation of β-myrcene into the nasal cavity during the gavage procedure, but a hematogenous effect of β-myrcene on the nose cannot be ruled out. In the forestomach, the incidence of chronic active inflammation in 0.5 g/kg males was increased relative to the vehicle control incidence (Table A4), suggesting that the chemical is an irritant. In females, the incidence of thyroid gland C-cell adenoma was significantly increased in the 0.25 g/kg group; the incidences did not increase with increasing dose (Tables B1 and B2). The incidence of cystic endometrial hyperplasia of the uterus was significantly increased in the 1 g/kg group (Table B4).

Mice

3-Month Study

All male and female 4 g/kg mice died during week 1; nine 2 g/kg males and eight 2 g/kg females died by week 4 (Table 11). The final mean body weights and body weight gains of 1 g/kg males and 0.5 g/kg females were significantly less than those of the vehicle controls. Clinical findings for those animals that did not survive to the end of the study included thinness, lethargy, and abnormal breathing.

Table 11
Survival and Body Weights of Mice in the 3-Month Gavage Study of β-Myrcene

Dose (g/kg)	Survival[a]	Mean Body Weight[b] (g) Initial	Final	Change	Final Weight Relative to Controls (%)
Male					
0	10/10	23.8 ± 0.3	34.6 ± 1.0	10.7 ± 0.8	
0.25	10/10	23.1 ± 0.3	35.8 ± 0.6	12.7 ± 0.6	103
0.5	10/10	23.7 ± 0.3	34.6 ± 0.9	10.9 ± 0.9	100
1	10/10	23.4 ± 0.3	31.6 ± 0.8*	8.2 ± 0.7*	91
2	1/10[c]	23.3 ± 0.2	26.43.5	76	
4	0/10[d]	23.5 ± 0.3	——	—	
Female					
0	10/10	19.4 ± 0.3	28.0 ± 0.7	8.5 ± 0.5	
0.25	10/10	19.0 ± 0.3	26.3 ± 0.6	7.3 ± 0.5	94
0.5	10/10	18.9 ± 0.2	25.7 ± 0.4*	6.8 ± 0.3*	92
1	10/10	18.6 ± 0.4	27.1 ± 0.4	8.5 ± 0.3	97
2	2/10[e]	19.4 ± 0.3	22.0 ± 0.2**	2.7 ± 1.2**	78
4	0/10[d]	18.7 ± 0.2	——	—	

* Significantly different (P≤0.05) from the vehicle control group by Williams' or Dunnett's test
** P≤0.01
[a] Number of animals surviving at 3 months/number initially in group.
[b] Weights and weight changes are given as mean ± standard error. Subsequent calculations are based on animals surviving to the end of the study. No statistical analysis performed for 2 g/kg males because less than two measurements available.
[c] Weeks of death: 1, 1, 3, 3, 3, 3, 4, 4, 4
[d] Week of deaths: 1
[e] Weeks of death: 1, 1, 1, 1, 4, 4, 4, 4

Hematology findings of 2 and 4 g/kg mice were not presented due to the large number of early deaths. At week 14, an approximately 15% to 20% decrease in hematocrit, hemoglobin, and erythrocyte count values occurred in the 1 g/kg male and female mice (Tables 12 and F2). The erythron effect may be secondary to the decreased body weights.

The relative liver weights of 0.5 and 1 g/kg males and the absolute and relative liver weights of 0.25 g/kg males were significantly greater than those of the vehicle controls (Tables 13 and G2). The absolute and relative liver weights of 0.5 and 1 g/kg females, the absolute and relative right kidney weights of 1 g/kg females, and the relative kidney weights or 0.25 and 0.5 g/kg females were significantly greater than those of the vehicle controls. There were no significant changes seen in the weights of the reproductive organs nor in the sperm parameters or estrous cyclicity of the male or female mice at any dose concentration (Tables H3 and H4).

TABLE 12
Selected Hematology Data for Mice in the 3-Month Gavage Study of β-Myrcene[a]

	Vehicle Control	0.25 g/kg	0.5 g/kg	1 g/kg
Male				
n	10	10	9	10
Hematocrit (auto) (%)	48.7 ± 0.3	48.7 ± 0.5	48.4 ± 0.4	47.0 ± 0.4**
Hemoglobin (g/dL)	16.4 ± 0.1	16.4 ± 0.1	16.3 ± 0.1	15.8 ± 0.2**
Erythrocytes (10^6/μL)	10.78 ± 0.10	10.65 ± 0.11	10.50 ± 0.07	10.11 ± 0.10**
Female				
n	10	10	10	10
Hematocrit (auto) (%)	47.5 ± 0.5	48.0 ± 0.6	47.4 ± 0.4	46.8 ± 0.2
Hemoglobin (g/dL)	16.1 ± 0.2	16.3 ± 0.2	16.1 ± 0.1	15.7 ± 0.1
Erythrocytes (10^6/μL)	10.37 ± 0.11	10.50 ± 0.14	10.29 ± 0.10	9.92 ± 0.07*

* Significantly different ($P \leq 0.05$) from the vehicle control group by Dunn's or Shirley's test
** $P \leq 0.01$
[a] Mean ± standard error. Statistical tests were performed on unrounded data. No data presented for 2 or 4 g/kg males or females due to early mortality.

TABLE 13
Selected Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 3-Month Gavage Study of β-Myrcene[a]

	Vehicle Control	0.25 g/kg	0.5 g/kg	1 g/kg
Male				
n	10	10	10	10
Necropsy body wt	35.8 ± 1.0	36.6 ± 0.7	35.8 ± 0.9	32.7 ± 0.8*
Liver				
Absolute	1.453 ± 0.043	1.593 ± 0.036*	1.537 ± 0.042	1.557 ± 0.038
Relative	40.689 ± 0.735	43.580 ± 0.850*	43.104 ± 1.201*	47.687 ± 0.516**
Female				
n	10	10	10	10
Necropsy body wt	27.9 ± 0.7	26.1 ± 0.5	26.1 ± 0.5	26.9 ± 0.3
R. Kidney				
Absolute	0.164 ± 0.004	0.175 ± 0.004	0.173 ± 0.003	0.193 ± 0.004**
Relative	5.901 ± 0.150	6.719 ± 0.178**	6.621 ± 0.084**	7.197 ± 0.116**
Liver				
Absolute	1.127 ± 0.024	1.143 ± 0.036	1.236 ± 0.025*	1.369 ± 0.019**
Relative	40.604 ± 1.024	43.862 ± 1.481	47.458 ± 1.076**	50.985 ± 0.531**

* Significantly different ($P \leq 0.05$) from the vehicle control group by Williams' or Dunnett's test
** $P \leq 0.01$
[a] Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error). No data presented for 2 or 4 g/kg males or females due to early mortality.

Due to high, early mortality (i.e. acute toxicity), the 2 and 4 g/kg males and females were omitted from the analysis and summary tables. There were no significant lesions in the 0.25 to 1 g/kg male and female groups.

Dose Selection Rationale: Based on adverse effects on survival and body weight gains at 2 and 4 g/kg, the highest dose selected for the 2-year gavage study in mice was 1 g/kg. There were no significant histopathologic lesions observed at this dose.

2-Year Study

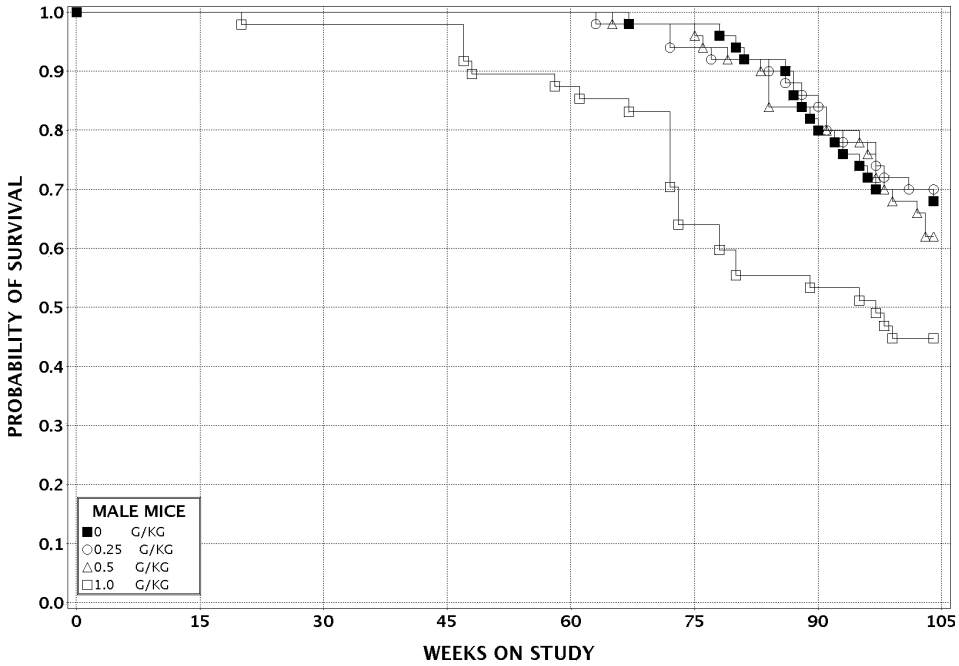
Survival

Estimates of 2-year survival probabilities for male and female mice are shown in Table 14 and in the Kaplan-Meier survival curves (Figure 4). Survival of 0.25 and 0.5 g/kg mice was similar to that of the vehicle controls. Survival of 1 g/kg mice was significantly less than that of the vehicle controls; the cause of deaths was uncertain.

Table 14
Survival of Mice in the 2-Year Gavage Study of β-Myrcene

	Vehicle Control	0.25 g/kg	0.5 g/kg	1 g/kg
Male				
Animals initially in study	50	50	50	50
Accidental deaths[a]	0	0	0	3
Moribund	7	6	8	12
Natural deaths	8	9	11	14
Animals surviving to study termination	35[d]	35	31	21
Percent probability of survival at end of study[b]	68	70	62	45
Mean survival (days)[c]	692	691	689	577
Survival analysis[e]	P=0.001	P=0.969N	P=0.719	P=0.008
Female				
Animals initially in study	50	50	50	50
Accidental deaths	0	1	1	0
Moribund	4	5	6	5
Natural deaths	7	10	8	28
Animals surviving to study termination	39	34	35	17
Percent probability of survival at end of study	78	69	72	34
Mean survival (days)	705	680	664	552
Survival analysis	P<0.001	P=0.434	P=0.457	P<0.001

[a] Censored from survival analyses
[b] Kaplan-Meier determinations
[c] Mean of all deaths (uncensored, censored, and terminal sacrifice).
[d] Includes one animal that died during the last week of the study
[e] The result of the life table trend test (Tarone, 1975) is in the vehicle control column, and the results of the life table pairwise comparisons (Cox, 1972) with the vehicle controls are in the dosed group columns. A lower mortality in a dose group is indicated by **N**



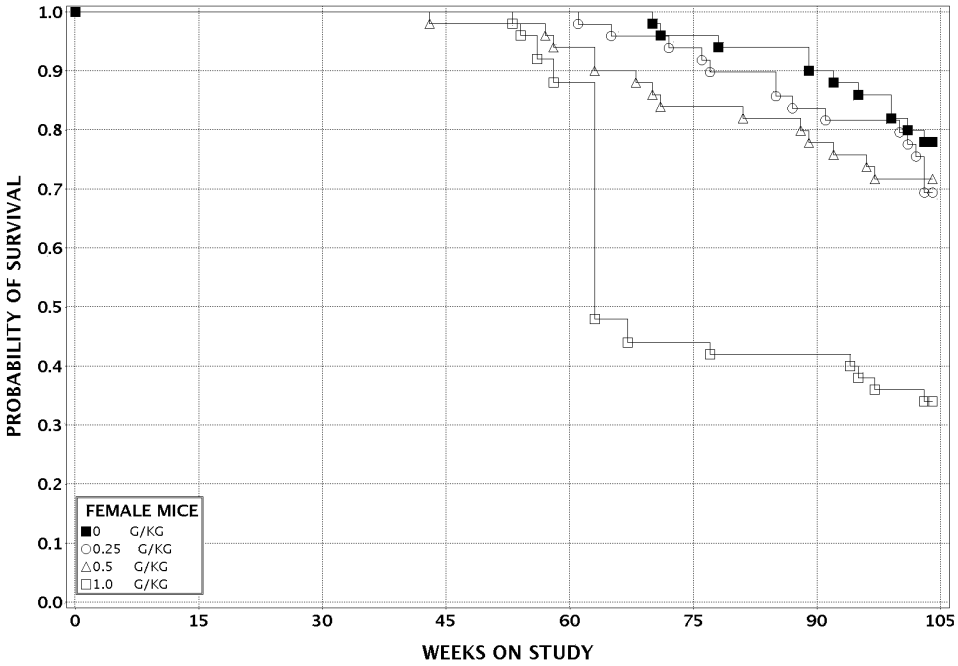
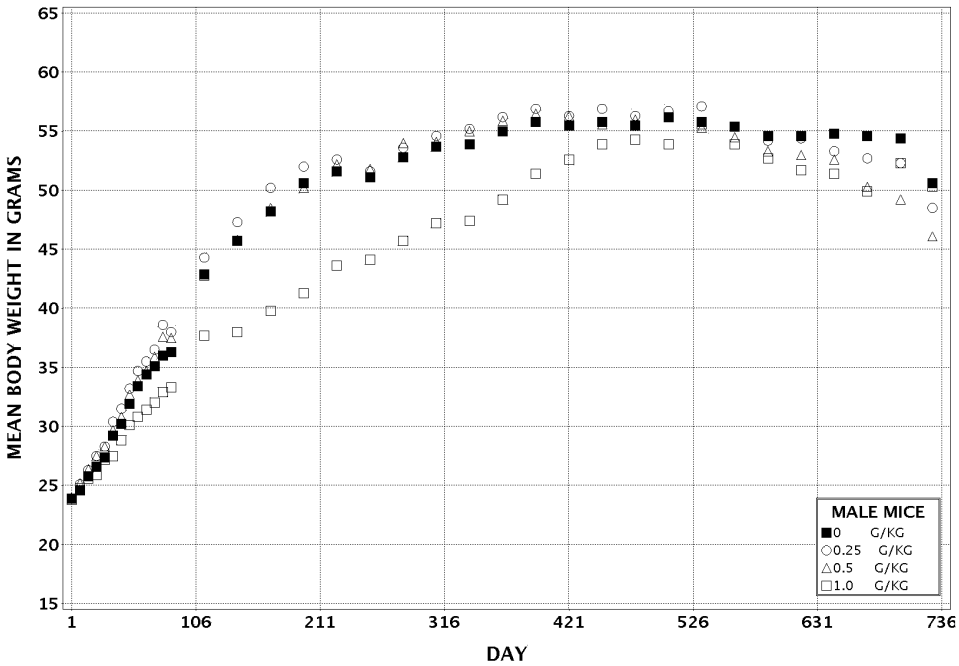


FIGURE 4
Kaplan-Meier Survival Curves for Male and Female Mice Administered β-Myrcene by Gavage for 2 Years

Body Weights and Clinical Findings

Mean body weights of 0.25 g/kg males and females and 0.5 g/kg males were generally similar to vehicle controls throughout the study (Figure 5; Tables 15 and 16). Mean body weights of 1 g/kg males, 0.5 g/kg females, and 1 g/kg females were less than those of the vehicle controls after weeks 8, 17, and 11, respectively. No clinical findings related to β-myrcene administration were observed.



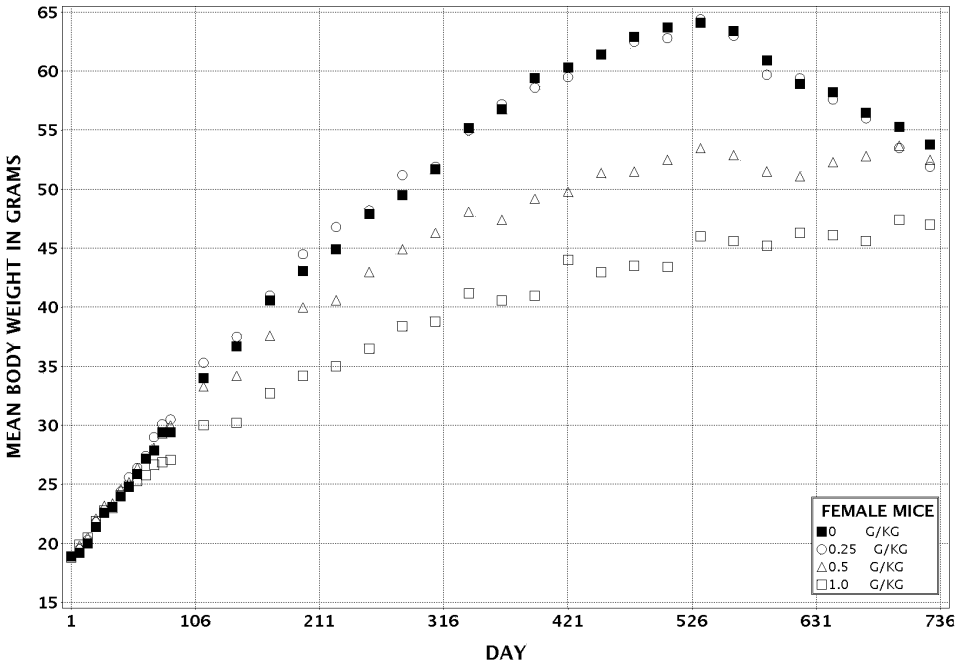


FIGURE 5
Growth Curves for Male and Female Mice Administered β-Myrcene by Gavage for 2 Years

Table 15
Mean Body Weights and Survival of Male Mice in the 2-Year Gavage Study of β-Myrcene

Days on Study	Vehicle Control		0.25 g/kg			0.5 g/kg			1 g/kg		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	23.9	50	23.9	100	50	24.0	100	50	23.8	100	50
8	24.6	50	25.1	102	50	25.2	103	50	24.8	101	50
15	25.8	50	26.3	102	50	26.4	103	50	25.6	99	50
22	26.6	50	27.5	103	50	27.5	103	50	25.9	97	50
29	27.4	50	28.3	103	50	28.3	103	50	27.2	99	50
36	29.2	50	30.4	104	50	29.7	102	50	27.5	94	50
43	30.2	50	31.5	104	50	30.8	102	50	28.8	95	50
50	31.9	50	33.2	104	50	32.7	103	50	30.1	95	50
57	33.4	50	34.7	104	50	33.9	101	50	30.8	92	50
64	34.4	50	35.5	103	50	34.7	101	50	31.4	91	50
71	35.1	50	36.5	104	50	35.9	102	50	32.0	91	50
78	36.0	50	38.6	107	50	37.6	105	50	32.9	91	50
85	36.3	50	38.0	105	50	37.5	103	50	33.3	92	50
113	42.9	50	44.3	103	50	42.8	100	50	37.7	88	49
141	45.7	50	47.3	104	50	45.8	100	50	38.0	83	48
169	48.2	50	50.2	104	50	48.5	101	50	39.8	83	48
197	50.6	50	52.0	103	50	50.2	99	50	41.3	82	48
225	51.6	50	52.6	102	50	52.2	101	50	43.6	85	48
253	51.1	50	51.6	101	50	51.8	101	50	44.1	86	48
281	52.8	50	53.6	101	50	54.0	102	50	45.7	87	48
309	53.7	50	54.6	102	50	54.1	101	50	47.2	88	47
337	53.9	50	55.2	102	50	55.0	102	50	47.4	88	42
365	55.0	50	56.2	102	50	55.9	102	50	49.2	89	42
393	55.8	50	56.9	102	50	56.5	101	50	51.4	92	42
421	55.5	50	56.3	101	50	56.3	101	50	52.6	95	41
449	55.8	50	56.9	102	49	55.6	100	50	53.9	97	40
477	55.5	49	56.3	101	49	56.0	101	49	54.3	98	39
505	56.2	49	56.7	101	47	56.2	100	49	53.9	96	30
533	55.8	49	57.1	102	46	55.3	99	47	55.6	100	30
561	55.4	47	55.4	100	46	54.5	98	46	53.9	97	26
589	54.6	46	54.2	99	45	53.3	98	42	52.7	96	26
617	54.6	42	54.4	100	43	53.0	97	42	51.7	95	26
645	54.8	38	53.3	97	40	52.6	96	40	51.4	94	25
673	54.6	35	52.7	97	37	50.3	92	37	49.9	91	23
701	54.4	35	52.3	96	36	49.2	90	34	52.3	96	21
Mean for weeks											
1-13	30.4		31.5	104		31.1	102		28.8	95	
14-52	50.1		51.3	102		50.5	101		42.8	85	
53-101	55.2		55.3	100		54.2	98		52.5	95	

TABLE 16
Mean Body Weights and Survival of Female Mice in the 2-Year Gavage Study of β-Myrcene

Days on Study	Vehicle Control		0.25 g/kg			0.5 g/kg			1 g/kg		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	18.9	50	18.9	100	50	18.9	100	50	18.8	99	50
8	19.2	50	19.5	101	50	19.8	103	50	19.9	104	50
15	20.0	50	20.3	101	50	20.5	102	50	20.5	102	50
22	21.4	50	21.8	102	50	22.1	103	50	21.9	102	50
29	22.6	50	22.8	101	50	23.2	103	50	22.8	101	50
36	23.1	50	23.1	100	50	23.4	101	50	23.0	100	50
43	24.0	50	24.4	102	50	24.6	103	50	24.1	100	50
50	24.8	50	25.6	103	50	25.2	101	50	24.9	100	50
57	25.9	50	26.4	102	50	26.4	102	50	25.3	98	50
64	27.2	50	27.4	101	50	27.2	100	50	25.8	95	50
71	27.9	50	29.0	104	50	28.1	101	50	26.7	96	50
78	29.4	50	30.1	102	50	29.3	100	50	26.9	92	50
85	29.4	50	30.5	103	50	30.0	102	50	27.1	92	50
113	34.0	50	35.3	104	50	33.3	98	50	30.0	88	50
141	36.7	50	37.5	102	49	34.2	93	50	30.2	82	50
169	40.6	50	41.0	101	49	37.6	93	50	32.7	81	50
197	43.1	50	44.5	103	49	40.0	93	50	34.2	79	50
225	44.9	50	46.8	104	49	40.6	90	50	35.0	78	50
253	47.9	50	48.2	101	49	43.0	90	50	36.5	76	50
281	49.5	50	51.2	103	49	44.9	91	50	38.4	78	50
309	51.7	50	51.9	100	49	46.3	90	49	38.8	75	50
337	55.2	50	55.0	100	49	48.1	87	49	41.2	75	50
365	56.8	50	57.2	101	49	47.4	84	49	40.6	72	50
393	59.4	50	58.6	99	49	49.2	83	49	41.0	69	46
421	60.3	50	59.5	99	49	49.8	83	47	44.0	73	44
449	61.4	50	61.4	100	48	51.4	84	45	43.0	70	24
477	62.9	50	62.5	100	47	51.5	82	44	43.5	69	22
505	63.7	48	62.8	99	46	52.5	82	42	43.4	68	22
533	64.1	48	64.4	100	44	53.5	83	42	46.0	72	22
561	63.4	47	63.0	99	44	52.9	84	41	45.6	72	21
589	60.9	47	59.7	98	44	51.5	85	40	45.2	74	21
617	58.9	47	59.4	101	41	51.1	87	39	46.3	79	21
645	58.2	44	57.6	99	40	52.3	90	37	46.1	79	21
673	56.5	43	56.0	99	40	52.8	93	36	45.6	81	18
701	55.3	41	53.5	97	39	53.7	97	35	47.4	86	18
Mean for weeks											
1-13	24.1		24.6	102		24.5	102		23.7	99	
14-52	44.8		45.7	102		40.9	92		35.2	79	
53-101	60.1		59.7	99		51.5	86		44.4	74	

Pathology and Statistical Analyses

This section describes the statistically significant or biologically noteworthy changes in the incidences of neoplasms and/or nonneoplastic lesions of the liver, bone marrow, spleen, mandibular lymph node, forestomach, pancreatic islets, and uterus. Due to the early mortality in 1 g/kg mice, data from this group are not presented in this section. Summaries of the incidences of neoplasms and nonneoplastic lesions, statistical analyses of primary neoplasms that occurred with an incidence of at least 5% in at least one animal group, and historical incidences for neoplasms mentioned in this section are presented in Appendix C for male mice and Appendix D for female mice.

Liver: In male mice, the incidences of hepatocellular adenoma in the 0.25 and 0.5 g/kg groups and hepatocellular carcinoma and hepatoblastoma in the 0.5 g/kg group were significantly greater than the vehicle control incidences (Tables 17 and C1). The combined incidences of hepatocellular adenoma or hepatocellular carcinoma and of hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma in the 0.25 and 0.5 g/kg males were significantly greater than those in the vehicle controls. A significantly increased incidence of hepatocellular carcinoma or hepatoblastoma (combined) also occurred in 0.5 g/kg males.

In female mice, significantly increased incidences of hepatocellular adenoma, hepatocellular carcinoma, and hepatocellular adenoma or carcinoma (combined) occurred in the 0.25 g/kg group compared to those in the vehicle controls (Tables 17 and D1).

A significantly decreased incidence of mixed cell focus occurred in the 0.25 g/kg males, and a significantly increased incidence of mixed cell focus occurred in the 0.5 g/kg females (Tables 17, C4, and D4).

Foci of cellular alteration are putative preneoplastic lesions, and these lesions had an appearance typical of that seen in B6C3F1 mice. Mixed foci were small to moderately large lesions composed of hepatocytes with eosinophilic, basophilic, or clear cytoplasm. The hepatocytes were arranged in normal hepatic cords that merged with the surrounding normal hepatocytes. Most foci had little or no compression of the surrounding normal

TABLE 17
Incidences of Neoplasms and Nonneoplastic Lesions of the Liver in Mice in the 2-Year Gavage Study of β-Myrcene[a]

	Vehicle Control	0.25 g/kg	0.5 g/kg
Male			
Number Examined Microscopically	50	50	50
Clear Cell Focus[b]	15	21	21
Eosinophilic Focus	16	23	21
Mixed Cell Focus	13	3**	6
Hepatocyte, Hypertrophy	1 (1.0)[c]	2 (1.5)	16** (1.7)
Fatty Change	25 (1.6)	18 (1.6)	16* (1.6)
Inflammation, Chronic Active	26 (1.0)	24 (1.1)	23 (1.0)
Hepatocellular Adenoma, Multiple	15	31**	30**
Hepatocellular Adenoma (includes multiple)[d]			
Overall rate[e]	26/50 (52%)	41/50 (82%)	43/50 (86%)
Adjusted rate[f]	57.8%	88.2%	89.3%
Terminal rate[g]	23/34 (68%)	33/35 (94%)	29/31 (94%)
First incidence (days)	468	533	525
Poly-3 test[h]	P<0.001	P<0.001	P<0.001
Hepatocellular Carcinoma, Multiple	1	4	9**
Hepatocellular Carcinoma, (includes multiple)[i]			
Overall rate	14/50 (28%)	20/50 (40%)	28/50 (56%)
Adjusted rate	30.6%	42.8%	58.8%
Terminal rate	8/34 (24%)	13/35 (37%)	16/31 (52%)
First incidence (days)	611	533	450
Poly-3 test	P=0.003	P=0.158	P=0.004
Hepatocellular Adenoma or Carcinoma[j]			
Overall rate	33/50 (66%)	44/50 (88%)	48/50 (96%)
Adjusted rate	71.0%	92.6%	96.6%
Terminal rate	25/34 (74%)	33/35 (94%)	30/31 (97%)
First incidence (days)	468	533	450
Poly-3 test	P<0.001	P=0.003	P<0.001
Hepatoblastoma[k]			
Overall rate	4/50 (8%)	6/50 (12%)	11/50 (22%)
Adjusted rate	9.0%	13.3%	25.0%
Terminal rate	3/34 (9%)	3/35 (9%)	7/31 (23%)
First incidence (days)	599	582	660
Poly-3 test	P=0.027	P=0.382	P=0.041

TABLE 17
Incidences of Neoplasms and Nonneoplastic Lesions of the Liver in Mice in the 2-Year Gavage Study of β-Myrcene

	Vehicle Control	0.25 g/kg	0.5 g/kg
Male (continued)			
Hepatocellular Carcinoma or Hepatoblastoma[l]			
Overall rate	16/50 (32%)	22/50 (44%)	31/50 (62%)
Adjusted rate	34.7%	46.7%	65.1%
Terminal rate	9/34 (27%)	14/35 (40%)	19/31 (61%)
First incidence (days)	599	533	450
Poly-3 test	P=0.002	P=0.163	P=0.002
Hepatocellular Adenoma, Hepatocellular Carcinoma, or Hepatoblastoma[m]			
Overall rate	34/50 (68%)	45/50 (90%)	48/50 (96%)
Adjusted rate	72.5%	94.0%	96.6%
Terminal rate	25/34 (74%)	33/35 (94%)	30/31 (97%)
First incidence (days)	468	533	450
Poly-3 test	P<0.001	P=0.003	P<0.001
Female			
Number Examined Microscopically	50	50	50
Clear Cell Focus	0	1	1
Eosinophilic Focus	4	5	6
Mixed Cell Focus	1	4	6*
Hepatocyte, Hypertrophy	0	0	6* (1.5)
Fatty Change	29 (1.6)	35 (1.4)	16* (1.3)
Inflammation, Chronic Active	43 (1.0)	35* (1.1)	34 (1.0)
Hepatocellular Adenoma, Multiple	0	2	0
Hepatocellular Adenoma (includes multiple)[n]			
Overall rate	6/50 (12%)	13/50 (26%)	6/50 (12%)
Adjusted rate	13.0%	29.8%	14.6%
Terminal rate	6/39 (15%)	12/34 (35%)	6/35 (17%)
First incidence (days)	727 (T)	709	727 (T)
Poly-3 test	P=0.418	P=0.042	P=0.534
Hepatocellular Carcinoma[o]			
Overall rate	1/50 (2%)	7/50 (14%)	2/50 (4%)
Adjusted rate	2.2%	15.9%	4.8%
Terminal rate	1/39 (3%)	5/34 (15%)	1/35 (3%)
First incidence (days)	727 (T)	608	640
Poly-3 test	P=0.334	P=0.025	P=0.461

TABLE 17
Incidences of Neoplasms and Nonneoplastic Lesions of the Liver in Mice in the 2-Year Gavage Study of β-Myrcene

	Vehicle Control	0.25 g/kg	0.5 g/kg
Female (continued)			
Hepatocellular Adenoma or Carcinoma[p]			
Overall rate	7/50 (14%)	18/50 (36%)	8/50 (16%)
Adjusted rate	15.1%	40.9%	19.3%
Terminal rate	7/39 (18%)	15/34 (44%)	7/35 (20%)
First incidence (days)	727 (T)	608	640
Poly-3 test	P=0.297	P=0.005	P=0.406

* Significantly different (P≤0.05) from the vehicle control group by the Poly-3 test
** P≤0.01
(T) Terminal sacrifice
[a] Data for the 1 g/kg mice are not presented due to high mortality.
[b] Number of animals with lesion
[c] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked
[d] Historical incidence for 2-year gavage studies with corn oil vehicle control groups (mean ± standard deviation): 94/200 (47.0% ± 3.8%), range 44%-52%; all routes: 733/1,447 (50.7% ± 13.9%), range 22%-72%
[e] Number of animals with neoplasm per number of animals with liver examined microscopically
[f] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[g] Observed incidence at terminal kill
[h] Beneath the vehicle control incidence is the P value associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal sacrifice.
[i] Historical incidence for corn oil gavage studies: 52/200 (26.0% ± 7.5%), range 16%-34%; all routes: 415/1,447 (28.7% ± 8.8%), range 16%-52%
[j] Historical incidence for corn oil gavage studies: 124/200 (62.0% ± 5.9%), range 56%-68%; all routes: 961/1,447 (66.4% ± 12.3%), range 36%-84%
[k] Historical incidence for corn oil gavage studies: 10/200 (5.0% ± 2.6%), range 2%-8%; all routes: 48/1,447 (3.3% ± 6.4%), range 0%-34%
[l] Historical incidence for corn oil gavage studies: 59/200 (29.5% ± 7.0%), range 22%-38%; all routes: 446/1,447 (30.8% ± 9.7%), range 16%-54%
[m] Historical incidence for corn oil gavage studies: 127/200 (63.5% ± 5.3%), range 58%-68%; all routes: 972/1,447 (67.2% ± 13.1%), range 36%-92%
[n] Historical incidence for corn oil gavage studies: 33/197 (16.8% ± 9.4%), range 6%-27%; all routes: 396/1,494 (26.5% ± 15.2%), range 2%-54%
[o] Historical incidence for corn oil gavage studies: 11/197 (5.6% ± 3.5%), range 2%-10%; all routes: 137/1,494 (9.2% ± 6.7%), range 0%-28%
[p] Historical incidence for corn oil gavage studies: 41/197 (20.9% ± 12.0%); range 8%-35%; all routes: 481/1,494 (32.2% ± 17.3%); range 6%-64%

hepatocytes, although some degree of compression was present in some larger foci. Adenomas were discrete masses with distinct borders that caused compression of the surrounding normal hepatic parenchyma. Adenomas usually were composed of hepatocytes that appeared similar to those seen in eosinophilic foci, except that in adenomas, the normal lobular architecture was not apparent, and plates of hepatocytes intersected the surrounding normal hepatocytes at sharp angles rather than merging with them as in foci. Carcinomas were discrete masses that generally had irregular borders due to localized areas of growth of neoplastic hepatocytes into the surrounding normal parenchyma. The neoplastic hepatocytes often were somewhat atypical, but the major distinguishing feature of carcinomas was the presence of abnormal patterns of growth. The most abnormal growth pattern was formation of trabeculae of neoplastic hepatocytes that were three or more cell layers thick, while less commonly the neoplastic cells formed glandular structures or solid masses. Hepatoblastomas appeared as irregular masses comprised of multiple rows of cells palisading around blood vessels or blood-filled cystic or necrotic spaces. The cells were small and irregular with scant cytoplasm and hyperchromatic nuclei.

In both males and females, the incidences of hepatocellular hypertrophy were significantly increased in the 0.5 g/kg groups (Tables 17, C4, and D4). Also, there was a dose-related increase in the average severity of hepatocellular hypertrophy in males.

The incidences of fatty change were significantly decreased in the 0.5 g/kg groups of males and females, and the incidence of chronic active inflammation was significantly decreased in 0.25 g/kg females (Tables 17, C4, and D4).

Other Findings: In female mice, a significantly increased incidence of bone marrow atrophy, manifested as a decrease in the density of hematopoietic cells, occurred in the 0.5 g/kg group (Table D4). A significantly increased incidence of lymphoid follicle atrophy occurred in the spleen of 0.5 g/kg females, and dose-related increases in severity were found in males and females. Lymphoid follicle atrophy was characterized by the decrease in the size and number of lymphoid follicles with total or near total loss of the white pulp in severe cases. A significantly increased incidence of atrophy in the mandibular lymph node occurred in 0.5 g/kg females. The features of lymph

node atrophy were loss of lymphocytes from follicles and paracortical areas and a decrease in the size of the cross-section of the lymph node in some cases. In females, significantly increased incidences of inflammation and epithelial hyperplasia of the forestomach occurred in the 0.5 g/kg group (Table D4). The inflammation was mainly chronic active, and epithelial hyperplasia was characterized by increased numbers of basal cells and/or layers of squamous cells with occasional mitotic figures and hyperkeratosis. These lesions were considered part of the same process and were typically seen together. The incidence of pancreatic islet hyperplasia was significantly decreased in 0.5 g/kg male mice (Table C4). The incidences of uterine endometrial hyperplasia were decreased in 0.25 and 0.5 g/kg females when compared to the vehicle controls; the average severity in these groups was also decreased (Table D4).

Genetic Toxicology

β-Myrcene did not show evidence of genotoxicity in assays conducted by the NTP. No mutagenic activity was observed in any of several strains of *Salmonella typhimurium* (TA97, TA98, TA100, and TA1535) or *Escherichia coli* strain WP2 *uvrA* pKM101 exposed to β-myrcene concentrations ranging up to 10,000 μg/plate in two independent Ames assays conducted with and without exogenous metabolic activation provided by Aroclor 1254-induced rat or hamster liver enzymes (Table E1). In addition, no significant increases in the frequencies of micronucleated normochromatic erythrocytes, biomarkers of chromosomal damage, were observed in male or female B6C3F1 mice administered β-myrcene (0.25 to 2 g/kg) for 3 months by gavage (Table E2). The percentage of reticulocytes among total erythrocytes (% polychromatic erythrocytes) increased slightly with dose, but remained within the normal range, suggesting an absence of β-myrcene-induced bone marrow toxicity over this dose range (Table E2).

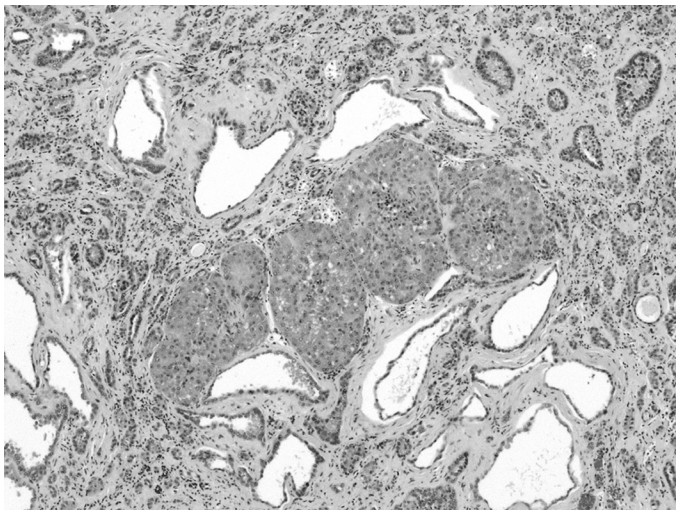


Plate 1
Renal tubule adenoma in a male F344/N rat administered 1 g/kg β-myrcene by gavage for 2 years. The adenoma has extended beyond the confines of a single tubule with 7 or 8 tubular profiles. There is also central necrosis of the neoplastic cells. H&E

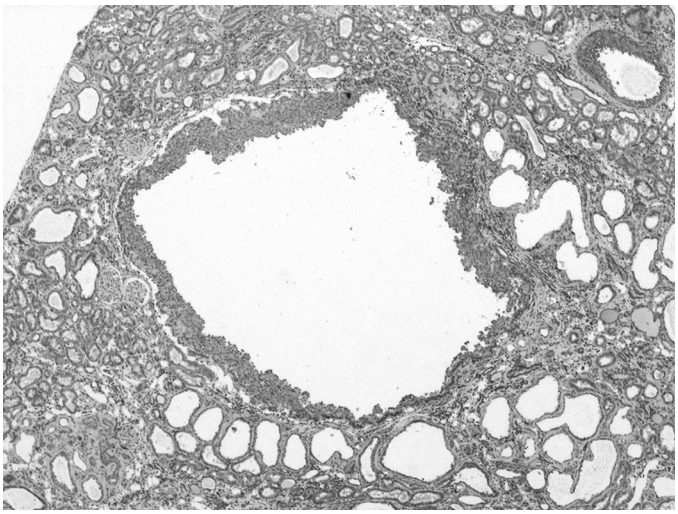


Plate 2
Renal tubule adenoma in a male F344/N rat administered 0.5 g/kg β-myrcene by gavage for 2 years. This is an example of a cystic adenoma. H&E

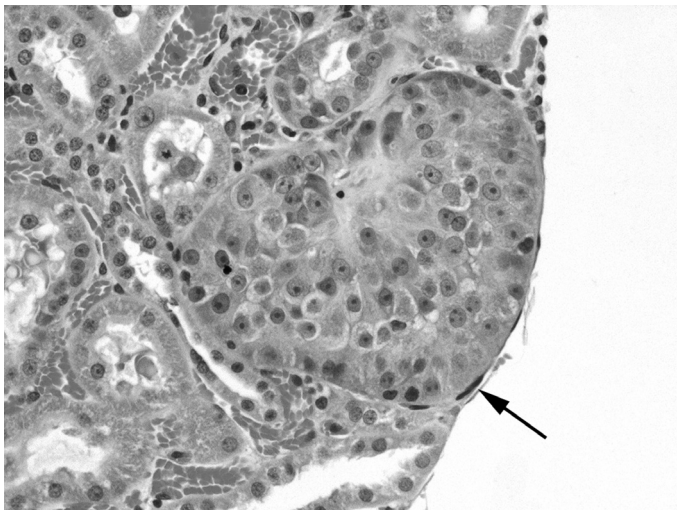


Plate 3
Renal tubule hyperplasia in a male F344/N rat administered 0.25 g/kg β-myrcene by gavage for 2 years. There are fibroblasts in intimate contact with the tubule margin (arrow), and the cells, which have a glassy, basophilic sheen, have well-demarcated borders and well-developed cytoplasm. H&E

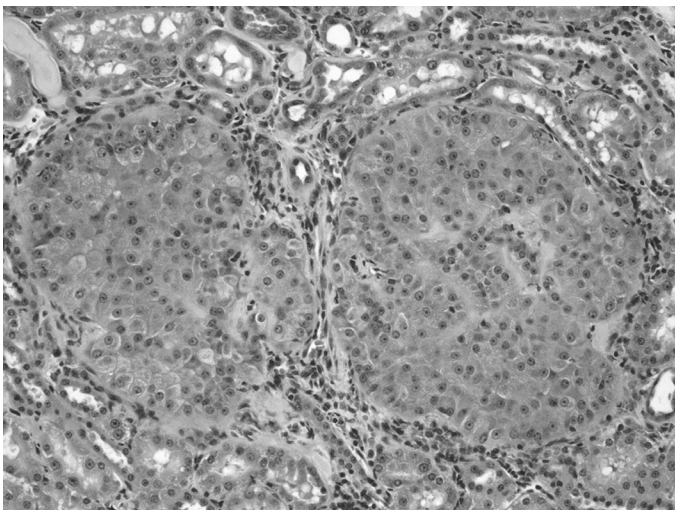


Plate 4
Renal tubule hyperplasia in a male F344/N rat administered 0.25 g/kg β-myrcene by gavage for 2 years. Compared to the example in Plate 3, this lesion is larger and more complex. H&E

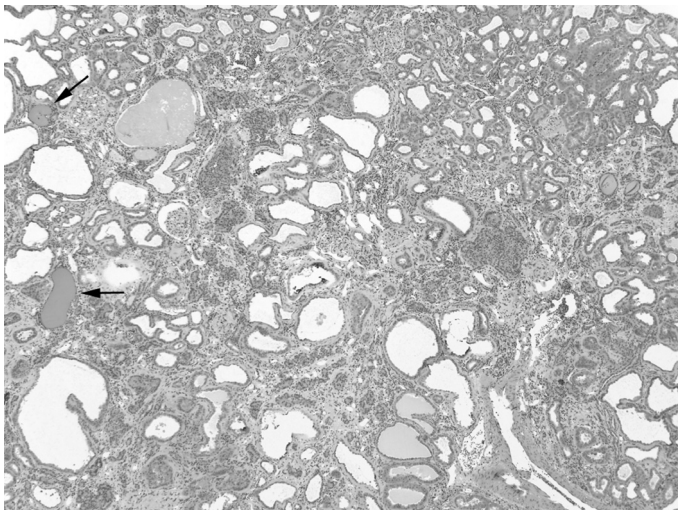


PLATE 5
Renal tubule nephrosis in a male F344/N rat administered 1 g/kg β-myrcene by gavage for 2 years. This plate shows many of the features of nephrosis, including renal tubule dilation and hyperplasia, karyomegaly of renal tubule epithelial cells, and interstitial fibrosis. The proteinaceous tubule casts (arrows), however, are more consistent with chronic progressive nephropathy. H&E

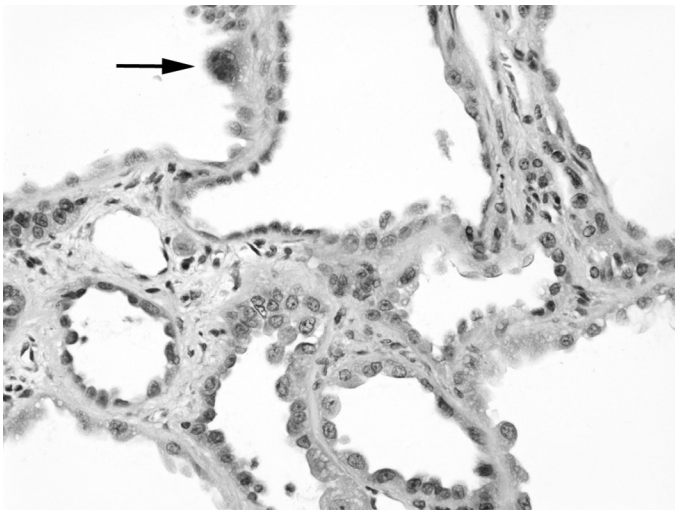


PLATE 6
Renal tubule nephrosis in a male F344/N rat administered 1 g/kg β-myrcene by gavage for 2 years. This photomicrograph of a lesion consistent with nephrosis highlights the karyomegaly of the renal tubule epithelium (arrow). H&E

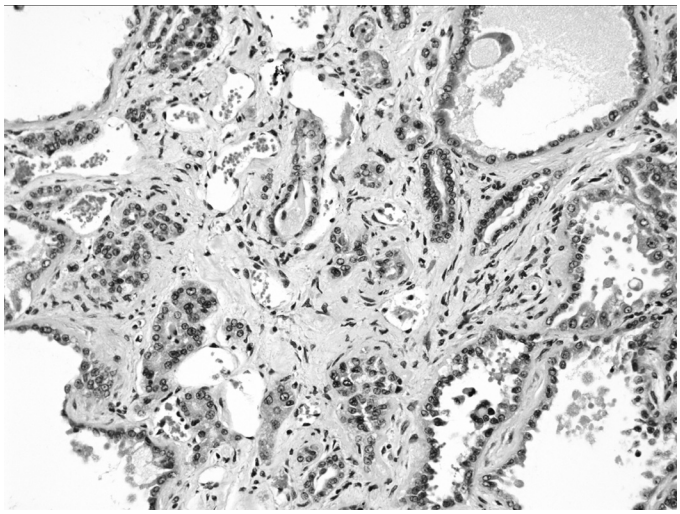


PLATE 7
Renal tubule nephrosis in a male F344/N rat administered 1 g/kg β-myrcene by gavage for 2 years. This plate illustrates interstitial fibrosis, a feature of both nephrosis and chronic progressive nephropathy. H&E

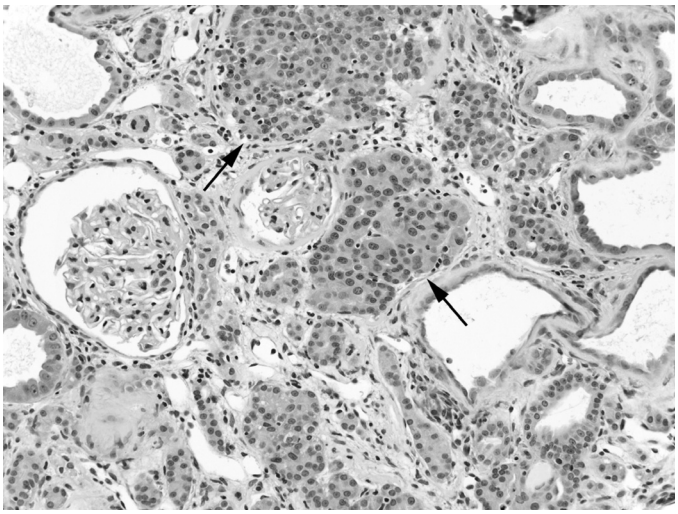


PLATE 8
Renal tubule nephrosis in a male F344/N rat administered 0.5 g/kg β-myrcene by gavage for 2 years. This plate shows the hyperplasia of the renal tubule epithelium (arrows) that is associated with nephrosiss. H&E

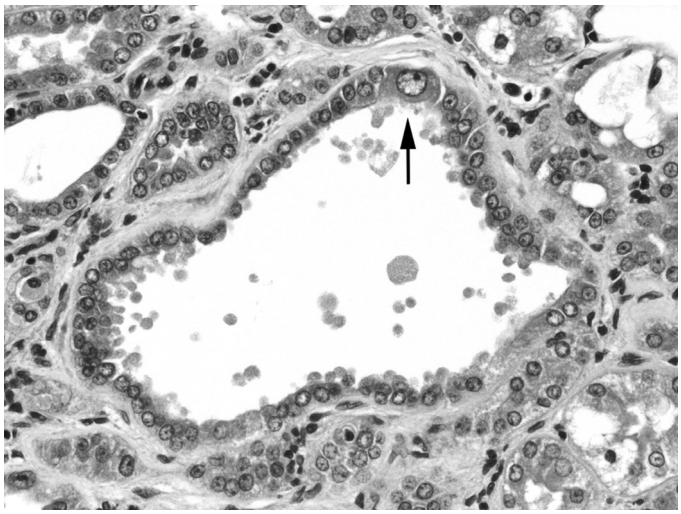


PLATE 9
Renal tubule nephrosis in a male F344/N rat administered 1 g/kg β-myrcene by gavage for 2 years. This is an example of simple tubule hyperplasia associated with nephrosis. Note the karyomegalic tubule epithelial cell (arrow). H&E

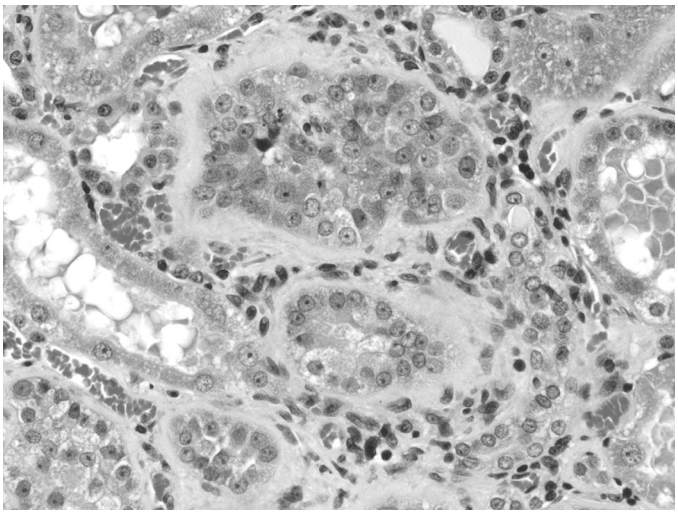


PLATE 10
Nephropathy in the kidney of a male F344/N rat administered 0.5 g/kg β-myrcene by gavage for 2 years. Note the tubule hyperplasia that is typically associated with nephropathy. Compared to the lesions in Plates 8 and 9, the basement membranes around these tubules are moderately thickened. H&E

DISCUSSION AND CONCLUSIONS

β-Myrcene is a ubiquitous chemical in the environment produced by many plant species. It is found in verbena, galbanum, and lemongrass oils and is the major constituent of hop and bay oils used as flavoring additives in food and alcoholic beverages (Madyastha and Srivatsan, 1987; Lorente *et al.*, 1989; Delgado *et al.*, 1993b). It is used commercially as an intermediate in the production of terpene alcohols (geraniol, nerol, and linalool), which serve as intermediates for the production of scents in cosmetics, soaps, and detergents (Kuney, 1994). β-myrcene was nominated for study based on its estimated high production volume, widespread human exposure, and lack of toxicity and carcinogenicity data. It is structurally related to *d*-limonene, which induced neoplasms in the kidneys of male rats in association with α2u-globulin (hyaline droplet) nephropathy (NTP, 1990).

The 3-month toxicity studies were conducted without any preceding dose-range-finding studies; the highest dose concentration was selected based on the published toxicity information for β-myrcene. In the absence of a range-finding study, it was expected that there would be frank toxicity at the high dose concentration and a gradation of adverse effects at the lower dose concentrations. However, all 4 g/kg rats and mice, most 2 g/kg mice, and a few 2 g/kg rats died by week 4, most during week 1. At 1 g/kg, one male and one female rat died due to dosing accidents. The final mean body weights of the surviving 1 g/kg male and female rats were 89% and 94%, respectively, relative to those of the vehicle controls; those of the 1 g/kg male and female mice were 91% and 97%, respectively, relative to those of the vehicle controls. The renal tubule necrosis observed in the 1 g/kg male and female rats in the 3-month study was considered mild and not life threatening to the animals. Thus, 1 g/kg was selected as the high dose concentration for rats and mice in the 2-year studies.

In the 2-year studies, all 1 g/kg male rats died early, and survival of 1 g/kg male and female mice was significantly decreased. Early deaths occurred primarily between 60 weeks and 85 weeks of administration. Deaths were

attributed to renal toxicity [nephrosis and chronic progressive nephropathy (CPN)] for male rats, but the cause of deaths for male and female mice was uncertain. Mortality in the 1 g/kg mice was not predicted based on the data from the corresponding 3-month studies. The 1 g/kg dose in the 2-year studies was considered to have exceeded the maximum tolerated dose for male rats and male and female mice.

In the 3-month and 2-year rat studies, the kidney was a primary target of β-myrcene-induced toxicity and carcinogenicity. Nephrotoxicity was not observed in male or female mice in the 3-month or 2-year studies. Three distinct renal lesions occurred in the male rats, including α2u-globulin nephropathy, CPN, and nephrosis. The latter two renal lesions were also observed in female rats. α2u-Globulin nephropathy is a renal syndrome in male rats characterized by the accumulation of a protein, α2u-globulin, in the form of brightly stained eosinophilic hyaline droplets in the cytoplasm of the proximal tubule epithelium (Swenberg *et al.*, 1989; Hard *et al.*, 1993; Swenberg and Lehman-McKeeman, 1999). The proposed sequence of events in the pathogenesis of α2u-globulin nephropathy involves binding of a chemical or its metabolites to α2u-globulin, which changes the conformation of the protein and decreases the rate of or prevents its degradation, ultimately resulting in accumulation within phagolysosomes of the renal tubule epithelial cells. The accumulation of α2u-globulin is thought to cause lysosomal dysfunction and subsequent release of lysosomal enzymes into the cytoplasm, resulting in a cycle of cytotoxicity, cell death, and a compensatory increase in cell proliferation that, if chronic, may lead to the promotion of neoplastic lesions (Swenberg *et al.*, 1989; Borghoff *et al.*, 1990). Female F344/N rats and mice of either sex do not produce α2u-globulin and thus do not develop α2u-globulin nephropathy (MacInnes *et al.*, 1986; Chatterjee *et al.*, 1989; Lehman-McKeeman and Caudill, 1992). CPN is one of the most commonly observed spontaneous lesions in the rat and as a syndrome is more prevalent and severe in male rats (Seely *et al.*, 2002). However, this syndrome can be exacerbated by chemical exposure resulting in increased incidences and average severities (Lock and Hard, 2004). Nephrosis is an uncommon lesion defined as renal tubule epithelial degeneration and regeneration.

In the 3-month β-myrcene studies, hyaline droplets were observed in the renal tubule epithelium of all vehicle controls and dose groups of male rats on day 23 when kidneys were stained with the Mallory-Heidenhain method. The histopathologic changes in the kidneys of these animals, including the amount of hyaline droplets and the changes in the hyaline droplet pattern, were consistent with α2u-globulin (hyaline droplet) nephropathy (Hard *et al.*, 1993; Hard, 2008). Hyaline droplets were not observed in female rats or male or female mice. The rats from the 4 g/kg dose group died prior to the end of the study and were not evaluated. In the 2 g/kg male rats from both studies, the incidence of hyaline droplet accumulation and the size and number of the hyaline droplets in the renal tubule epithelium were decreased, suggesting that the renal lesions in the 2 g/kg and greater groups were not related to hyaline droplet nephropathy or an α2u-globulin-associated mechanism.

In the 21-day *d*-limonene studies (NTP, 1990), there was a dose-related increase in the incidences of hyaline droplet accumulation in the kidneys of male rats as well as changes in the hyaline droplet pattern similar to those seen in the current β-myrcene studies. In the 3-month studies, there was no increase in the incidences of hyaline droplets in the dosed male rats compared to the vehicle controls; but there were other changes in these animals consistent with hyaline droplet nephropathy, including granular casts in the outer medulla and degeneration and regeneration of the epithelium of the convoluted tubules (NTP, 1990; Hard *et al.*, 1993). The droplets in the *d*-limonene study stained immunohistochemically for α2u-globulin (NTP, 1990). *d*-Limonene induced renal neoplasms in male rats only, presumably through an α2u-globulin-associated mechanism (NTP, 1990; Hard *et al.*, 1993).

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 (combined) in the 0.25 and 0.5 g/kg groups. In the females, the incidence of renal tubule adenoma was not significantly increased relative to controls, but was slightly above the historical control range in the 1 g/kg group. In females, the marginally increased incidence of renal tubule adenoma was considered to be equivocal evidence of carcinogenicity. β-Myrcene administration also resulted in the increased incidence and/or severity of a number of nonneoplastic renal lesions, including nephrosis

and exacerbation of CPN in both sexes, and papillary mineralization in the males. The papillary mineralization had a linear appearance and was found in the loops of Henle in the medulla. This type of mineralization, which is considered a chronic manifestation of α2u-globulin nephropathy, was also seen in NTP chronic studies of the structurally related compound *d*-limonene (NTP, 1990; Hard *et al.*, 1993).

Nephrosis was a 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 colocalization 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 *d*-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 and female rats is not clear. The observation of α2u-globulin nephropathy and linear papillary mineralization in male rats suggests 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, α2u-globulin nephropathy. The incidence and severity of linear papillary mineralization were greatest in the 0.25 g/kg males but slightly decreased in the 0.5 g/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 α2u-globulin mechanism.

In male and female mice, the liver was a primary target of β-myrcene toxicity. In the 3-month study, absolute and relative liver weights were increased in male and female mice. In the 2-year study, there was clear evidence of

carcinogenicity in male mice based on increased incidences of liver neoplasms. The incidences of hepatocellular adenoma were significantly increased in 0.25 and 0.5 g/kg males, and the incidences of hepatocellular carcinoma and of hepatoblastoma were significantly increased in the 0.5 g/kg group. When these neoplasm types were combined, the increases were statistically significant in the 0.25 and 0.5 g/kg groups.

In female mice, the incidence of hepatocellular adenoma or carcinoma (combined) was increased in the 0.25 g/kg group compared to the vehicle controls, while those in the 0.5 g/kg group were similar to vehicle controls. The incidences of these neoplasms were within or slightly higher than the historical control range for 2-year corn oil gavage studies. The lack of a dose-response in the 1 g/kg group is likely due to the reduction in body weight gain combined with somewhat shorter survival in this dose group. The marginally increased incidence in the 0.5 g/kg group was considered to be equivocal evidence of carcinogenicity in female mice. In contrast, administration of *d*-limonene did not induce liver lesions in mice in the 3-month study, nor did it induce significant increases in liver neoplasms in the 2-year study (NTP, 1990).

β-Myrcene appears to be more irritating and toxic than *d*-limonene (NTP, 1990) in rats. β-Myrcene-induced lesions in the nose (olfactory epithelium degeneration, suppurative inflammation, and chronic inflammation) of male and female rats and forestomach inflammation of female rats in the 3-month study and inflammation in the nose and forestomach of male rats in the 2-year study; these lesions were not seen in the *d*-limonene study (NTP, 1990).

Further studies are needed to understand the mechanism of action of β-myrcene-induced toxicity and carcinogenesis in rats and mice. β-Myrcene and *d*-limonene are not mutagenic nor clastogenic. *d*-Limonene interacts with α2u-globulin to induce kidney neoplasms in male rats (NTP, 1990). There is no evidence that *d*-limonene reacts with DNA and induces neoplasms at any other organ site. β-Myrcene may be metabolized by P450 to an epoxide, which may have the ability to alkylate DNA. The NTP could not procure labeled β-myrcene, and no disposition or metabolism studies were conducted.

CONCLUSIONS

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 *equivocal evidence of carcinogenic activity* of β-myrcene in female F344/N rats based on increased incidences of renal tubule adenoma. There was *clear evidence of carcinogenic activity* of β-myrcene in male B6C3F1 mice based on increased incidences of liver neoplasms. There was *equivocal evidence of carcinogenic activity* of β-myrcene in female B6C3F1 mice based on marginally increased incidences of hepatocellular neoplasms.

Administration of β-myrcene induced nonneoplastic lesions in the kidney of male and female rats, nose of male rats, and liver of male and female mice.

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* Explanation of Levels of Evidence of Carcinogenic Activity is on page 12.

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APPENDIX A
SUMMARY OF LESIONS IN MALE RATS IN THE 2-YEAR GAVAGE STUDY OF β-MYRCENE

TABLE A1 Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Gavage Study of β-Myrcene A-2
TABLE A2 Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Gavage Study of β-Myrcene A-6
TABLE A3 Historical Incidence of Renal Tubule Neoplasms in Control Male F344/N Rats A-10
TABLE A4 Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Gavage Study of β-Myrcene A-11

Table A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Gavage Study of β-Myrcene[a]

	Vehicle Control	0.25 g/kg	0.5 g/kg	1 g/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Accidental deaths		1		1
Moribund	18	8	13	24
Natural deaths	3	5	9	25
Survivors				
Terminal sacrifice	29	36	28	
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Intestine large, cecum	(50)	(50)	(50)	(50)
Intestine large, colon	(50)	(50)	(50)	(50)
Intestine large, rectum	(50)	(50)	(50)	(48)
Intestine small, duodenum	(49)	(50)	(50)	(50)
Intestine small, ileum	(50)	(50)	(50)	(50)
Intestine small, jejunum	(50)	(50)	(50)	(50)
Liver	(50)	(50)	(50)	(50)
Carcinoma, metastatic, kidney		1 (2%)		
Hepatocellular adenoma			1 (2%)	
Mesentery	(13)	(8)	(5)	(3)
Carcinoma, metastatic, kidney		1 (13%)		
Sarcoma		1 (13%)		
Oral mucosa	(2)	(0)	(0)	(0)
Squamous cell papilloma	1 (50%)			
Pancreas	(50)	(50)	(50)	(50)
Adenoma	1 (2%)		2 (4%)	
Salivary glands	(50)	(50)	(50)	(48)
Stomach, forestomach	(50)	(50)	(50)	(50)
Stomach, glandular	(50)	(50)	(50)	(50)
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Osteosarcoma, metastatic, bone			1 (2%)	
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Adenoma	1 (2%)	1 (2%)		
Carcinoma, metastatic, kidney		1 (2%)		
Adrenal medulla	(50)	(50)	(50)	(50)
Ganglioneuroma	1 (2%)			
Pheochromocytoma benign	10 (20%)	5 (10%)	4 (8%)	
Pheochromocytoma benign, multiple		1 (2%)		
Pheochromocytoma malignant	2 (4%)			
Bilateral, pheochromocytoma benign	1 (2%)		1 (2%)	
Islets, pancreatic	(50)	(50)	(50)	(50)
Adenoma	2 (4%)			
Carcinoma	1 (2%)			

Table A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Gavage Study of β-Myrcene

	Vehicle Control	0.25 g/kg	0.5 g/kg	1 g/kg
Endocrine System (continued)				
Parathyroid gland	(45)	(47)	(48)	(45)
Adenoma	1 (2%)			1 (2%)
Pituitary gland	(50)	(49)	(50)	(50)
Pars distalis, adenoma	6 (12%)	9 (18%)	5 (10%)	1 (2%)
Pars distalis, adenoma, multiple		1 (2%)		
Thyroid gland	(50)	(50)	(50)	(49)
Bilateral, C-cell, adenoma	3 (6%)	1 (2%)		
C-cell, adenoma	8 (16%)	5 (10%)	4 (8%)	1 (2%)
C-cell, adenoma, multiple	1 (2%)			
Follicle, adenoma	1 (2%)	2 (4%)	3 (6%)	2 (4%)
Follicle, carcinoma	2 (4%)	2 (4%)	1 (2%)	
General Body System				
None				
Genital System				
Epididymis	(50)	(50)	(50)	(48)
Carcinoma, metastatic, kidney		1 (2%)		
Preputial gland	(50)	(50)	(50)	(47)
Adenoma	4 (8%)	1 (2%)		
Adenoma, multiple	1 (2%)			
Carcinoma		1 (2%)		
Prostate	(50)	(50)	(50)	(50)
Adenoma			1 (2%)	
Seminal vesicle	(50)	(50)	(50)	(50)
Mesenchymal tumor malignant, metastatic, kidney		1 (2%)		
Testes	(50)	(50)	(50)	(48)
Bilateral, interstitial cell, adenoma	39 (78%)	43 (86%)	40 (80%)	18 (38%)
Interstitial cell, adenoma	8 (16%)	2 (4%)	6 (12%)	7 (15%)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Lymph node	(3)	(5)	(6)	(0)
Lymph node, mandibular	(0)	(0)	(1)	(0)
Lymph node, mesenteric	(50)	(50)	(50)	(50)
Mesenchymal tumor malignant, metastatic, kidney		1 (2%)		
Spleen	(50)	(50)	(50)	(50)
Thymus	(47)	(50)	(48)	(47)
Thymoma benign				1 (2%)
Integumentary System				
Mammary gland	(50)	(50)	(49)	(50)
Fibroadenoma		2 (4%)	4 (8%)	
Skin	(50)	(50)	(50)	(50)
Basal cell adenoma		1 (2%)	1 (2%)	
Basal cell carcinoma	1 (2%)			
Keratoacanthoma	2 (4%)	6 (12%)	2 (4%)	1 (2%)
Trichoepithelioma		1 (2%)		

Table A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Gavage Study of β-Myrcene

	Vehicle Control	0.25 g/kg	0.5 g/kg	1 g/kg
Integumentary System (continued)				
Skin (continued)	(50)	(50)	(50)	(50)
Sebaceous gland, adenoma	1 (2%)	1 (2%)		
Subcutaneous tissue, fibroma	3 (6%)	4 (8%)	3 (6%)	
Subcutaneous tissue, fibrosarcoma		1 (2%)	1 (2%)	
Subcutaneous tissue, fibrous histiocytoma			1 (2%)	
Subcutaneous tissue, lipoma		2 (4%)		
Subcutaneous tissue, sarcoma	1 (2%)			
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Chordoma	1 (2%)			
Osteoma			1 (2%)	
Osteosarcoma	1 (2%)		1 (2%)	
Skeletal muscle	(1)	(1)	(1)	(0)
Fibrous histiocytoma			1 (100%)	
Hemangiosarcoma		1 (100%)		
Rhabdomyosarcoma	1 (100%)			
Nervous System				
Brain	(50)	(50)	(50)	(50)
Ependymoma benign	1 (2%)			
Oligodendroglioma benign	1 (2%)			
Cranial nerve, schwannoma malignant		1 (2%)		
Peripheral nerve	(2)	(0)	(0)	(0)
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma	3 (6%)	6 (12%)	1 (2%)	
Alveolar/bronchiolar adenoma, multiple		1 (2%)		
Carcinoma, metastatic, kidney		1 (2%)		
Carcinoma, metastatic, preputial gland		1 (2%)		
Chordoma, metastatic, bone	1 (2%)			
Fibrous histiocytoma, metastatic, skeletal muscle			1 (2%)	
Mesenchymal tumor malignant, metastatic, kidney		1 (2%)		
Osteosarcoma, metastatic, bone	1 (2%)		1 (2%)	
Sarcoma, metastatic, skin	1 (2%)			
Nose	(50)	(50)	(50)	(50)
Trachea	(50)	(50)	(50)	(50)
Special Senses System				
Eye	(50)	(50)	(50)	(50)
Harderian gland	(50)	(50)	(50)	(49)
Adenoma			1 (2%)	
Carcinoma		1 (2%)		
Zymbal's gland	(0)	(1)	(0)	(0)
Carcinoma		1 (100%)		

Table A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Gavage Study of β-Myrcene

	Vehicle Control	0.25 g/kg	0.5 g/kg	1 g/kg
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Hemangioma			1 (2%)	
Lipoma	1 (2%)			
Mesenchymal tumor malignant		1 (2%)		
Nephroblastoma			1 (2%)	
Renal tubule, adenoma		2 (4%)	7 (14%)	2 (4%)
Renal tubule, adenoma, multiple		2 (4%)	1 (2%)	
Renal tubule, carcinoma		3 (6%)	1 (2%)	
Transitional epithelium, carcinoma	1 (2%)			
Urinary bladder	(50)	(50)	(50)	(49)
Papilloma	1 (2%)			
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Histiocytic sarcoma	1 (2%)			
Leukemia mononuclear	9 (18%)	10 (20%)	15 (30%)	
Lymphoma malignant	1 (2%)	1 (2%)		
Mesothelioma benign			1 (2%)	
Mesothelioma malignant	2 (4%)	1 (2%)	1 (2%)	
Neoplasm Summary				
Total animals with primary neoplasms[c]	50	49	49	27
Total primary neoplasms	126	124	113	34
Total animals with benign neoplasms	49	47	47	27
Total benign neoplasms	102	99	89	34
Total animals with malignant neoplasms	23	22	23	
Total malignant neoplasms	24	25	23	
Total animals with metastatic neoplasms	4	3	2	
Total metastatic neoplasms	4	9	3	

[a] Number of animals examined microscopically at the site and the number of animals with neoplasm
[b] Number of animals with any tissue examined microscopically
[c] Primary neoplasms: all neoplasms except metastatic neoplasms

TABLE A2
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Gavage Study of β-Myrcene[a]

	Vehicle Control	0.25 g/kg	0.5 g/kg
Adrenal Medulla: Benign Pheochromocytoma			
Overall rate[b]	11/50 (22%)	6/50 (12%)	5/50 (10%)
Adjusted rate[c]	25.5%	13.3%	11.9%
Terminal rate[d]	7/29 (24%)	6/36 (17%)	4/28 (14%)
First incidence (days)	674	729 (T)	709
Poly-3 test[e]	P=0.063N	P=0.117N	P=0.091N
Adrenal Medulla: Benign or Malignant Pheochromocytoma			
Overall rate	13/50 (26%)	6/50 (12%)	5/50 (10%)
Adjusted rate	30.1%	13.3%	11.9%
Terminal rate	9/29 (31%)	6/36 (17%)	4/28 (14%)
First incidence (days)	674	729 (T)	709
Poly-3 test	P=0.020N	P=0.045N	P=0.034N
Kidney (Renal Tubule): Adenoma (Single Section)			
Overall rate	0/50 (0%)	4/50 (8%)	8/50 (16%)
Adjusted rate	0.0%	8.8%	18.7%
Terminal rate	0/29 (0%)	3/36 (8%)	5/28 (18%)
First incidence (days)	—[f]	717	551
Poly-3 test	P=0.002	P=0.068	P=0.003
Kidney (Renal Tubule): Adenoma (Step Section)			
Overall rate	0/50 (0%)	8/50 (16%)	7/50 (14%)
Adjusted rate	0.0%	17.7%	16.4%
Terminal rate	0/29 (0%)	8/36 (22%)	4/28 (14%)
First incidence (days)	—	729 (T)	551
Poly-3 test	P=0.013	P=0.005	P=0.007
Kidney (Renal Tubule): Adenoma (Single and Step Sections)			
Overall rate	0/50 (0%)	12/50 (24%)	13/50 (26%)
Adjusted rate	0.0%	26.5%	30.2%
Terminal rate	0/29 (0%)	11/36 (31%)	9/28 (32%)
First incidence (days)	—	717	551
Poly-3 test	P<0.001	P<0.001	P<0.001
Kidney (Renal Tubule): Carcinoma (Single Section)			
Overall rate	0/50 (0%)	3/50 (6%)	1/50 (2%)
Adjusted rate	0.0%	6.6%	2.4%
Terminal rate	0/29 (0%)	2/36 (6%)	1/28 (4%)
First incidence (days)	—	652	729 (T)
Poly-3 test	P=0.369	P=0.130	P=0.496
Kidney (Renal Tubule): Carcinoma (Step Section)			
Overall rate	0/50 (0%)	3/50 (6%)	0/50 (0%)
Adjusted rate	0.0%	6.6%	0.0%
Terminal rate	0/29 (0%)	2/36 (6%)	0/28 (0%)
First incidence (days)	—	652	—
Poly-3 test	P=0.631	P=0.130	—[g]
Kidney (Renal Tubule): Carcinoma (Single and Step Sections)			
Overall rate	0/50 (0%)	3/50 (6%)	1/50 (2%)
Adjusted rate	0.0%	6.6%	2.4%
Terminal rate	0/29 (0%)	2/36 (6%)	1/28 (4%)
First incidence (days)	—	652	729 (T)
Poly-3 test	P=0.369	P=0.130	P=0.496

Table A2
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Gavage Study of β-Myrcene

	Vehicle Control	0.25 g/kg	0.5 g/kg
Kidney (Renal Tubule): Adenoma or Carcinoma (Single Section)			
Overall rate	0/50 (0%)	7/50 (14%)	9/50 (18%)
Adjusted rate	0.0%	15.4%	21.0%
Terminal rate	0/29 (0%)	5/36 (14%)	6/28 (21%)
First incidence (days)	—	652	551
Poly-3 test	P=0.002	P=0.010	P=0.002
Kidney (Renal Tubule): Adenoma or Carcinoma (Step Section)			
Overall rate	0/50 (0%)	10/50 (20%)	7/50 (14%)
Adjusted rate	0.0%	22.0%	16.4%
Terminal rate	0/29 (0%)	9/36 (25%)	4/28 (14%)
First incidence (days)	—	652	551
Poly-3 test	P=0.017	P<0.001	P=0.007
Kidney (Renal Tubule): Adenoma or Carcinoma (Single and Step Sections)			
Overall rate	0/50 (0%)	14/50 (28%)	13/50 (26%)
Adjusted rate	0.0%	30.8%	30.2%
Terminal rate	0/29 (0%)	12/36 (33%)	9/28 (32%)
First incidence (days)	—	652	551
Poly-3 test	P<0.001	P<0.001	P<0.001
Lung: Alveolar/bronchiolar Adenoma			
Overall rate	3/50 (6%)	7/50 (14%)	1/50 (2%)
Adjusted rate	7.0%	15.5%	2.4%
Terminal rate	1/29 (3%)	6/36 (17%)	0/28 (0%)
First incidence (days)	667	704	674
Poly-3 test	P=0.299N	P=0.177	P=0.314N
Mammary Gland: Fibroadenoma			
Overall rate	0/50 (0%)	2/50 (4%)	4/50 (8%)
Adjusted rate	0.0%	4.4%	9.6%
Terminal rate	0/29 (0%)	2/36 (6%)	4/28 (14%)
First incidence (days)	—	729 (T)	729 (T)
Poly-3 test	P=0.034	P=0.250	P=0.057
Pancreatic Islets: Adenoma or Carcinoma			
Overall rate	3/50 (6%)	0/50 (0%)	0/50 (0%)
Adjusted rate	7.0%	0.0%	0.0%
Terminal rate	2/29 (7%)	0/36 (0%)	0/28 (0%)
First incidence (days)	688	—	—
Poly-3 test	P=0.036N	P=0.109N	P=0.123N
Pituitary Gland (Pars Distalis): Adenoma			
Overall rate	6/50 (12%)	10/49 (20%)	5/50 (10%)
Adjusted rate	14.0%	22.4%	11.9%
Terminal rate	5/29 (17%)	9/36 (25%)	3/28 (11%)
First incidence (days)	726	611	666
Poly-3 test	P=0.454N	P=0.232	P=0.509N
Preputial Gland: Adenoma			
Overall rate	5/50 (10%)	1/50 (2%)	0/50 (0%)
Adjusted rate	11.4%	2.2%	0.0%
Terminal rate	0/29 (0%)	1/36 (3%)	0/28 (0%)
First incidence (days)	667	729 (T)	—
Poly-3 test	P=0.011N	P=0.093N	P=0.034N

Table A2
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Gavage Study of β-Myrcene

	Vehicle Control	0.25 g/kg	0.5 g/kg
Preputial Gland: Adenoma or Carcinoma			
Overall rate	5/50 (10%)	2/50 (4%)	0/50 (0%)
Adjusted rate	11.4%	4.4%	0.0%
Terminal rate	0/29 (0%)	1/36 (3%)	0/28 (0%)
First incidence (days)	667	578	—
Poly-3 test	P=0.017N	P=0.198N	P=0.034N
Skin: Keratoacanthoma			
Overall rate	2/50 (4%)	6/50 (12%)	2/50 (4%)
Adjusted rate	4.7%	13.3%	4.8%
Terminal rate	2/29 (7%)	6/36 (17%)	2/28 (7%)
First incidence (days)	729 (T)	729 (T)	729 (T)
Poly-3 test	P=0.566	P=0.151	P=0.687
Skin: Keratoacanthoma, Trichoepithelioma, Basal Cell Adenoma, or Basal Cell Carcinoma			
Overall rate	3/50 (6%)	8/50 (16%)	3/50 (6%)
Adjusted rate	6.9%	17.7%	7.1%
Terminal rate	2/29 (7%)	8/36 (22%)	2/28 (7%)
First incidence (days)	429	729 (T)	560
Poly-3 test	P=0.548	P=0.110	P=0.651
Skin (Subcutaneous Tissue): Fibroma			
Overall rate	3/50 (6%)	4/50 (8%)	3/50 (6%)
Adjusted rate	7%	8.9%	7.1%
Terminal rate	3/29 (10%)	4/36 (11%)	2/28 (7%)
First incidence (days)	729 (T)	729 (T)	666
Poly-3 test	P=0.572	P=0.531	P=0.656
Skin (Subcutaneous Tissue): Fibroma, Fibrous Histiocytoma, Fibrosarcoma, or Sarcoma			
Overall rate	4/50 (8%)	5/50 (10%)	5/50 (10%)
Adjusted rate	9.3%	11.0%	11.9%
Terminal rate	3/29 (10%)	4/36 (11%)	4/28 (14%)
First incidence (days)	611	611	666
Poly-3 test	P=0.418	P=0.535	P=0.485
Testes: Adenoma			
Overall rate	47/50 (94%)	45/50 (90%)	46/50 (92%)
Adjusted rate	96.8%	93.1%	97.9%
Terminal rate	28/29 (97%)	33/36 (92%)	28/28 (100%)
First incidence (days)	429	544	486
Poly-3 test	P=0.509	P=0.346N	P=0.642
Thyroid Gland (Follicular Cell): Adenoma			
Overall rate	1/50 (2%)	2/50 (4%)	3/50 (6%)
Adjusted rate	2.3%	4.4%	7.1%
Terminal rate	1/29 (3%)	2/36 (6%)	2/28 (7%)
First incidence (days)	729 (T)	729 (T)	674
Poly-3 test	P=0.219	P=0.520	P=0.299
Thyroid Gland (Follicular Cell): Adenoma or Carcinoma			
Overall rate	3/50 (6%)	4/50 (8%)	4/50 (8%)
Adjusted rate	7.0%	8.9%	9.5%
Terminal rate	2/29 (7%)	4/36 (11%)	3/28 (11%)
First incidence (days)	674	729 (T)	674
Poly-3 test	P=0.414	P=0.528	P=0.489

TABLE A2
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Gavage Study of β-Myrcene

	Vehicle Control	0.25 g/kg	0.5 g/kg
Thyroid Gland (C-Cell): Adenoma			
Overall rate	12/50 (24%)	6/50 (12%)	4/50 (8%)
Adjusted rate	27.3%	13.3%	9.5%
Terminal rate	9/29 (31%)	6/36 (17%)	3/28 (11%)
First incidence (days)	528	729 (T)	666
Poly-3 test	P=0.018N	P=0.080N	P=0.030N
All Organs: Mononuclear Cell Leukemia			
Overall rate	9/50 (18%)	10/50 (20%)	15/50 (30%)
Adjusted rate	20.1%	21.0%	33.3%
Terminal rate	3/29 (10%)	4/36 (11%)	5/28 (18%)
First incidence (days)	528	486	449
Poly-3 test	P=0.094	P=0.562	P=0.119
All Organs: Benign Neoplasms			
Overall rate	49/50 (98%)	47/50 (94%)	47/50 (94%)
Adjusted rate	99.4%	97.2%	99.5%
Terminal rate	29/29 (100%)	35/36 (97%)	28/28 (100%)
First incidence (days)	429	544	486
Poly-3 test	P=0.714N	P=0.469N	P=0.996
All Organs: Malignant Neoplasms			
Overall rate	23/50 (46%)	22/50 (44%)	23/50 (46%)
Adjusted rate	48.0%	45.2%	49.7%
Terminal rate	8/29 (28%)	10/36 (28%)	9/28 (32%)
First incidence (days)	429	486	449
Poly-3 test	P=0.478	P=0.472N	P=0.515
All Organs: Benign or Malignant Neoplasms			
Overall rate	50/50 (100%)	49/50 (98%)	48/50 (96%)
Adjusted rate	100%	99.9%	100%
Terminal rate	29/29 (100%)	36/36 (100%)	28/28 (100%)
First incidence (days)	429	486	449
Poly-3 test	P=1.000N	P=1.000N	P=1.000N

(T) Terminal sacrifice
[a] The 1 g/kg group has been excluded from the statistical analyses due to early mortality.
[b] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal medulla, kidney, lung, pancreatic islets, pituitary gland, preputial gland, testes, and thyroid gland; for other tissues, denominator is number of animals necropsied.
[c] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[d] Observed incidence at terminal kill
[e] Beneath the vehicle control incidence is the P value associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for the differential mortality in animals that do not reach terminal sacrifice. A negative trend or a lower incidence in a dose group is indicated by **N**.
[f] Not applicable; no neoplasms in animal group
[g] Value of statistic cannot be computed.

TABLE A3
Historical Incidence of Renal Tubule Neoplasms in Control Male F344/N Rats

Study (Study Start)	Incidence in Controls		
	Adenoma	Carcinoma	Adenoma or Carcinoma
Historical Incidence: Corn Oil Gavage Studies			
β-Myrcene (March, 2002)	0/50	0/50	0/50
Isoeugenol (April, 2002)	0/50	0/50	0/50
Pulegone (April, 2003)	1/50	0/50	1/50
Total (%)	1/150 (0.7%)	0/150 (0%)	1/150 (0.7%)
Mean standard deviation	0.7% ± 1.2%		0.7% ± 1.2%
Range	0%-2%		0%-2%
Overall Historical Incidence: All Routes			
Total	8/1394 (0.6%)	2/1394 (0.1%)	10/1394 (0.7%)
Mean ± standard deviation	0.6% ± 0.9%	0.1% ± 0.5%	0.7% ± 1.2%
Range	0%-2%	0%-2%	0%-4%

[a] Data as of November 17, 2008

Table A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Gavage Study of β-Myrcene[a]

	Vehicle Control	0.25 g/kg	0.5 g/kg	1 g/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Accidental deaths		1		1
Moribund	18	8	13	24
Natural deaths	3	5	9	25
Survivors				
Terminal sacrifice	29	36	28	
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Inflammation, chronic			1 (2%)	
Perforation		1 (2%)		1 (2%)
Muscularis, periesophageal tissue, hemorrhage				1 (2%)
Intestine large, cecum	(50)	(50)	(50)	(50)
Inflammation, chronic			1 (2%)	
Intestine large, colon	(50)	(50)	(50)	(50)
Inflammation, chronic			1 (2%)	
Parasite metazoan	1 (2%)	1 (2%)	2 (4%)	
Intestine large, rectum	(50)	(50)	(50)	(48)
Inflammation, chronic			1 (2%)	
Parasite metazoan	6 (12%)	4 (8%)	3 (6%)	
Ulcer				1 (2%)
Intestine small, duodenum	(49)	(50)	(50)	(50)
Intestine small, ileum	(50)	(50)	(50)	(50)
Parasite metazoan	1 (2%)			
Intestine small, jejunum	(50)	(50)	(50)	(50)
Liver	(50)	(50)	(50)	(50)
Angiectasis	2 (4%)	4 (8%)	3 (6%)	
Basophilic focus	23 (46%)	12 (24%)	5 (10%)	2 (4%)
Clear cell focus	14 (28%)	19 (38%)	9 (18%)	
Degeneration, cystic	1 (2%)	1 (2%)		
Eosinophilic focus	6 (12%)	5 (10%)	3 (6%)	
Fatty change	4 (8%)	4 (8%)	2 (4%)	
Hemorrhage				1 (2%)
Hepatodiaphragmatic nodule	3 (6%)	3 (6%)	2 (4%)	3 (6%)
Inflammation, chronic	34 (68%)	36 (72%)	19 (38%)	1 (2%)
Mixed cell focus	6 (12%)	1 (2%)		
Necrosis		4 (8%)	4 (8%)	4 (8%)
Regeneration		1 (2%)		
Bile duct, hyperplasia	39 (78%)	42 (84%)	39 (78%)	6 (12%)
Centrilobular, degeneration	3 (6%)	1 (2%)	1 (2%)	
Hepatocyte, hypertrophy	1 (2%)			30 (60%)
Serosa, hyperplasia		1 (2%)		
Serosa, inflammation, suppurative			1 (2%)	
Mesentery	(13)	(8)	(5)	(3)
Fat, necrosis	12 (92%)	7 (88%)	4 (80%)	3 (100%)
Oral mucosa	(2)	(0)	(0)	(0)
Inflammation, chronic	1 (50%)			

[a] Number of animals examined microscopically at the site and the number of animals with lesion

Table A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Gavage Study of β-Myrcene

	Vehicle Control	0.25 g/kg	0.5 g/kg	1 g/kg
Alimentary System (continued)				
Pancreas	(50)	(50)	(50)	(50)
Inflammation, granulomatous	1 (2%)			
Necrosis	1 (2%)			
Acinus, atrophy	9 (18%)	14 (28%)	11 (22%)	5 (10%)
Acinus, hyperplasia	8 (16%)	7 (14%)	2 (4%)	
Artery, inflammation, chronic			1 (2%)	
Artery, thrombosis				1 (2%)
Duct, cyst	2 (4%)	3 (6%)	5 (10%)	
Salivary glands	(50)	(50)	(50)	(48)
Atrophy				1 (2%)
Cyst		1 (2%)		
Stomach, forestomach	(50)	(50)	(50)	(50)
Erosion	1 (2%)			3 (6%)
Fibrosis			1 (2%)	
Inflammation, chronic active	5 (10%)	3 (6%)	12 (24%)	27 (54%)
Ulcer	2 (4%)	2 (4%)	5 (10%)	18 (36%)
Serosa, hyperplasia				1 (2%)
Stomach, glandular	(50)	(50)	(50)	(50)
Inflammation, chronic	3 (6%)	7 (14%)	2 (4%)	
Mineralization	1 (2%)		1 (2%)	
Ulcer	1 (2%)	2 (4%)	1 (2%)	1 (2%)
Epithelium, ectasia	14 (28%)	27 (54%)	33 (66%)	14 (28%)
Epithelium, hyperplasia	1 (2%)	2 (4%)	1 (2%)	
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Cardiomyopathy	39 (78%)	43 (86%)	42 (84%)	38 (76%)
Atrium, thrombosis	2 (4%)		2 (4%)	
Endocardium, hyperplasia				1 (2%)
Epicardium, hyperplasia				1 (2%)
Myocardium, mineralization			1 (2%)	1 (2%)
Valve, thrombosis			2 (4%)	
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Hyperplasia	3 (6%)	5 (10%)	9 (18%)	1 (2%)
Necrosis		2 (4%)		1 (2%)
Vacuolization cytoplasmic	13 (26%)	16 (32%)	15 (30%)	7 (14%)
Capsule, hyperplasia		1 (2%)		
Adrenal medulla	(50)	(50)	(50)	(50)
Hyperplasia	9 (18%)	9 (18%)	13 (26%)	
Islets, pancreatic	(50)	(50)	(50)	(50)
Hyperplasia	1 (2%)			
Parathyroid gland	(45)	(47)	(48)	(45)
Hyperplasia			1 (2%)	2 (4%)
Pituitary gland	(50)	(49)	(50)	(50)
Angiectasis	8 (16%)	8 (16%)	9 (18%)	1 (2%)
Cyst	6 (12%)	9 (18%)	5 (10%)	2 (4%)
Pars distalis, hyperplasia	15 (30%)	11 (22%)	10 (20%)	4 (8%)
Thyroid gland	(50)	(50)	(50)	(49)
Cyst	1 (2%)		1 (2%)	
Inflammation, granulomatous				1 (2%)
C-cell, hyperplasia	20 (40%)	18 (36%)	15 (30%)	1 (2%)
Follicle, hyperplasia	4 (8%)	2 (4%)	3 (6%)	

Table A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Gavage Study of β-Myrcene

	Vehicle Control	0.25 g/kg	0.5 g/kg	1 g/kg
General Body System				
None				
Genital System				
Epididymis	(50)	(50)	(50)	(48)
Granuloma sperm			1 (2%)	
Inflammation, chronic			1 (2%)	
Vacuolization cytoplasmic, focal	1 (2%)			
Preputial gland	(50)	(50)	(50)	(47)
Atrophy	1 (2%)			
Cyst	1 (2%)			
Hyperplasia	1 (2%)	2 (4%)		
Inflammation, chronic	42 (84%)	44 (88%)	43 (86%)	36 (77%)
Prostate	(50)	(50)	(50)	(50)
Atrophy				1 (2%)
Hyperplasia	7 (14%)	3 (6%)	6 (12%)	1 (2%)
Inflammation, chronic	4 (8%)	6 (12%)	8 (16%)	6 (12%)
Inflammation, chronic, active	1 (2%)			
Necrosis				1 (2%)
Seminal vesicle	(50)	(50)	(50)	(50)
Atrophy				1 (2%)
Testes	(50)	(50)	(50)	(48)
Interstitial cell, hyperplasia	6 (12%)	2 (4%)	2 (4%)	9 (19%)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Hyperplasia	8 (16%)	5 (10%)	7 (14%)	1 (2%)
Lymph node	(3)	(5)	(6)	(0)
Mediastinal, ectasia	1 (33%)			
Mediastinal, hemorrhage		1 (20%)	1 (17%)	
Mediastinal, hyperplasia, lymphoid		2 (40%)		
Mediastinal, pigmentation, hemosiderin		1 (20%)	1 (17%)	
Lymph node, mandibular	(0)	(0)	(1)	(0)
Lymph node, mesenteric	(50)	(50)	(50)	(50)
Pigmentation, hemosiderin				1 (2%)
Spleen	(50)	(50)	(50)	(50)
Atrophy	1 (2%)	2 (4%)	6 (12%)	46 (92%)
Congestion			2 (4%)	
Fibrosis	3 (6%)			
Hematopoietic cell proliferation	8 (16%)	6 (12%)	4 (8%)	
Infarct			1 (2%)	
Necrosis, focal		1 (2%)	1 (2%)	
Thymus	(47)	(50)	(48)	(47)
Atrophy	40 (85%)	42 (84%)	43 (90%)	44 (94%)
Inflammation, suppurative		1 (2%)		
Epithelial cell, hyperplasia			1 (2%)	
Integumentary System				
Mammary gland	(50)	(50)	(49)	(50)
Cyst			1 (2%)	
Inflammation, chronic			1 (2%)	

Table A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Gavage Study of β-Myrcene

	Vehicle Control	0.25 g/kg	0.5 g/kg	1 g/kg
Integumentary System (continued)				
Skin	(50)	(50)	(50)	(50)
Hyperkeratosis		1 (2%)	1 (2%)	
Inflammation, chronic			1 (2%)	
Ulcer			1 (2%)	
Subcutaneous tissue, inflammation, granulomatous		1 (2%)		
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Skeletal muscle	(1)	(1)	(1)	(0)
Nervous System				
Brain	(50)	(50)	(50)	(50)
Hydrocephalus	3 (6%)			
Cerebrum, compression		1 (2%)		
Hypothalamus, compression	1 (2%)			
Neuron, necrosis, focal			1 (2%)	
Peripheral nerve	(2)	(0)	(0)	(0)
Axon, degeneration	1 (50%)			
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Inflammation	32 (64%)	33 (66%)	18 (36%)	14 (28%)
Inflammation, chronic	1 (2%)			
Metaplasia, osseous	5 (10%)		1 (2%)	2 (4%)
Alveolar epithelium, hyperplasia	9 (18%)	9 (18%)	2 (4%)	4 (8%)
Alveolus, emphysema	1 (2%)			
Nose	(50)	(50)	(50)	(50)
Dysplasia			1 (2%)	
Inflammation, chronic active	14 (28%)	19 (38%)	27 (54%)	35 (70%)
Ulcer			1 (2%)	
Olfactory epithelium, degeneration	45 (90%)	49 (98%)	47 (94%)	49 (98%)
Trachea	(50)	(50)	(50)	(50)
Peritracheal tissue, inflammation, chronic		1 (2%)		
Special Senses System				
Eye	(50)	(50)	(50)	(50)
Cataract	1 (2%)			
Anterior chamber, inflammation, suppurative		1 (2%)		
Cornea, inflammation, suppurative		1 (2%)		
Retina, degeneration		1 (2%)		
Sclera, metaplasia, osseous	23 (46%)	25 (50%)	30 (60%)	6 (12%)
Harderian gland	(50)	(50)	(50)	(49)
Atrophy		1 (2%)		1 (2%)
Cyst	1 (2%)			
Hyperplasia	1 (2%)	2 (4%)		1 (2%)
Inflammation, chronic	2 (4%)	1 (2%)		2 (4%)
Pigmentation, porphyrin	50 (100%)	50 (100%)	50 (100%)	49 (100%)
Zymbal's gland	(0)	(1)	(0)	(0)

Table A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Gavage Study of β-Myrcene

	Vehicle Control	0.25 g/kg	0.5 g/kg	1 g/kg
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Hydronephrosis	1 (2%)			
Inflammation, suppurative, focal	1 (2%)	22 (44%)	22 (44%)	
Inflammation, chronic	1 (2%)			
Metaplasia, osseous			1 (2%)	
Mineralization		1 (2%)		
Necrosis	1 (2%)	2 (4%)	2 (4%)	
Nephropathy	45 (90%)	48 (96%)	48 (96%)	49 (98%)
Pigmentation	1 (2%)			
Papilla, mineralization	1 (2%)	48 (96%)	40 (80%)	4 (8%)
Renal tubule, cyst	1 (2%)	3 (6%)	2 (4%)	
Renal tubule, hyperplasia			2 (4%)	
Renal tubule, hyperplasia, oncocytic		3 (6%)		
Renal tubule, nephrosis		42 (84%)	46 (92%)	48 (96%)
Transitional epithelium, hyperplasia		21 (42%)	19 (38%)	18 (36%)
Vein, thrombosis			3 (6%)	3 (6%)
Urinary bladder	(50)	(50)	(50)	(49)
Inflammation				1 (2%)

APPENDIX B
SUMMARY OF LESIONS IN FEMALE RATS IN THE 2-YEAR GAVAGE STUDY OF β-MYRCENE

TABLE B1 Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Gavage Study of β-Myrcene B-2
TABLE B2 Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Gavage Study of β-Myrcene B-5
TABLE B3 Historical Incidence of Renal Tubule Adenoma in Control Female F344/N Rats B-8
TABLE B4 Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study of β-Myrcene B-9

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Gavage Study of β-Myrcene[a]

	Vehicle Control	0.25 g/kg	0.5 g/kg	1 g/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Accidental deaths		2		1
Moribund	11	9	12	7
Natural deaths	8	6	10	9
Survivors				
Terminal sacrifice	31	33	28	33
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(49)	(50)
Intestine large, colon	(50)	(50)	(50)	(50)
Intestine large, rectum	(50)	(50)	(50)	(50)
Intestine small, duodenum	(50)	(50)	(49)	(50)
Leiomyosarcoma				1 (2%)
Intestine small, ileum	(50)	(50)	(49)	(50)
Liver	(50)	(50)	(49)	(50)
Hepatocellular adenoma				1 (2%)
Hepatocellular carcinoma		1 (2%)		
Mesentery	(7)	(13)	(7)	(9)
Oral mucosa	(1)	(0)	(0)	(0)
Squamous cell carcinoma	1 (100%)			
Pancreas	(50)	(50)	(49)	(50)
Salivary glands	(50)	(49)	(49)	(50)
Myoepithelioma			1 (2%)	
Stomach, forestomach	(50)	(50)	(49)	(50)
Stomach, glandular	(50)	(50)	(49)	(50)
Tongue	(1)	(0)	(1)	(0)
Squamous cell papilloma	1 (100%)			
Cardiovascular System				
Heart	(50)	(50)	(49)	(50)
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Adrenal medulla	(50)	(50)	(50)	(50)
Hemangioma	1 (2%)			
Pheochromocytoma benign	1 (2%)			
Islets, pancreatic	(50)	(50)	(49)	(50)
Adenoma	1 (2%)	1 (2%)		
Parathyroid gland	(47)	(48)	(47)	(45)
Pituitary gland	(50)	(50)	(50)	(50)
Pars distalis, adenoma	24 (48%)	21 (42%)	13 (26%)	14 (28%)
Pars distalis, adenoma, multiple	1 (2%)	1 (2%)		1 (2%)
Pars intermedia, adenoma			1 (2%)	
Thyroid gland	(50)	(49)	(49)	(50)
Bilateral, C-cell, adenoma	1 (2%)			
C-cell, adenoma	4 (8%)	13 (27%)	4 (8%)	4 (8%)
Follicle, adenoma		2 (4%)		1 (2%)
Follicle, carcinoma			1 (2%)	

Table B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Gavage Study of β-Myrcene

	Vehicle Control	0.25 g/kg	0.5 g/kg	1 g/kg
General Body System				
None				
Genital System				
Clitoral gland	(50)	(50)	(50)	(50)
Adenoma	6 (12%)	2 (4%)	2 (4%)	1 (2%)
Carcinoma			1 (2%)	
Bilateral, adenoma	1 (2%)			
Ovary	(50)	(50)	(50)	(50)
Granulosa cell tumor benign	1 (2%)			
Granulosa cell tumor malignant		1 (2%)		1 (2%)
Thecoma benign		1 (2%)		
Uterus	(50)	(50)	(50)	(50)
Adenoma			1 (2%)	
Carcinoma	1 (2%)			
Deciduoma malignant		1 (2%)		
Polyp stromal	8 (16%)	3 (6%)	10 (20%)	6 (12%)
Polyp stromal, multiple			2 (4%)	
Sarcoma stromal		1 (2%)		1 (2%)
Bilateral, polyp stromal			1 (2%)	
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Lymph node	(5)	(3)	(2)	(6)
Mediastinal, schwannoma malignant, metastatic, skin			1 (50%)	
Mediastinal, squamous cell carcinoma, metastatic, oral mucosa	1 (20%)			
Lymph node, mesenteric	(50)	(50)	(50)	(50)
Spleen	(50)	(50)	(50)	(50)
Thymus	(49)	(48)	(49)	(50)
Integumentary System				
Mammary gland	(50)	(50)	(50)	(50)
Carcinoma		2 (4%)		
Fibroadenoma	13 (26%)	15 (30%)	8 (16%)	7 (14%)
Fibroadenoma, multiple	4 (8%)	5 (10%)	4 (8%)	
Skin	(50)	(50)	(50)	(50)
Basal cell adenoma	1 (2%)			
Basal cell carcinoma			1 (2%)	
Keratoacanthoma		1 (2%)		
Subcutaneous tissue, fibroma	2 (4%)		1 (2%)	
Subcutaneous tissue, fibrosarcoma	1 (2%)			
Subcutaneous tissue, lipoma		1 (2%)	3 (6%)	
Subcutaneous tissue, mast cell tumor, malignant			1 (2%)	
Subcutaneous tissue, schwannoma malignant			1 (2%)	
Musculoskeletal System				
Skeletal muscle	(0)	(0)	(1)	(0)

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Gavage Study of β-Myrcene

	Vehicle Control	0.25 g/kg	0.5 g/kg	1 g/kg
Nervous System				
Brain	(50)	(50)	(50)	(50)
Spinal cord	(1)	(0)	(0)	(0)
Astrocytoma benign	1 (100%)			
Respiratory System				
Lung	(50)	(50)	(49)	(50)
Alveolar/bronchiolar adenoma	4 (8%)	3 (6%)	2 (4%)	1 (2%)
Alveolar/bronchiolar carcinoma		1 (2%)		1 (2%)
Carcinoma, metastatic, clitoral gland			1 (2%)	
Fibrosarcoma, metastatic, skin	1 (2%)			
Schwannoma malignant, metastatic, skin			1 (2%)	
Squamous cell carcinoma	1 (2%)			
Nose	(50)	(50)	(50)	(50)
Special Senses System				
Ear	(0)	(3)	(0)	(0)
Pinna, neural crest tumor		1 (33%)		
Eye	(50)	(50)	(49)	(50)
Harderian gland	(50)	(50)	(49)	(50)
Zymbal's gland	(0)	(0)	(0)	(2)
Carcinoma				2 (100%)
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Nephroblastoma	1 (2%)			
Renal tubule, adenoma		1 (2%)		2 (4%)
Urinary bladder	(50)	(50)	(50)	(50)
Transitional epithelium, carcinoma			1 (2%)	
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Leukemia mononuclear	5 (10%)	8 (16%)	4 (8%)	11 (22%)
Lymphoma malignant				1 (2%)
Mesothelioma malignant	1 (2%)			
Neoplasm Summary				
Total animals with primary neoplasms[c]	45	44	39	37
Total primary neoplasms	86	86	63	56
Total animals with benign neoplasms	40	39	35	27
Total benign neoplasms	75	70	53	38
Total animals with malignant neoplasms	11	14	10	17
Total malignant neoplasms	11	15	10	18
Total animals with metastatic neoplasms	2		2	
Total metastatic neoplasms	2		3	
Total animals with uncertain neoplasms - benign or malignant		1		
Total uncertain neoplasms		1		

[a] Number of animals examined microscopically at the site and the number of animals with neoplasm
[b] Number of animals with any tissue examined microscopically
[c] Primary neoplasms: all neoplasms except metastatic neoplasms

Table B2
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Gavage Study of β-Myrcene

	Vehicle Control	0.25 g/kg	0.5 g/kg	1 g/kg
Clitoral Gland: Adenoma				
Overall rate[a]	7/50 (14%)	2/50 (4%)	2/50 (4%)	1/50 (2%)
Adjusted rate[b]	16.8%	4.8%	5.1%	2.4%
Terminal rate[c]	5/31 (16%)	2/33 (6%)	2/28 (7%)	0/33 (0%)
First incidence (days)	670	730 (T)	730 (T)	663
Poly-3 test[d]	P=0.020N	P=0.077N	P=0.091N	P=0.028N
Clitoral Gland: Adenoma or Carcinoma				
Overall rate	7/50 (14%)	2/50 (4%)	3/50 (6%)	1/50 (2%)
Adjusted rate	16.8%	4.8%	7.6%	2.4%
Terminal rate	5/31 (16%)	2/33 (6%)	2/28 (7%)	0/33 (0%)
First incidence (days)	670	730 (T)	637	663
Poly-3 test	P=0.027N	P=0.077N	P=0.175N	P=0.028N
Kidney (Renal Tubule): Adenoma (Single and Step Sections)				
Overall rate	0/50 (0%)	2/50 (4%)	1/50 (2%)	3/50 (6%)
Adjusted rate	0.0%	4.8%	2.5%	7.2%
Terminal rate	0/31 (0%)	1/33 (3%)	0/28 (0%)	3/33 (9%)
First incidence (days)	—[e]	689	701	730 (T)
Poly-3 test	P=0.105	P=0.239	P=0.491	P=0.121
Lung: Alveolar/bronchiolar Adenoma				
Overall rate	4/50 (8%)	3/50 (6%)	2/49 (4%)	1/50 (2%)
Adjusted rate	9.7%	7.2%	5.0%	2.4%
Terminal rate	4/31 (13%)	3/33 (9%)	1/28 (4%)	1/33 (3%)
First incidence (days)	730 (T)	730 (T)	283	730 (T)
Poly-3 test	P=0.112N	P=0.496N	P=0.350N	P=0.173N
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rate	4/50 (8%)	4/50 (8%)	2/49 (4%)	2/50 (4%)
Adjusted rate	9.7%	9.6%	5.0%	4.8%
Terminal rate	4/31 (13%)	4/33 (12%)	1/28 (4%)	2/33 (6%)
First incidence (days)	730 (T)	730 (T)	283	730 (T)
Poly-3 test	P=0.208N	P=0.640N	P=0.350N	P=0.330N
Mammary Gland: Fibroadenoma				
Overall rate	17/50 (34%)	20/50 (40%)	12/50 (24%)	7/50 (14%)
Adjusted rate	40.8%	45.9%	29.8%	16.4%
Terminal rate	15/31 (48%)	15/33 (46%)	8/28 (29%)	4/33 (12%)
First incidence (days)	670	462	595	578
Poly-3 test	P=0.002N	P=0.395	P=0.207N	P=0.010N
Mammary Gland: Fibroadenoma or Carcinoma				
Overall rate	17/50 (34%)	21/50 (42%)	12/50 (24%)	7/50 (14%)
Adjusted rate	40.8%	48.2%	29.8%	16.4%
Terminal rate	15/31 (48%)	16/33 (49%)	8/28 (29%)	4/33 (12%)
First incidence (days)	670	462	595	578
Poly-3 test	P<0.001N	P=0.315	P=0.207N	P=0.010N
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	25/50 (50%)	22/50 (44%)	13/50 (26%)	15/50 (30%)
Adjusted rate	56.5%	50.8%	31.4%	35.0%
Terminal rate	19/31 (61%)	16/33 (49%)	7/28 (25%)	10/33 (30%)
First incidence (days)	330	548	595	588
Poly-3 test	P=0.013N	P=0.371N	P=0.014N	P=0.032N

TABLE B2
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Gavage Study of β-Myrcene

	Vehicle Control	0.25 g/kg	0.5 g/kg	1 g/kg
Skin (Subcutaneous Tissue): Lipoma				
Overall rate	0/50 (0%)	1/50 (2%)	3/50 (6%)	0/50 (0%)
Adjusted rate	0.0%	2.4%	7.6%	0.0%
Terminal rate	0/31 (0%)	1/33 (3%)	3/28 (11%)	0/33 (0%)
First incidence (days)	—	730 (T)	730 (T)	—
Poly-3 test	P=0.627N	P=0.502	P=0.110	—[f]
Skin (Subcutaneous Tissue): Fibroma or Fibrosarcoma				
Overall rate	3/50 (6%)	0/50 (0%)	1/50 (2%)	0/50 (0%)
Adjusted rate	7.2%	0.0%	2.5%	0.0%
Terminal rate	1/31 (3%)	0/33 (0%)	1/28 (4%)	0/33 (0%)
First incidence (days)	656	—	730 (T)	—
Poly-3 test	P=0.085N	P=0.118N	P=0.325N	P=0.117N
Thyroid Gland (C-Cell): Adenoma				
Overall rate	5/50 (10%)	13/49 (27%)	4/49 (8%)	4/50 (8%)
Adjusted rate	12.0%	30.9%	10.1%	9.6%
Terminal rate	3/31 (10%)	9/33 (27%)	2/28 (7%)	4/33 (12%)
First incidence (days)	661	651	673	730 (T)
Poly-3 test	P=0.133N	P=0.030	P=0.534N	P=0.496N
Uterus: Stromal Polyp				
Overall rate	8/50 (16%)	3/50 (6%)	13/50 (26%)	6/50 (12%)
Adjusted rate	18.6%	7.2%	30.9%	16.7%
Terminal rate	5/31 (16%)	3/33 (9%)	7/28 (25%)	6/33 (18%)
First incidence (days)	330	730 (T)	366	730 (T)
Poly-3 test	P=0.512	P=0.107N	P=0.141	P=0.406N
Uterus: Stromal Polyp or Stromal Sarcoma				
Overall rate	8/50 (16%)	4/50 (8%)	13/50 (26%)	7/50 (14%)
Adjusted rate	18.6%	9.4%	30.9%	14.3%
Terminal rate	5/31 (16%)	3/33 (9%)	7/28 (25%)	7/33 (21%)
First incidence (days)	330	408	366	730 (T)
Poly-3 test	P=0.421	P=0.181N	P=0.141	P=0.522N
All Organs: Mononuclear Cell Leukemia				
Overall rate	5/50 (10%)	8/50 (16%)	4/50 (8%)	11/50 (22%)
Adjusted rate	11.9%	18.9%	9.7%	25.1%
Terminal rate	2/31 (7%)	6/33 (18%)	0/28 (0%)	6/33 (18%)
First incidence (days)	619	581	371	225
Poly-3 test	P=0.090	P=0.274	P=0.517N	P=0.095
All Organs: Benign Neoplasms				
Overall rate	40/50 (80%)	39/50 (78%)	35/50 (70%)	27/50 (54%)
Adjusted rate	87.8%	87.0%	78.0%	61.9%
Terminal rate	29/31 (94%)	30/33 (91%)	21/28 (75%)	20/33 (61%)
First incidence (days)	330	462	283	578
Poly-3 test	P<0.001N	P=0.587N	P=0.150N	P=0.003N
All Organs: Malignant Neoplasms				
Overall rate	11/50 (22%)	14/50 (28%)	10/50 (20%)	17/50 (34%)
Adjusted rate	24.4%	32.0%	24.0%	37.9%
Terminal rate	4/31 (13%)	9/33 (27%)	4/28 (14%)	9/33 (27%)
First incidence (days)	291	408	371	225
Poly-3 test	P=0.126	P=0.289	P=0.581N	P=0.123

TABLE B2
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Gavage Study of β-Myrcene

	Vehicle Control	0.25 g/kg	0.5 g/kg	1 g/kg
All Organs: Benign or Malignant Neoplasms				
Overall rate	45/50 (90%)	44/50 (88%)	39/50 (78%)	37/50 (74%)
Adjusted rate	91.7%	95.1%	84.2%	80.1%
Terminal rate	29/31 (94%)	32/33 (97%)	22/28 (79%)	24/33 (73%)
First incidence (days)	291	408	283	225
Poly-3 test	P=0.018N	P=0.397	P=0.192N	P=0.082N

(T) Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for clitoral gland, kidney, lung, pituitary gland, and thyroid gland; for other tissues, denominator is number of animals necropsied.
[b] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the vehicle control incidence is the P value associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for the differential mortality in animals that do not reach terminal sacrifice. A negative trend or a lower incidence in a dose group is indicated by **N**.
[e] Not applicable; no neoplasms in animal group
[f] Value of statistic cannot be computed.

TABLE B3
Historical Incidence of Renal Tubule Adenoma in Control Female F344/N Rats

Study	Incidence in Controls
Historical Incidence: Corn Oil Gavage Studies	
β-Myrcene (March, 2002)	0/50
Isoeugenol (April, 2002)	0/50
Pulegone (April, 2003)	0/50
Total	0/150
Overall Historical Incidence: All Routes	
Total (%)	1/1,340 (0.1%)
Mean ± standard deviation	0.1% ± 0.4%
Range	0%-2%

[a] Data as of November 17, 2008

Table B4
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study of β-Myrcene[a]

	Vehicle Control	0.25 g/kg	0.5 g/kg	1 g/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Accidental deaths		2		1
Moribund	11	9	12	7
Natural deaths	8	6	10	9
Survivors				
Terminal sacrifice	31	33	28	33
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(49)	(50)
Perforation		1 (2%)		
Muscularis, inflammation, chronic				1 (2%)
Intestine large, colon	(50)	(50)	(50)	(50)
Parasite metazoan		1 (2%)	1 (2%)	
Ulcer				1 (2%)
Intestine large, rectum	(50)	(50)	(50)	(50)
Parasite metazoan	1 (2%)	5 (10%)	5 (10%)	2 (4%)
Ulcer	1 (2%)			
Intestine small, duodenum	(50)	(50)	(49)	(50)
Intestine small, ileum	(50)	(50)	(49)	(50)
Epithelium, hyperplasia, focal				1 (2%)
Liver	(50)	(50)	(49)	(50)
Angiectasis	1 (2%)			1 (2%)
Basophilic focus	44 (88%)	43 (86%)	42 (86%)	31 (62%)
Clear cell focus	4 (8%)	6 (12%)	6 (12%)	8 (16%)
Eosinophilic focus	6 (12%)	10 (20%)	15 (31%)	24 (48%)
Fatty change	3 (6%)	3 (6%)	4 (8%)	1 (2%)
Fibrosis			2 (4%)	
Hemorrhage		1 (2%)		
Hepatodiaphragmatic nodule	6 (12%)	5 (10%)	6 (12%)	8 (16%)
Inflammation, chronic	41 (82%)	41 (82%)	41 (84%)	33 (66%)
Mineralization			1 (2%)	
Mixed cell focus	6 (12%)	5 (10%)	3 (6%)	6 (12%)
Necrosis	2 (4%)	1 (2%)	1 (2%)	1 (2%)
Regeneration	1 (2%)			
Bile duct, cyst				1 (2%)
Bile duct, hyperplasia	8 (16%)	11 (22%)	12 (24%)	11 (22%)
Centrilobular, degeneration	1 (2%)		2 (4%)	1 (2%)
Centrilobular, necrosis			2 (4%)	
Sinusoid, congestion	1 (2%)			
Sinusoid, infiltration cellular, histiocyte	1 (2%)			
Mesentery	(7)	(13)	(7)	(9)
Fat, necrosis	6 (86%)	13 (100%)	7 (100%)	9 (100%)
Oral mucosa	(1)	(0)	(0)	(0)
Pancreas	(50)	(50)	(49)	(50)
Acinus, atrophy	9 (18%)	9 (18%)	10 (20%)	3 (6%)
Acinus, hyperplasia	1 (2%)		1 (2%)	
Duct, cyst	3 (6%)	2 (4%)	3 (6%)	4 (8%)
Salivary glands	(50)	(49)	(49)	(50)
Hyperplasia				1 (2%)

[a] Number of animals examined microscopically at the site and the number of animals with lesion

Table B4
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study of β-Myrcene

	Vehicle Control	0.25 g/kg	0.5 g/kg	1 g/kg
Alimentary System (continued)				
Stomach, forestomach	(50)	(50)	(49)	(50)
Inflammation, chronic active	1 (2%)	1 (2%)	2 (4%)	3 (6%)
Ulcer			1 (2%)	1 (2%)
Stomach, glandular	(50)	(50)	(49)	(50)
Mineralization	1 (2%)	4 (8%)		1 (2%)
Epithelium, ectasia	38 (76%)	39 (78%)	36 (73%)	38 (76%)
Epithelium, hyperplasia				1 (2%)
Tongue	(1)	(0)	(1)	(0)
Cardiovascular System				
Heart	(50)	(50)	(49)	(50)
Cardiomyopathy	43 (86%)	31 (62%)	30 (61%)	34 (68%)
Fibrosis		1 (2%)		
Atrium, thrombosis			1 (2%)	
Myocardium, inflammation, chronic		1 (2%)		
Valve, inflammation, chronic		1 (2%)		
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Hyperplasia	10 (20%)	10 (20%)	12 (24%)	8 (16%)
Necrosis			1 (2%)	
Vacuolization cytoplasmic	17 (34%)	14 (28%)	14 (28%)	16 (32%)
Adrenal medulla	(50)	(50)	(50)	(50)
Hyperplasia	3 (6%)	1 (2%)	4 (8%)	1 (2%)
Islets, pancreatic	(50)	(50)	(49)	(50)
Hyperplasia		2 (4%)	4 (8%)	
Parathyroid gland	(47)	(48)	(47)	(45)
Cyst				1 (2%)
Pituitary gland	(50)	(50)	(50)	(50)
Angiectasis	17 (34%)	24 (48%)	16 (32%)	14 (28%)
Cyst	14 (28%)	22 (44%)	16 (32%)	9 (18%)
Fibrosis	1 (2%)	2 (4%)		
Pars distalis, hyperplasia	14 (28%)	13 (26%)	15 (30%)	10 (20%)
Pars intermedia, hyperplasia	1 (2%)			
Thyroid gland	(50)	(49)	(49)	(50)
Cyst			1 (2%)	
C-cell, hyperplasia	19 (38%)	20 (41%)	22 (45%)	17 (34%)
Follicle, hyperplasia	1 (2%)	1 (2%)	4 (8%)	4 (8%)
General Body System				
None				
Genital System				
Clitoral gland	(50)	(50)	(50)	(50)
Cyst	3 (6%)	1 (2%)	1 (2%)	
Hyperplasia	8 (16%)	8 (16%)	13 (26%)	4 (8%)
Inflammation, chronic	22 (44%)	11 (22%)	26 (52%)	14 (28%)
Ovary	(50)	(50)	(50)	(50)
Atrophy		1 (2%)		
Cyst	3 (6%)	7 (14%)	6 (12%)	4 (8%)

Table B4
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study of β-Myrcene

	Vehicle Control	0.25 g/kg	0.5 g/kg	1 g/kg
Genital System (continued)				
Uterus	(50)	(50)	(50)	(50)
Angiectasis				1 (2%)
Cyst		2 (4%)	2 (4%)	
Dilatation	2 (4%)	1 (2%)	2 (4%)	1 (2%)
Inflammation, suppurative		1 (2%)		1 (2%)
Inflammation, chronic	1 (2%)			1 (2%)
Necrosis			1 (2%)	
Endometrium, hyperplasia, cystic	4 (8%)	7 (14%)	7 (14%)	13 (26%)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Hyperplasia	3 (6%)	1 (2%)	2 (4%)	1 (2%)
Lymph node	(5)	(3)	(2)	(6)
Deep cervical, hyperplasia, lymphoid	1 (20%)	1 (33%)		
Deep cervical, infiltration cellular, histiocyte				1 (17%)
Deep cervical, pigmentation				1 (17%)
Mediastinal, ectasia		1 (33%)		
Mediastinal, hyperplasia, lymphoid	1 (20%)	2 (67%)		1 (17%)
Lymph node, mesenteric	(50)	(50)	(50)	(50)
Infiltration cellular, histiocyte				1 (2%)
Pigmentation, hemosiderin				1 (2%)
Spleen	(50)	(50)	(50)	(50)
Atrophy	6 (12%)	2 (4%)	3 (6%)	2 (4%)
Fibrosis			1 (2%)	
Hematopoietic cell proliferation	7 (14%)	12 (24%)	13 (26%)	9 (18%)
Infarct			1 (2%)	
Thymus	(49)	(48)	(49)	(50)
Atrophy	46 (94%)	45 (94%)	47 (96%)	44 (88%)
Cyst			1 (2%)	
Infiltration cellular, lymphocyte	1 (2%)	1 (2%)		
Inflammation, chronic				1 (2%)
Epithelial cell, hyperplasia	1 (2%)		1 (2%)	
Integumentary System				
Mammary gland	(50)	(50)	(50)	(50)
Cyst	2 (4%)	1 (2%)	2 (4%)	2 (4%)
Skin	(50)	(50)	(50)	(50)
Cyst epithelial inclusion		1 (2%)		
Musculoskeletal System				
Skeletal muscle	(0)	(0)	(1)	(0)
Inflammation, suppurative			1 (100%)	
Nervous System				
Brain	(50)	(50)	(50)	(50)
Hydrocephalus	3 (6%)	4 (8%)	6 (12%)	2 (4%)
Hypothalamus, compression	4 (8%)	4 (8%)	8 (16%)	5 (10%)
Meninges, inflammation, chronic			1 (2%)	2 (4%)
Spinal cord	(1)	(0)	(0)	(0)

Table B4
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study of β-Myrcene

	Vehicle Control	0.25 g/kg	0.5 g/kg	1 g/kg
Respiratory System				
Lung	(50)	(50)	(49)	(50)
Congestion	1 (2%)			
Edema		1 (2%)		
Inflammation	34 (68%)	43 (86%)	40 (82%)	32 (64%)
Metaplasia, osseous	1 (2%)			2 (4%)
Alveolar epithelium, hyperplasia	6 (12%)	3 (6%)	4 (8%)	7 (14%)
Nose	(50)	(50)	(50)	(50)
Erosion	1 (2%)			
Inflammation, chronic active	16 (32%)	10 (20%)	13 (26%)	10 (20%)
Olfactory epithelium, degeneration	45 (90%)	44 (88%)	45 (90%)	48 (96%)
Special Senses System				
Ear	(0)	(3)	(0)	(0)
Hyperplasia		1 (33%)		
Eye	(50)	(50)	(49)	(50)
Cataract	1 (2%)		1 (2%)	1 (2%)
Degeneration				1 (2%)
Optic nerve, atrophy	1 (2%)			1 (2%)
Retina, atrophy				1 (2%)
Sclera, metaplasia, osseous	1 (2%)			1 (2%)
Harderian gland	(50)	(50)	(49)	(50)
Atrophy	1 (2%)	1 (2%)	2 (4%)	
Hyperplasia	1 (2%)		1 (2%)	1 (2%)
Inflammation, chronic	7 (14%)	8 (16%)	7 (14%)	2 (4%)
Pigmentation, porphyrin	50 (100%)	48 (96%)	48 (98%)	45 (90%)
Zymbal's gland	(0)	(0)	(0)	(2)
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Inflammation, suppurative, focal		1 (2%)		1 (2%)
Mineralization	1 (2%)			
Necrosis	1 (2%)	1 (2%)	1 (2%)	
Nephropathy	26 (52%)	43 (86%)	41 (82%)	44 (88%)
Pigmentation	1 (2%)			
Papilla, inflammation, suppurative	1 (2%)			
Papilla, mineralization	5 (10%)	3 (6%)	1 (2%)	
Pelvis, inflammation, chronic	1 (2%)	3 (6%)		
Renal tubule, cyst	2 (4%)		1 (2%)	
Renal tubule, hyperplasia				1 (2%)
Renal tubule, nephrosis		2 (4%)	27 (54%)	45 (90%)
Transitional epithelium, hyperplasia	1 (2%)	12 (24%)	15 (30%)	19 (38%)
Urinary bladder	(50)	(50)	(50)	(50)
Inflammation	2 (4%)		1 (2%)	

APPENDIX C
SUMMARY OF LESIONS IN MALE MICE IN THE 2-YEAR GAVAGE STUDY OF β-MYRCENE

TABLE C1 Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Gavage Study of β-Myrcene C-2
TABLE C2 Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Gavage Study of β-Myrcene C-6
TABLE C3 Historical Incidence of Liver Neoplasms in Control Male B6C3F1 Mice C-9
TABLE C4 Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Gavage Study of β-Myrcene C-10

Table C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Gavage Study of β-Myrcene[a]

	Vehicle Control	0.25 g/kg	0.5 g/kg	1 g/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Accidental deaths				3
Moribund	7	6	8	12
Natural deaths	8	9	11	14
Survivors				
Died last week of study	1			
Terminal sacrifice	34	35	31	21
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(49)
Gallbladder	(49)	(49)	(49)	(49)
Squamous cell carcinoma, metastatic, stomach, forestomach				1 (2%)
Intestine large, cecum	(49)	(48)	(49)	(47)
Intestine small, duodenum	(49)	(47)	(47)	(48)
Adenoma		1 (2%)		
Intestine small, ileum	(49)	(47)	(47)	(47)
Carcinoma		1 (2%)		
Intestine small, jejunum	(49)	(47)	(49)	(45)
Liver	(50)	(50)	(50)	(50)
Hemangiosarcoma	5 (10%)	1 (2%)	1 (2%)	
Hemangiosarcoma, metastatic, spleen	1 (2%)			
Hemangiosarcoma, multiple		2 (4%)	1 (2%)	
Hepatoblastoma	4 (8%)	6 (12%)	11 (22%)	1 (2%)
Hepatocellular adenoma	11 (22%)	10 (20%)	13 (26%)	8 (16%)
Hepatocellular adenoma, multiple	15 (30%)	31 (62%)	30 (60%)	10 (20%)
Hepatocellular carcinoma	13 (26%)	16 (32%)	19 (38%)	9 (18%)
Hepatocellular carcinoma, multiple	1 (2%)	4 (8%)	9 (18%)	1 (2%)
Ito cell tumor malignant	2 (4%)			
Mesentery	(4)	(7)	(5)	(3)
Hepatoblastoma, metastatic, liver		1 (14%)		
Hepatocellular carcinoma, metastatic, liver			1 (20%)	
Squamous cell carcinoma, metastatic, stomach, forestomach				1 (33%)
Pancreas	(50)	(50)	(50)	(50)
Salivary glands	(50)	(50)	(50)	(50)
Stomach, forestomach	(50)	(50)	(49)	(50)
Squamous cell carcinoma			1 (2%)	1 (2%)
Squamous cell papilloma		2 (4%)	1 (2%)	
Stomach, glandular	(50)	(50)	(50)	(50)
Tongue	(1)	(0)	(0)	(0)
Squamous cell carcinoma	1 (100%)			
Tooth	(38)	(33)	(35)	(13)
Peridontal tissue, fibrosarcoma		1 (3%)		
Cardiovascular System				
Blood vessel	(0)	(2)	(1)	(2)
Heart	(50)	(50)	(50)	(50)
Alveolar/bronchiolar carcinoma, metastatic, lung				1 (2%)
Hepatocellular carcinoma, metastatic, liver			1 (2%)	

Table C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Gavage Study of β-Myrcene

	Vehicle Control	0.25 g/kg	0.5 g/kg	1 g/kg
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Squamous cell carcinoma, metastatic, stomach, forestomach				1 (2%)
Subcapsular, adenoma	2 (4%)	4 (8%)		
Adrenal medulla	(50)	(50)	(50)	(50)
Pheochromocytoma benign			1 (2%)	
Pheochromocytoma malignant		1 (2%)		
Islets, pancreatic	(50)	(50)	(50)	(50)
Adenoma		1 (2%)		
Pituitary gland	(49)	(49)	(50)	(49)
Pars intermedia, adenoma			1 (2%)	
Thyroid gland	(50)	(50)	(50)	(50)
Follicular cell, adenoma		1 (2%)	1 (2%)	
Follicular cell, carcinoma	1 (2%)		1 (2%)	
General Body System				
None				
Genital System				
Epididymis	(50)	(50)	(50)	(50)
Hepatoblastoma, metastatic, liver		1 (2%)		
Squamous cell carcinoma, metastatic, stomach, forestomach				1 (2%)
Preputial gland	(50)	(50)	(50)	(50)
Hemangiosarcoma	1 (2%)			
Prostate	(50)	(50)	(50)	(50)
Seminal vesicle	(50)	(50)	(50)	(50)
Hepatoblastoma, metastatic, liver		1 (2%)		
Squamous cell carcinoma, metastatic, stomach, forestomach				1 (2%)
Testes	(50)	(50)	(50)	(50)
Interstitial cell, adenoma		1 (2%)	1 (2%)	
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Hemangiosarcoma		1 (2%)		
Hemangiosarcoma, metastatic, spleen	1 (2%)			
Lymph node	(2)	(0)	(5)	(1)
Bronchial, alveolar/bronchiolar carcinoma, metastatic, lung	1 (50%)			
Bronchial, hepatocellular carcinoma, metastatic, liver			1 (20%)	
Bronchial, squamous cell carcinoma, metastatic, stomach, forestomach				1 (100%)
Mediastinal, squamous cell carcinoma, metastatic, stomach, forestomach				1 (100%)
Lymph node, mandibular	(49)	(50)	(48)	(49)
Melanoma malignant, metastatic, eye			1 (2%)	
Lymph node, mesenteric	(49)	(50)	(47)	(44)
Hepatoblastoma, metastatic, liver		1 (2%)		
Pheochromocytoma malignant, metastatic, adrenal medulla		1 (2%)		

Table C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Gavage Study of β-Myrcene

	Vehicle Control	0.25 g/kg	0.5 g/kg	1 g/kg
Hematopoietic System (continued)				
Spleen	(49)	(50)	(48)	(49)
Hemangiosarcoma	1 (2%)	2 (4%)	2 (4%)	
Squamous cell carcinoma, metastatic, stomach, forestomach				1 (2%)
Thymus	(47)	(47)	(48)	(47)
Alveolar/bronchiolar carcinoma, metastatic, lung				1 (2%)
Hepatoblastoma, metastatic, liver		1 (2%)		
Hepatocellular carcinoma, metastatic, liver			1 (2%)	
Integumentary System				
Skin	(50)	(50)	(50)	(50)
Hemangiosarcoma	1 (2%)			
Squamous cell carcinoma			1 (2%)	
Subcutaneous tissue, sarcoma	1 (2%)			
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Chondroma		1 (2%)		
Skeletal muscle	(0)	(2)	(1)	(1)
Nervous System				
Brain	(50)	(50)	(50)	(50)
Peripheral nerve	(0)	(0)	(1)	(0)
Spinal cord	(0)	(0)	(1)	(0)
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma	5 (10%)	9 (18%)	6 (12%)	13 (26%)
Alveolar/bronchiolar adenoma, multiple	3 (6%)	4 (8%)	3 (6%)	1 (2%)
Alveolar/bronchiolar carcinoma	5 (10%)	5 (10%)	2 (4%)	2 (4%)
Alveolar/bronchiolar carcinoma, metastatic, lung	1 (2%)	2 (4%)	1 (2%)	1 (2%)
Carcinoma, metastatic, harderian gland			1 (2%)	
Carcinoma, metastatic, thyroid gland	1 (2%)		1 (2%)	
Hepatoblastoma, metastatic, liver		1 (2%)		
Hepatocellular carcinoma, metastatic, liver	4 (8%)	8 (16%)	10 (20%)	5 (10%)
Melanoma malignant, metastatic, eye			1 (2%)	
Mediastinum, hemangiosarcoma			1 (2%)	
Nose	(50)	(50)	(50)	(50)
Carcinoma, metastatic, harderian gland			1 (2%)	
Special Senses System				
Eye	(50)	(50)	(50)	(50)
Melanoma malignant			1 (2%)	
Harderian gland	(50)	(50)	(50)	(50)
Adenoma	8 (16%)	8 (16%)	10 (20%)	6 (12%)
Adenoma, multiple	1 (2%)			
Carcinoma	1 (2%)	2 (4%)	2 (4%)	1 (2%)
Bilateral, adenoma		2 (4%)		

Table C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Gavage Study of β-Myrcene

	Vehicle Control	0.25 g/kg	0.5 g/kg	1 g/kg
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Hepatocellular carcinoma, metastatic, liver			2 (4%)	
Renal tubule, adenoma	1 (2%)	1 (2%)	1 (2%)	1 (2%)
Urinary bladder	(50)	(50)	(49)	(49)
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Histiocytic sarcoma		1 (2%)	3 (6%)	
Lymphoma malignant	1 (2%)	1 (2%)		
Neoplasm Summary				
Total animals with primary neoplasms[c]	43	49	50	29
Total primary neoplasms	84	120	123	54
Total animals with benign neoplasms	33	45	44	24
Total benign neoplasms	46	76	68	39
Total animals with malignant neoplasms	30	33	41	13
Total malignant neoplasms	38	44	55	15
Total animals with metastatic neoplasms	7	12	14	6
Total metastatic neoplasms	9	17	22	16

[a] Number of animals examined microscopically at the site and the number of animals with neoplasm
[b] Number of animals with any tissue examined microscopically
[c] Primary neoplasms: all neoplasms except metastatic neoplasms

TABLE C2
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Gavage Study of β-Myrcene[a]

	Vehicle Control	0.25 g/kg	0.5 g/kg
Adrenal Cortex: Adenoma			
Overall rate[b]	2/50 (4%)	4/50 (8%)	0/50 (0%)
Adjusted rate[c]	4.6%	9.1%	0.0%
Terminal rate[d]	2/34 (6%)	4/35 (11%)	0/31 (0%)
First incidence (days)	728 (T)	728 (T)	—[f]
Poly-3 test[e]	P=0.224N	P=0.337	P=0.239N
Harderian Gland: Adenoma			
Overall rate	9/50 (18%)	10/50 (20%)	10/50 (20%)
Adjusted rate	20.3%	22.0%	22.8%
Terminal rate	7/34 (21%)	7/35 (20%)	9/31 (29%)
First incidence (days)	666	503	587
Poly-3 test	P=0.441	P=0.525	P=0.492
Harderian Gland: Adenoma or Carcinoma			
Overall rate	9/50 (18%)	12/50 (24%)	12/50 (24%)
Adjusted rate	20.3%	26.4%	26.9%
Terminal rate	7/34 (21%)	9/35 (26%)	9/31 (29%)
First incidence (days)	666	503	586
Poly-3 test	P=0.276	P=0.332	P=0.315
Liver: Hemangiosarcoma			
Overall rate	5/50 (10%)	3/50 (6%)	2/50 (4%)
Adjusted rate	11.3%	6.8%	4.6%
Terminal rate	4/34 (12%)	2/35 (6%)	2/31 (7%)
First incidence (days)	644	706	728 (T)
Poly-3 test	P=0.163N	P=0.358N	P=0.222N
Liver: Hepatocellular Adenoma			
Overall rate	26/50 (52%)	41/50 (82%)	43/50 (86%)
Adjusted rate	57.8%	88.2%	89.3%
Terminal rate	23/34 (68%)	33/35 (94%)	29/31 (94%)
First incidence (days)	468	533	525
Poly-3 test	P<0.001	P<0.001	P<0.001
Liver: Hepatocellular Carcinoma			
Overall rate	14/50 (28%)	20/50 (40%)	28/50 (56%)
Adjusted rate	30.6%	42.8%	58.8%
Terminal rate	8/34 (24%)	13/35 (37%)	16/31 (52%)
First incidence (days)	611	533	450
Poly-3 test	P=0.003	P=0.158	P=0.004
Liver: Hepatocellular Adenoma or Carcinoma			
Overall rate	33/50 (66%)	44/50 (88%)	48/50 (96%)
Adjusted rate	71.0%	92.6%	96.6%
Terminal rate	25/34 (74%)	33/35 (94%)	30/31 (97%)
First incidence (days)	468	533	450
Poly-3 test	P<0.001	P=0.003	P<0.001
Liver: Hepatoblastoma			
Overall rate	4/50 (8%)	6/50 (12%)	11/50 (22%)
Adjusted rate	9.0%	13.3%	25.0%
Terminal rate	3/34 (9%)	3/35 (9%)	7/31 (23%)
First incidence (days)	599	582	660
Poly-3 test	P=0.027	P=0.382	P=0.041

Table C2
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Gavage Study of β-Myrcene

	Vehicle Control	0.25 g/kg	0.5 g/kg
Liver: Hepatocellular Carcinoma or Hepatoblastoma			
Overall rate	16/50 (32%)	22/50 (44%)	31/50 (62%)
Adjusted rate	34.7%	46.7%	65.1%
Terminal rate	9/34 (27%)	14/35 (40%)	19/31 (61%)
First incidence (days)	599	533	450
Poly-3 test	P=0.002	P=0.163	P=0.002
Liver: Hepatocellular Adenoma, Hepatocellular Carcinoma, or Hepatoblastoma			
Overall rate	34/50 (68%)	45/50 (90%)	48/50 (96%)
Adjusted rate	72.5%	94.0%	96.6%
Terminal rate	25/34 (74%)	33/35 (94%)	30/31 (97%)
First incidence (days)	468	533	450
Poly-3 test	P<0.001	P=0.003	P<0.001
Lung: Alveolar/bronchiolar Adenoma			
Overall rate	8/50 (16%)	13/50 (26%)	9/50 (18%)
Adjusted rate	17.9%	28.5%	19.8%
Terminal rate	7/34 (21%)	8/35 (23%)	5/31 (16%)
First incidence (days)	468	533	549
Poly-3 test	P=0.473	P=0.173	P=0.518
Lung: Alveolar/bronchiolar Carcinoma			
Overall rate	5/50 (10%)	5/50 (10%)	2/50 (4%)
Adjusted rate	11.2%	11.4%	4.5%
Terminal rate	3/34 (9%)	5/35 (14%)	1/31 (3%)
First incidence (days)	567	728 (T)	450
Poly-3 test	P=0.183N	P=0.619	P=0.220N
Lung: Alveolar/bronchiolar Adenoma or Carcinoma			
Overall rate	13/50 (26%)	17/50 (34%)	11/50 (22%)
Adjusted rate	28.6%	37.2%	23.8%
Terminal rate	10/34 (29%)	12/35 (34%)	6/31 (19%)
First incidence (days)	468	533	450
Poly-3 test	P=0.343N	P=0.254	P=0.388N
All Organs: Hemangiosarcoma			
Overall rate	7/50 (14%)	3/50 (6%)	4/50 (8%)
Adjusted rate	15.7%	6.8%	9.2%
Terminal rate	4/34 (12%)	2/35 (6%)	3/31 (10%)
First incidence (days)	621	706	673
Poly-3 test	P=0.201N	P=0.161N	P=0.271N
All Organs: Histiocytic Sarcoma			
Overall rate	0/50 (0%)	1/50 (2%)	3/50 (6%)
Adjusted rate	0.0%	2.3%	6.7%
Terminal rate	0/34 (0%)	1/35 (3%)	0/31 (0%)
First incidence (days)	—	728 (T)	531
Poly-3 test	P=0.062	P=0.500	P=0.123
All Organs: Benign Neoplasms			
Overall rate	33/50 (66%)	45/50 (90%)	44/50 (88%)
Adjusted rate	72.6%	93.3%	91.3%
Terminal rate	28/34 (82%)	34/35 (97%)	30/31 (97%)
First incidence (days)	468	503	525
Poly 3 test	P=0.003	P=0.003	P=0.009

TABLE C2
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Gavage Study of β-Myrcene

	Vehicle Control	0.25 g/kg	0.5 g/kg
All Organs: Malignant Neoplasms			
Overall rate	30/50 (60%)	33/50 (66%)	41/50 (82%)
Adjusted rate	62.0%	68.8%	82.4%
Terminal	18/34 (53%)	23/35 (66%)	23/31 (74%)
First incidence (days)	468	436	450
Poly-3 test	P=0.015	P=0.313	P=0.018
All Organs: Benign or Malignant Neoplasms			
Overall rate	43/50 (86%)	49/50 (98%)	50/50 (100%)
Adjusted rate	88.5%	98.0%	100.0%
Terminal	30/34 (88%)	34/35 (97%)	31/31 (100%)
First incidence (days)	468	436	450
Poly-3 test	P=0.003	P=0.057	P=0.013

(T) Terminal sacrifice
[a] The 1 g/kg group has been excluded from the statistical analyses due to early mortality.
[b] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal cortex, liver, and lung; for other tissues, denominator is number of animals necropsied.
[c] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[d] Observed incidence at terminal kill
[e] Beneath the vehicle control incidence is the P value associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for the differential mortality in animals that do not reach terminal sacrifice. A negative trend or a lower incidence in a dose group is indicated by **N**.
[f] Not applicable; no neoplasms in animal group

TABLE C3
Historical Incidence of Liver Neoplasms in Control Male B6C3F1 Mice

Study (Study Start)	Incidence in Controls		
	Hepatocellular Adenoma	Hepatocellular Carcinoma	Hepatocellular Adenoma or Carcinoma
Historical Incidence: Corn Oil Gavage Studies			
β-Myrcene (April, 2002)	26/50	14/50	33/50
Isoeugenol (May, 2002)	24/50	8/50	28/50
Pulegone (April, 2003)	22/50	13/50	29/50
3,3′,4,4′-Tetrachloroazobenzene (February, 2003)	22/50	17/50	34/50
Total (%)	94/200 (47.0%)	52/200 (26.0%)	124/200 (62.0%)
Mean ± standard deviation	47.0% ± 3.8%	26.0% ± 7.5%	62.0% ± 5.9%
Range	44%-52%	16%-34%	56%-68%
Overall Historical Incidence: All Routes			
Total (%)	733/1,447 (50.7%)	415/1,447 (28.7%)	961/1,447 (66.4%)
Mean ± standard deviation	50.7% ± 13.9%	28.7% ± 8.8%	66.4% ± 12.3%
Range	22%-72%	16%-52%	36%-84%
	Hepatoblastoma	Hepatocellular Carcinoma or Hepatoblastoma	Hepatocellular Adenoma, Hepatocellular Carcinoma, or Hepatoblastoma
Historical Incidence: Corn Oil Gavage Studies			
β-Myrcene	4/50	16/50	34/50
Isoeugenol	3/50	11/50	30/50
Pulegone (April, 2003)	1/50	13/50	29/50
3,3′,4,4′-Tetrachloroazobenzene (February, 2003)	2/50	19/50	34/50
Total (%)	10/200 (5.0%)	59/200 (29.5%)	127/200 (63.5%)
Mean ± standard deviation	5.0% ± 2.6%	29.5% ± 7.0%	63.5% ± 5.3%
Range	2%-8%	22%-38%	58%-68%
Overall Historical Incidence: All Routes			
Total (%)	48/1,447 (3.3%)	446/1,447 (30.8%)	972/1,447 (67.2%)
Mean ± standard deviation	3.3% ± 6.4%	30.8% ± 9.7%	67.2% ± 13.1%
Range	0%-34%	16%-54%	36%-92%

[a] Data as of November 19, 2008

Table C4
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Gavage Study of β-Myrcene[a]

	Vehicle Control	0.25 g/kg	0.5 g/kg	1 g/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Accidental deaths				3
Moribund	7	6	8	12
Natural deaths	8	9	11	14
Survivors				
Died last week of study	1			
Terminal sacrifice	34	35	31	21
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(49)
Inflammation	2 (4%)	6 (12%)	3 (6%)	8 (16%)
Necrosis				5 (10%)
Muscularis, degeneration		3 (6%)		
Gallbladder	(49)	(49)	(49)	(49)
Intestine large, cecum	(49)	(48)	(49)	(47)
Edema			1 (2%)	
Lymphoid tissue, hyperplasia		2 (4%)		
Intestine small, duodenum	(49)	(47)	(47)	(48)
Inflammation, chronic active				1 (2%)
Necrosis				2 (4%)
Intestine small, ileum	(49)	(47)	(47)	(47)
Intestine small, jejunum	(49)	(47)	(49)	(45)
Peyer's patch, hyperplasia, lymphoid			2 (4%)	
Liver	(50)	(50)	(50)	(50)
Abscess				1 (2%)
Amyloid deposition	1 (2%)		1 (2%)	
Angiectasis		1 (2%)	2 (4%)	
Basophilic focus	3 (6%)	7 (14%)	6 (12%)	2 (4%)
Clear cell focus	15 (30%)	21 (42%)	21 (42%)	2 (4%)
Degeneration, cystic			1 (2%)	
Eosinophilic focus	16 (32%)	23 (46%)	21 (42%)	18 (36%)
Fatty change	25 (50%)	18 (36%)	16 (32%)	29 (58%)
Hematopoietic cell proliferation	3 (6%)	2 (4%)	2 (4%)	1 (2%)
Infarct		2 (4%)		
Inflammation, chronic active	26 (52%)	24 (48%)	23 (46%)	17 (34%)
Mineralization			2 (4%)	7 (14%)
Mixed cell focus	13 (26%)	3 (6%)	6 (12%)	3 (6%)
Necrosis	7 (14%)	10 (20%)	13 (26%)	17 (34%)
Pigmentation, ceroid	1 (2%)			
Pigmentation, hemosiderin	2 (4%)	4 (8%)		2 (4%)
Tension lipidosis	3 (6%)	5 (10%)	4 (8%)	7 (14%)
Thrombosis	1 (2%)			
Vacuolization cytoplasmic	3 (6%)	3 (6%)	4 (8%)	23 (46%)
Hepatocyte, hypertrophy	1 (2%)	2 (4%)	16 (32%)	38 (76%)
Hepatocyte, pigmentation, hemosiderin			1 (2%)	
Oval cell, hyperplasia		3 (6%)	2 (4%)	
Mesentery	(4)	(7)	(5)	(3)
Necrosis		1 (14%)		
Fat, necrosis	4 (100%)	5 (71%)	4 (80%)	2 (67%)

[a] Number of animals examined microscopically at the site and the number of animals with lesion

Table C4
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Gavage Study of β-Myrcene

	Vehicle Control	0.25 g/kg	0.5 g/kg	1 g/kg
Alimentary System (continued)				
Pancreas	(50)	(50)	(50)	(50)
Atrophy	1 (2%)	1 (2%)		2 (4%)
Cytoplasmic alteration	1 (2%)		1 (2%)	2 (4%)
Inflammation				1 (2%)
Necrosis, focal				1 (2%)
Duct, cyst				1 (2%)
Salivary glands	(50)	(50)	(50)	(50)
Inflammation	1 (2%)		1 (2%)	1 (2%)
Stomach, forestomach	(50)	(50)	(49)	(50)
Inflammation	10 (20%)	9 (18%)	13 (27%)	23 (46%)
Mineralization	1 (2%)			
Necrosis	1 (2%)	1 (2%)	1 (2%)	6 (12%)
Ulcer	4 (8%)	4 (8%)	7 (14%)	9 (18%)
Epithelium, hyperplasia	12 (24%)	17 (34%)	16 (33%)	28 (56%)
Epithelium, metaplasia		1 (2%)		
Stomach, glandular	(50)	(50)	(50)	(50)
Dysplasia	1 (2%)			
Hyperplasia	2 (4%)	1 (2%)	1 (2%)	2 (4%)
Inflammation	1 (2%)		1 (2%)	2 (4%)
Metaplasia, squamous		1 (2%)	1 (2%)	1 (2%)
Necrosis			1 (2%)	2 (4%)
Muscularis, hypertrophy	1 (2%)			
Tongue	(1)	(0)	(0)	(0)
Tooth	(38)	(33)	(35)	(13)
Dysplasia	38 (100%)	31 (94%)	35 (100%)	13 (100%)
Gingiva, inflammation	1 (3%)			
Peridontal tissue, inflammation	1 (3%)	1 (3%)		
Pulp, inflammation	1 (3%)	3 (9%)	1 (3%)	
Cardiovascular System				
Blood vessel	(0)	(2)	(1)	(2)
Mineralization		1 (50%)		
Heart	(50)	(50)	(50)	(50)
Cardiomyopathy	15 (30%)	12 (24%)	13 (26%)	19 (38%)
Fibrosis		1 (2%)		
Inflammation	2 (4%)	1 (2%)		
Mineralization	2 (4%)	1 (2%)	4 (8%)	7 (14%)
Necrosis		1 (2%)		
Atrium, thrombosis	1 (2%)		2 (4%)	1 (2%)
Coronary artery, inflammation	3 (6%)			
Vein, venule, thrombosis		1 (2%)		
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Amyloid deposition	1 (2%)			
Hypertrophy	7 (14%)	4 (8%)	10 (20%)	
Necrosis				1 (2%)
Thrombosis			1 (2%)	
Subcapsular, hyperplasia	46 (92%)	47 (94%)	46 (92%)	43 (86%)
Zona fasciculata, atrophy		1 (2%)		
Zona fasciculata, hyperplasia		1 (2%)	3 (6%)	2 (4%)

Table C4
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Gavage Study of β-Myrcene

	Vehicle Control	0.25 g/kg	0.5 g/kg	1 g/kg
Endocrine System (continued)				
Adrenal medulla	(50)	(50)	(50)	(50)
Hyperplasia	1 (2%)	2 (4%)		
Islets, pancreatic	(50)	(50)	(50)	(50)
Hyperplasia	38 (76%)	36 (72%)	28 (56%)	18 (36%)
Infiltration cellular, mixed cell			1 (2%)	
Pituitary gland	(49)	(49)	(50)	(49)
Pars distalis, hyperplasia	1 (2%)	1 (2%)		
Thyroid gland	(50)	(50)	(50)	(50)
Inflammation				1 (2%)
Mineralization	1 (2%)			
C-cell, hyperplasia	1 (2%)		1 (2%)	1 (2%)
Follicle, cyst	1 (2%)	3 (6%)		1 (2%)
Follicle, degeneration	1 (2%)	1 (2%)		2 (4%)
Follicular cell, hyperplasia		4 (8%)		2 (4%)
Follicular cell, hypertrophy	1 (2%)	1 (2%)		
General Body System				
None				
Genital System				
Epididymis	(50)	(50)	(50)	(50)
Cyst	1 (2%)		1 (2%)	
Fibrosis	1 (2%)			
Granuloma sperm	1 (2%)			
Inflammation			2 (4%)	
Mineralization				1 (2%)
Preputial gland	(50)	(50)	(50)	(50)
Ectasia	4 (8%)	5 (10%)	4 (8%)	3 (6%)
Inflammation	5 (10%)	3 (6%)	2 (4%)	2 (4%)
Prostate	(50)	(50)	(50)	(50)
Atrophy			1 (2%)	
Hyperplasia		2 (4%)		
Inflammation	25 (50%)	15 (30%)	22 (44%)	12 (24%)
Seminal vesicle	(50)	(50)	(50)	(50)
Atrophy			1 (2%)	
Inflammation			1 (2%)	
Testes	(50)	(50)	(50)	(50)
Germinal epithelium, degeneration	5 (10%)	2 (4%)	5 (10%)	18 (36%)
Germinal epithelium, mineralization			2 (4%)	2 (4%)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Atrophy	1 (2%)		1 (2%)	18 (36%)
Myelofibrosis	1 (2%)		1 (2%)	1 (2%)
Necrosis, focal		1 (2%)		
Pigmentation		1 (2%)	1 (2%)	1 (2%)
Myeloid cell, hyperplasia	1 (2%)	3 (6%)	3 (6%)	1 (2%)
Lymph node	(2)	(0)	(5)	(1)
Inguinal, atrophy			1 (20%)	
Inguinal, infiltration cellular, histiocyte			1 (20%)	
Mediastinal, inflammation, granulomatous			1 (20%)	

Table C4
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Gavage Study of β-Myrcene

	Vehicle Control	0.25 g/kg	0.5 g/kg	1 g/kg
Hematopoietic System (continued)				
Lymph node, mandibular	(49)	(50)	(48)	(49)
Atrophy	3 (6%)	6 (12%)	9 (19%)	23 (47%)
Hyperplasia, lymphoid		3 (6%)	2 (4%)	
Infiltration cellular, plasma cell	1 (2%)	3 (6%)	1 (2%)	
Lymph node, mesenteric	(49)	(50)	(47)	(44)
Amyloid deposition	1 (2%)			
Atrophy	12 (24%)	12 (24%)	13 (28%)	28 (64%)
Hyperplasia, lymphoid		2 (4%)	1 (2%)	
Infiltration cellular, plasma cell		1 (2%)		
Inflammation	1 (2%)			
Inflammation, granulomatous			1 (2%)	
Pigmentation, hemosiderin	1 (2%)			
Spleen	(49)	(50)	(48)	(49)
Amyloid deposition	1 (2%)		1 (2%)	
Hematopoietic cell proliferation	33 (67%)	43 (86%)	41 (85%)	10 (20%)
Hyperplasia, lymphoid	1 (2%)		1 (2%)	2 (4%)
Infiltration cellular, histiocyte			1 (2%)	1 (2%)
Pigmentation, hemosiderin	1 (2%)	1 (2%)		2 (4%)
Lymphoid follicle, atrophy	7 (14%)	5 (10%)	10 (21%)	31 (63%)
Lymphoid follicle, hyperplasia			1 (2%)	
Thymus	(47)	(47)	(48)	(47)
Amyloid deposition	1 (2%)			
Atrophy	45 (96%)	40 (85%)	44 (92%)	41 (87%)
Hyperplasia, lymphoid				1 (2%)
Infiltration cellular, plasma cell				1 (2%)
Necrosis				1 (2%)
Integumentary System				
Skin	(50)	(50)	(50)	(50)
Infiltration cellular, mast cell, focal		1 (2%)		
Inflammation	1 (2%)		2 (4%)	
Ulcer	1 (2%)	2 (4%)	3 (6%)	
Hair follicle, inflammation	1 (2%)			
Sebaceous gland, hyperplasia				1 (2%)
Subcutaneous tissue, edema			1 (2%)	
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Fibrous osteodystrophy				1 (2%)
Osteoporosis		1 (2%)		
Joint, arthrosis	1 (2%)			
Skeletal muscle	(0)	(2)	(1)	(1)
Inflammation		1 (50%)		1 (100%)
Nervous System				
Brain	(50)	(50)	(50)	(50)
Vein, infiltration cellular, lymphocyte			1 (2%)	
Peripheral nerve	(0)	(0)	(1)	(0)
Infiltration cellular, lymphocyte			1 (100%)	
Spinal cord	(0)	(0)	(1)	(0)

Table C4
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Gavage Study of β-Myrcene

	Vehicle Control	0.25 g/kg	0.5 g/kg	1 g/kg
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Abscess				1 (2%)
Foreign body				1 (2%)
Hematopoietic cell proliferation			1 (2%)	
Inflammation, suppurative			1 (2%)	1 (2%)
Inflammation, chronic active	2 (4%)	1 (2%)	1 (2%)	3 (6%)
Thrombosis			1 (2%)	1 (2%)
Alveolar epithelium, hyperplasia	4 (8%)	8 (16%)	5 (10%)	5 (10%)
Alveolus, infiltration cellular, histiocyte	4 (8%)	9 (18%)	1 (2%)	3 (6%)
Arteriole, degeneration		1 (2%)		
Bronchiole, hyperplasia	1 (2%)	2 (4%)		1 (2%)
Glands, inflammation		2 (4%)	1 (2%)	
Nose	(50)	(50)	(50)	(50)
Inflammation	3 (6%)	2 (4%)	2 (4%)	3 (6%)
Polyp, inflammatory	6 (12%)			
Glands, dilatation		3 (6%)	1 (2%)	
Olfactory epithelium, degeneration	5 (10%)	5 (10%)	7 (14%)	6 (12%)
Olfactory epithelium, metaplasia, respiratory	7 (14%)	2 (4%)	1 (2%)	
Respiratory epithelium, hyperplasia	1 (2%)	1 (2%)		
Special Senses System				
Eye	(50)	(50)	(50)	(50)
Fibrosis			1 (2%)	
Thrombosis				1 (2%)
Cornea, hyperplasia, squamous		2 (4%)		
Cornea, inflammation		2 (4%)		1 (2%)
Lens, cataract		2 (4%)		
Optic nerve, fibrosis	1 (2%)	1 (2%)		
Retina, degeneration		2 (4%)		
Harderian gland	(50)	(50)	(50)	(50)
Fibrosis	1 (2%)	1 (2%)		
Hyperplasia	1 (2%)	3 (6%)	2 (4%)	2 (4%)
Inflammation				1 (2%)
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Accumulation, hyaline droplet			3 (6%)	
Infarct	6 (12%)	6 (12%)	7 (14%)	1 (2%)
Mineralization	31 (62%)	31 (62%)	29 (58%)	12 (24%)
Nephropathy	44 (88%)	44 (88%)	43 (86%)	27 (54%)
Cortex, cyst	6 (12%)	6 (12%)	4 (8%)	3 (6%)
Cortex, inflammation	1 (2%)	2 (4%)		
Cortex, metaplasia, osseous	1 (2%)			
Papilla, inflammation	1 (2%)	2 (4%)		1 (2%)
Papilla, necrosis	1 (2%)	1 (2%)	1 (2%)	
Pelvis, dilatation			1 (2%)	
Pelvis, inflammation	1 (2%)		2 (4%)	
Renal tubule, hyperplasia	14 (28%)	16 (32%)	14 (28%)	6 (12%)
Renal tubule, necrosis			3 (6%)	18 (36%)
Renal tubule, pigmentation, hemosiderin	1 (2%)	2 (4%)	1 (2%)	
Renal tubule, vacuolization cytoplasmic	39 (78%)	41 (82%)	20 (40%)	
Urinary bladder	(50)	(50)	(49)	(49)
Inflammation			1 (2%)	

APPENDIX D
SUMMARY OF LESIONS IN FEMALE MICE IN THE 2-YEAR GAVAGE STUDY OF β-MYRCENE

TABLE D1 Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Gavage Study of β-Myrcene D-2
TABLE D2 Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Gavage Study of β-Myrcene D-6
TABLE D3 Historical Incidence of Hepatocellular Neoplasms in Control Female B6C3F1 Mice D-8
TABLE D4 Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Gavage Study of β-Myrcene D-9

Table D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Gavage Study of β-Myrcene[a]

	Vehicle Control	0.25 g/kg	0.5 g/kg	1 g/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Accidental deaths		1	1	
Moribund	4	5	6	5
Natural deaths	7	10	8	28
Survivors				
Terminal sacrifice	39	34	35	17
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Carcinoma, metastatic, uterus			1 (2%)	
Nerve, schwannoma malignant, metastatic, uncertain primary site		1 (2%)		
Gallbladder	(47)	(50)	(50)	(49)
Sarcoma, metastatic, skin		1 (2%)		
Intestine large, cecum	(49)	(50)	(50)	(47)
Intestine large, colon	(50)	(50)	(50)	(47)
Intestine large, rectum	(50)	(49)	(50)	(49)
Intestine small, jejunum	(50)	(48)	(49)	(40)
Carcinoma	1 (2%)			
Sarcoma, metastatic, skin			1 (2%)	
Liver	(50)	(50)	(50)	(50)
Carcinoma, metastatic, uterus			1 (2%)	
Hemangiosarcoma			1 (2%)	
Hemangiosarcoma, metastatic, skin	1 (2%)			
Hepatocellular adenoma	6 (12%)	11 (22%)	6 (12%)	7 (14%)
Hepatocellular adenoma, multiple		2 (4%)		
Hepatocellular carcinoma	1 (2%)	7 (14%)	2 (4%)	1 (2%)
Sarcoma, metastatic, skin		1 (2%)		
Mesentery	(15)	(18)	(3)	(0)
Hemangioma	1 (7%)			
Hepatocellular carcinoma, metastatic, liver		1 (6%)		
Sarcoma, metastatic, skin		1 (6%)		
Schwannoma malignant, metastatic, uncertain primary site		1 (6%)		
Pancreas	(50)	(49)	(50)	(48)
Sarcoma, metastatic, skin			1 (2%)	
Schwannoma malignant, metastatic, uncertain primary site		1 (2%)		
Salivary glands	(50)	(50)	(50)	(50)
Stomach, forestomach	(50)	(49)	(50)	(47)
Carcinoma, metastatic, uterus			1 (2%)	
Squamous cell papilloma	1 (2%)	1 (2%)	1 (2%)	1 (2%)
Serosa, schwannoma malignant, metastatic, uncertain primary site		1 (2%)		
Stomach, glandular	(50)	(50)	(50)	(49)
Tooth	(1)	(3)	(4)	(0)

Table D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Gavage Study of β-Myrcene

	Vehicle Control	0.25 g/kg	0.5 g/kg	1 g/kg
Cardiovascular System				
Blood vessel	(2)	(2)	(2)	(1)
Heart	(50)	(50)	(50)	(50)
Carcinoma, metastatic, uterus			1 (2%)	
Hemangioma			1 (2%)	
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Sarcoma, metastatic, skin		1 (2%)		
Subcapsular, adenoma	1 (2%)			
Adrenal medulla	(50)	(50)	(50)	(50)
Hepatocellular carcinoma, metastatic, liver		1 (2%)		
Parathyroid gland	(47)	(34)	(43)	(44)
Pituitary gland	(50)	(50)	(50)	(50)
Pars distalis, adenoma	1 (2%)	1 (2%)	1 (2%)	
Thyroid gland	(50)	(50)	(50)	(50)
Follicular cell, adenoma	1 (2%)		1 (2%)	
General Body System				
None				
Genital System				
Clitoral gland	(49)	(48)	(50)	(49)
Hemangiosarcoma		1 (2%)		
Ovary	(50)	(50)	(49)	(49)
Carcinoma, metastatic, uterus			1 (2%)	
Cystadenoma	2 (4%)	1 (2%)		
Granulosa-theca tumor benign				1 (2%)
Luteoma	1 (2%)		1 (2%)	
Oviduct	(0)	(0)	(1)	(0)
Uterus	(50)	(50)	(50)	(50)
Hemangiosarcoma		1 (2%)		
Polyp stromal		1 (2%)	1 (2%)	1 (2%)
Endometrium, carcinoma			1 (2%)	
Hematopoietic System				
Bone marrow	(50)	(49)	(49)	(50)
Hemangiosarcoma		1 (2%)		
Hemangiosarcoma, metastatic, skin	1 (2%)			
Sarcoma, metastatic, skin		1 (2%)		
Lymph node	(7)	(8)	(7)	(0)
Bronchial, alveolar/bronchiolar carcinoma, metastatic, lung		1 (13%)		
Bronchial, sarcoma, metastatic, skin		1 (13%)		
Mediastinal, alveolar/bronchiolar carcinoma, metastatic, lung		1 (13%)		
Mediastinal, carcinoma, metastatic, uterus			1 (14%)	
Mediastinal, fibrosarcoma, metastatic, skin			1 (14%)	

Table D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Gavage Study of β-Myrcene

	Vehicle Control	0.25 g/kg	0.5 g/kg	1 g/kg
Hematopoietic System (continued)				
Lymph node (continued)	(7)	(8)	(7)	(0)
Mediastinal, hepatocellular carcinoma, metastatic, liver		1 (13%)		
Mediastinal, sarcoma, metastatic, skin		1 (13%)		
Pancreatic, carcinoma, metastatic, uterus			1 (14%)	
Lymph node, mandibular	(50)	(50)	(49)	(49)
Lymph node, mesenteric	(47)	(48)	(44)	(39)
Sarcoma, metastatic, skin		1 (2%)		
Spleen	(49)	(50)	(50)	(50)
Hemangiosarcoma, metastatic, skin	1 (2%)			
Thymus	(49)	(48)	(47)	(48)
Alveolar/bronchiolar carcinoma, metastatic, lung		1 (2%)		
Hemangioma			1 (2%)	
Hepatocellular carcinoma, metastatic, liver		1 (2%)		
Sarcoma, metastatic, skin		2 (4%)		
Integumentary System				
Mammary gland	(50)	(50)	(50)	(50)
Skin	(50)	(50)	(50)	(50)
Basosquamous tumor benign	1 (2%)			
Subcutaneous tissue, fibroma	1 (2%)			
Subcutaneous tissue, fibrosarcoma	1 (2%)		1 (2%)	1 (2%)
Subcutaneous tissue, hemangioma	1 (2%)			
Subcutaneous tissue, hemangiosarcoma	1 (2%)			
Subcutaneous tissue, sarcoma	1 (2%)	4 (8%)	1 (2%)	
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Skeletal muscle	(0)	(3)	(0)	(0)
Sarcoma, metastatic, skin		1 (33%)		
Nervous System				
Brain	(50)	(50)	(50)	(50)
Peripheral nerve	(0)	(1)	(0)	(0)
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma	4 (8%)	1 (2%)	5 (10%)	3 (6%)
Alveolar/bronchiolar adenoma, multiple				2 (4%)
Alveolar/bronchiolar carcinoma	2 (4%)	1 (2%)	1 (2%)	
Carcinoma, metastatic, uterus			1 (2%)	
Fibrosarcoma, metastatic, skin			1 (2%)	
Hepatocellular carcinoma, metastatic, liver		2 (4%)	1 (2%)	
Sarcoma, metastatic, skin		2 (4%)		
Sarcoma, metastatic, uncertain primary site		1 (2%)		
Mediastinum, fibrosarcoma, metastatic, skin			1 (2%)	
Nose	(50)	(50)	(50)	(50)
Trachea	(50)	(50)	(50)	(50)

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Gavage Study of β-Myrcene

	Vehicle Control	0.25 g/kg	0.5 g/kg	1 g/kg
Special Senses System				
Ear	(0)	(0)	(1)	(0)
Eye	(50)	(50)	(50)	(49)
Harderian gland	(50)	(50)	(50)	(49)
Adenoma	4 (8%)	6 (12%)	5 (10%)	5 (10%)
Carcinoma			1 (2%)	
Bilateral, adenoma		1 (2%)		
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Carcinoma, metastatic, uterus			1 (2%)	
Ureter	(0)	(0)	(1)	(0)
Urinary bladder	(50)	(50)	(50)	(49)
Carcinoma, metastatic, uterus			1 (2%)	
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Histiocytic sarcoma			2 (4%)	1 (2%)
Lymphoma malignant	7 (14%)	6 (12%)	3 (6%)	1 (2%)
Neoplasm Summary				
Total animals with primary neoplasms[c]	30	28	27	21
Total primary neoplasms	39	46	36	24
Total animals with benign neoplasms	21	19	20	18
Total benign neoplasms	25	25	23	20
Total animals with malignant neoplasms	14	18	12	4
Total malignant neoplasms	14	21	13	4
Total animals with metastatic neoplasms	1	7	4	
Total metastatic neoplasms	3	27	16	
Total animals with malignant neoplasms of uncertain primary site		2		

[a] Number of animals examined microscopically at the site and the number of animals with neoplasm
[b] Number of animals with any tissue examined microscopically
[c] Primary neoplasms: all neoplasms except metastatic neoplasms

TABLE D2
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Gavage Study of β-Myrcene[a]

	Vehicle Control	0.25 g/kg	0.5 g/kg
Harderian Gland: Adenoma			
Overall rate[b]	4/50 (8%)	7/50 (14%)	5/50 (10%)
Adjusted rate[c]	8.6%	15.9%	12.2%
Terminal rate[d]	3/39 (8%)	6/34 (18%)	5/35 (14%)
First incidence (days)	706	594	727 (T)
Poly-3 test[e]	P=0.346	P=0.230	P=0.424
Harderian Gland: Adenoma or Carcinoma			
Overall rate	4/50 (8%)	7/50 (14%)	6/50 (12%)
Adjusted rate	8.6%	15.9%	14.6%
Terminal rate	3/39 (8%)	6/34 (18%)	6/35 (17%)
First incidence (days)	706	594	727 (T)
Poly-3 test	P=0.239	P=0.230	P=0.295
Liver: Hepatocellular Adenoma			
Overall rate	6/50 (12%)	13/50 (26%)	6/50 (12%)
Adjusted rate	13.0%	29.8%	14.6%
Terminal rate	6/39 (15%)	12/34 (35%)	6/35 (17%)
First incidence (days)	727 (T)	709	727 (T)
Poly-3 test	P=0.418	P=0.042	P=0.534
Liver: Hepatocellular Carcinoma			
Overall rate	1/50 (2%)	7/50 (14%)	2/50 (4%)
Adjusted rate	2.2%	15.9%	4.8%
Terminal rate	1/39 (3%)	5/34 (15%)	1/35 (3%)
First incidence (days)	727 (T)	608	640
Poly-3 test	P=0.334	P=0.025	P=0.461
Liver: Hepatocellular Adenoma or Carcinoma			
Overall rate	7/50 (14%)	18/50 (36%)	8/50 (16%)
Adjusted rate	15.1%	40.9%	19.3%
Terminal rate	7/39 (18%)	15/34 (44%)	7/35 (20%)
First incidence (days)	727 (T)	608	640
Poly-3 test	P=0.297	P=0.005	P=0.406
Lung: Alveolar/bronchiolar Adenoma			
Overall rate	4/50 (8%)	1/50 (2%)	5/50 (10%)
Adjusted rate	8.5%	2.3%	12.0%
Terminal rate	3/39 (8%)	0/34 (0%)	4/35 (11%)
First incidence (days)	540	594	549
Poly-3 test	P=0.384	P=0.199N	P=0.426
Lung: Alveolar/bronchiolar Adenoma or Carcinoma			
Overall rate	6/50 (12%)	2/50 (4%)	6/50 (12%)
Adjusted rate	12.8%	4.5%	14.4%
Terminal rate	5/39 (13%)	0/34 (0%)	5/35 (14%)
First incidence (days)	540	594	549
Poly-3 test	P=0.518	P=0.155N	P=0.535
Skin (Subcutaneous Tissue): Sarcoma			
Overall rate	1/50 (2%)	4/50 (8%)	1/50 (2%)
Adjusted rate	2.2%	9.0%	2.4%
Terminal rate	0/39 (0%)	0/34 (0%)	0/35 (0%)
First incidence (days)	693	594	566
Poly-3 test	P=0.540	P=0.165	P=0.735

TABLE D2
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Gavage Study of β-Myrcene

	Vehicle Control	0.25 g/kg	0.5 g/kg
Skin (Subcutaneous Tissue): Fibrosarcoma or Sarcoma			
Overall rate	2/50 (4%)[f]	4/50 (8%)	2/50 (4%)
Adjusted rate	4.3%	9.0%	4.8%
Terminal rate	0/39 (0%)	0/34 (0%)	0/35 (0%)
First incidence (days)	622	594	566
Poly-3 test	P=0.529	P=0.313	P=0.654
All Organs: Hemangioma or Hemangiosarcoma			
Overall rate	3/50 (6%)	2/50 (4%)	3/50 (6%)
Adjusted rate	6.5%	4.6%	7.2%
Terminal rate	3/39 (8%)	2/34 (6%)	1/35 (3%)
First incidence (days)	727 (T)	727 (T)	619
Poly-3 test	P=0.549	P=0.529N	P=0.612
All Organs: Malignant Lymphoma			
Overall rate	7/50 (14%)	6/50 (12%)	3/50 (6%)
Adjusted rate	14.9%	13.8%	7.1%
Terminal rate	6/39 (15%)	5/34 (15%)	0/35 (0%)
First incidence (days)	497	706	394
Poly-3 test	P=0.173N	P=0.558N	P=0.201N
All Organs: Benign Neoplasms			
Overall rate	21/50 (42%)	19/50 (38%)	20/50 (40%)
Adjusted rate	44.0%	43.2%	47.3%
Terminal rate	17/39 (44%)	17/34 (50%)	17/35 (49%)
First incidence (days)	540	594	549
Poly-3 test	P=0.424	P=0.553N	P=0.457
All Organs: Malignant Neoplasms			
Overall rate	14/50 (28%)	20/50 (40%)	12/50 (24%)
Adjusted rate	29.4%	43.8%	26.8%
Terminal rate	10/39 (26%)	11/34 (32%)	4/35 (11%)
First incidence (days)	497	503	394
Poly-3 test	P=0.465N	P=0.109	P=0.482N
All Organs: Benign or Malignant Neoplasms			
Overall rate	30/50 (60%)	30/50 (60%)	27/50 (54%)
Adjusted rate	61.7%	65.7%	59.6%
Terminal rate	23/39 (59%)	21/34 (62%)	18/35 (51%)
First incidence (days)	497	503	394
Poly-3 test	P=0.469N	P=0.425	P=0.501N

(T) Terminal sacrifice
[a] The 1 g/kg group has been excluded from the statistical analyses due to early mortality.
[b] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for liver and lung; for other tissues, denominator is number of animals necropsied.
[c] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[d] Observed incidence at terminal kill
[e] Beneath the vehicle control incidence is the P value associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for the differential mortality in animals that do not reach terminal sacrifice. A negative trend or a lower incidence in a dose group is indicated by **N**.
[f] One animal that had a fibrosarcoma also had a fibroma.

TABLE D3
Historical Incidence of Hepatocellular Neoplasms in Control Female B6C3F1 Mice

Study (Study Start)	Incidence in Controls		
	Adenoma	Carcinoma	Adenoma or Carcinoma
Historical Incidence: Corn Oil Gavage Studies			
β-Myrcene (April, 2002)	6/50	1/50	7/50
Isoeugenol (May, 2002	11/49	3/49	13/49
Pulegone (April, 2003)	13/49	5/49	17/49
3,3′,4,4′-Tetrachloroazobenzene (February, 2003)	3/49	2/49	4/49
Total (%)	33/197 (16.8%)	11/197 (5.6%)	41/197 (20.8%)
Mean ± standard deviation	16.8% ± 9.4%	5.6% ± 3.5%	20.9% ± 12.0%
Range	6%-27%	2%-10%	8%-35%
Overall Historical Incidence: All Routes			
Total (%)	396/1,494 (26.5%)	137/1,494 (9.2%)	481/1,494 (32.2%)
Mean ± standard deviation	26.5% ± 15.2%	9.2% ± 6.7%	32.2% ± 17.3%
Range	2%-54%	0%-28%	6%-64%

[a] Data as of November 19, 2008

Table D4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Gavage Study of β-Myrcene[a]

	Vehicle Control	0.25 g/kg	0.5 g/kg	1 g/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Accidental deaths		1	1	
Moribund	4	5	6	5
Natural deaths	7	10	8	28
Survivors				
Terminal sacrifice	39	34	35	17
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Inflammation	2 (4%)	1 (2%)	4 (8%)	
Necrosis			2 (4%)	
Muscularis, degeneration	1 (2%)		1 (2%)	
Gallbladder	(47)	(50)	(50)	(49)
Intestine large, cecum	(49)	(50)	(50)	(47)
Edema			1 (2%)	
Inflammation			1 (2%)	
Epithelium, metaplasia			1 (2%)	
Intestine large, colon	(50)	(50)	(50)	(47)
Parasite metazoan				1 (2%)
Epithelium, inflammation			1 (2%)	
Intestine large, rectum	(50)	(49)	(50)	(49)
Inflammation			1 (2%)	
Necrosis			1 (2%)	
Intestine small, jejunum	(50)	(48)	(49)	(40)
Peyer's patch, hyperplasia, lymphoid			3 (6%)	
Serosa, fibrosis		1 (2%)		
Liver	(50)	(50)	(50)	(50)
Angiectasis	1 (2%)	1 (2%)	2 (4%)	
Basophilic focus	2 (4%)	1 (2%)	4 (8%)	1 (2%)
Clear cell focus		1 (2%)	1 (2%)	
Cytoplasmic alteration				1 (2%)
Eosinophilic focus	4 (8%)	5 (10%)	6 (12%)	9 (18%)
Fatty change	29 (58%)	35 (70%)	16 (32%)	15 (30%)
Hematopoietic cell proliferation	6 (12%)	1 (2%)	1 (2%)	1 (2%)
Infiltration cellular, lymphocyte		4 (8%)	1 (2%)	
Inflammation, suppurative				1 (2%)
Inflammation, chronic active	43 (86%)	35 (70%)	34 (68%)	14 (28%)
Mineralization				1 (2%)
Mixed cell focus	1 (2%)	4 (8%)	6 (12%)	1 (2%)
Necrosis	3 (6%)	2 (4%)	3 (6%)	7 (14%)
Pigmentation, hemosiderin	2 (4%)		2 (4%)	
Regeneration		1 (2%)		
Tension lipidosis	12 (24%)	3 (6%)	10 (20%)	4 (8%)
Vacuolization cytoplasmic	6 (12%)	11 (22%)	8 (16%)	30 (60%)
Bile duct, crystals			1 (2%)	
Bile duct, hyperplasia		1 (2%)	1 (2%)	
Hepatocyte, hypertrophy			6 (12%)	40 (80%)
Oval cell, hyperplasia	1 (2%)		1 (2%)	
Mesentery	(15)	(18)	(3)	(0)
Fat, necrosis	14 (93%)	15 (83%)	1 (33%)	

[a] Number of animals examined microscopically at the site and the number of animals with lesion

Table D4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Gavage Study of β-Myrcene

	Vehicle Control	0.25 g/kg	0.5 g/kg	1 g/kg
Alimentary System (continued)				
Pancreas	(50)	(49)	(50)	(48)
Atrophy	2 (4%)	1 (2%)	3 (6%)	1 (2%)
Basophilic focus			1 (2%)	
Inflammation, granulomatous			1 (2%)	
Mineralization				1 (2%)
Necrosis				1 (2%)
Acinus, cytoplasmic alteration, focal		1 (2%)		
Duct, cytoplasmic alteration			1 (2%)	
Salivary glands	(50)	(50)	(50)	(50)
Infiltration cellular, lymphocyte		1 (2%)		
Stomach, forestomach	(50)	(49)	(50)	(47)
Inflammation	2 (4%)	5 (10%)	8 (16%)	19 (40%)
Mineralization				1 (2%)
Necrosis			4 (8%)	6 (13%)
Ulcer	3 (6%)	2 (4%)	3 (6%)	8 (17%)
Epithelium, hyperplasia	7 (14%)	10 (20%)	17 (34%)	24 (51%)
Stomach, glandular	(50)	(50)	(50)	(49)
Cytoplasmic alteration, focal	1 (2%)			
Inflammation	1 (2%)			2 (4%)
Mineralization				1 (2%)
Necrosis				2 (4%)
Tooth	(1)	(3)	(4)	(0)
Dysplasia	1 (100%)	3 (100%)	4 (100%)	
Cardiovascular System				
Blood vessel	(2)	(2)	(2)	(1)
Inflammation, chronic active	1 (50%)			
Mineralization	1 (50%)	2 (100%)		
Aorta, inflammation				1 (100%)
Carotid artery, intima, hyperplasia			1 (50%)	
Media, degeneration	1 (50%)			
Heart	(50)	(50)	(50)	(50)
Cardiomyopathy	7 (14%)	1 (2%)	4 (8%)	4 (8%)
Degeneration		1 (2%)		
Fibrosis			1 (2%)	1 (2%)
Inflammation	1 (2%)	2 (4%)		
Mineralization	1 (2%)	2 (4%)	2 (4%)	5 (10%)
Pigmentation, hemosiderin		1 (2%)		
Atrium, thrombosis			1 (2%)	
Coronary artery, inflammation	1 (2%)		1 (2%)	
Epicardium, inflammation		1 (2%)		
Valve, thrombosis	2 (4%)	2 (4%)		1 (2%)
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Hypertrophy			3 (6%)	
Capsule, fibrosis		1 (2%)		
Capsule, infiltration cellular, lymphocyte		1 (2%)		
Subcapsular, hyperplasia	50 (100%)	50 (100%)	49 (98%)	50 (100%)
Zona fasciculata, hyperplasia	1 (2%)	2 (4%)		
Adrenal medulla	(50)	(50)	(50)	(50)
Hyperplasia	1 (2%)	2 (4%)		

Table D4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Gavage Study of β-Myrcene

	Vehicle Control	0.25 g/kg	0.5 g/kg	1 g/kg
Endocrine System (continued)				
Parathyroid gland	(47)	(34)	(43)	(44)
Cyst		1 (3%)		
Hyperplasia	1 (2%)			
Infiltration cellular, lymphocyte	1 (2%)			
Pituitary gland	(50)	(50)	(50)	(50)
Pars distalis, hyperplasia	1 (2%)	2 (4%)	5 (10%)	2 (4%)
Pars intermedia, hyperplasia			1 (2%)	
Pars nervosa, inflammation		1 (2%)		
Thyroid gland	(50)	(50)	(50)	(50)
Inflammation	2 (4%)	2 (4%)		1 (2%)
C-cell, hyperplasia			1 (2%)	
Follicle, cyst	1 (2%)	1 (2%)	3 (6%)	3 (6%)
Follicle, degeneration	1 (2%)			
Follicular cell, hyperplasia	2 (4%)	1 (2%)	3 (6%)	
General Body System				
None				
Genital System				
Clitoral gland	(49)	(48)	(50)	(49)
Ovary	(50)	(50)	(49)	(49)
Amyloid deposition			1 (2%)	
Angiectasis	1 (2%)	2 (4%)	1 (2%)	2 (4%)
Atrophy	43 (86%)	44 (88%)	45 (92%)	43 (88%)
Hyperplasia, tubular			1 (2%)	2 (4%)
Inflammation			1 (2%)	
Inflammation, granulomatous		1 (2%)		
Thrombosis	1 (2%)			1 (2%)
Corpus luteum, cyst		2 (4%)	1 (2%)	
Follicle, cyst	5 (10%)	4 (8%)	4 (8%)	
Follicle, cyst, multiple			1 (2%)	
Germinal epithelium, cyst	1 (2%)	2 (4%)	1 (2%)	1 (2%)
Germinal epithelium, cyst, multiple			1 (2%)	
Periovarian tissue, cyst		1 (2%)	1 (2%)	
Rete ovarii, cyst		1 (2%)		2 (4%)
Oviduct	(0)	(0)	(1)	(0)
Uterus	(50)	(50)	(50)	(50)
Angiectasis		1 (2%)	1 (2%)	2 (4%)
Thrombosis			1 (2%)	
Endometrium, hyperplasia	43 (86%)	33 (66%)	28 (56%)	23 (46%)
Hematopoietic System				
Bone marrow	(50)	(49)	(49)	(50)
Atrophy	1 (2%)		7 (14%)	29 (58%)
Myelofibrosis	16 (32%)	9 (18%)	4 (8%)	3 (6%)
Necrosis		1 (2%)		
Myeloid cell, hyperplasia	2 (4%)	1 (2%)	1 (2%)	
Lymph node	(7)	(8)	(7)	(0)
Bronchial, hyperplasia, lymphoid	1 (14%)		1 (14%)	
Inguinal, infiltration cellular, plasma cell	1 (14%)			
Renal, amyloid deposition	1 (14%)			
Renal, ectasia	1 (14%)			
Renal, infiltration cellular, plasma cell	1 (14%)			

Table D4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Gavage Study of β-Myrcene

	Vehicle Control	0.25 g/kg	0.5 g/kg	1 g/kg
Hematopoietic System (continued)				
Lymph node, mandibular	(50)	(50)	(49)	(49)
Amyloid deposition	1 (2%)			
Atrophy	4 (8%)	8 (16%)	11 (22%)	22 (45%)
Hyperplasia, lymphoid	3 (6%)	2 (4%)	1 (2%)	1 (2%)
Infiltration cellular, plasma cell	1 (2%)			
Lymph node, mesenteric	(47)	(48)	(44)	(39)
Atrophy	9 (19%)	7 (15%)	5 (11%)	25 (64%)
Hyperplasia, lymphoid	1 (2%)			
Spleen	(49)	(50)	(50)	(50)
Amyloid deposition	1 (2%)			
Hematopoietic cell proliferation	39 (80%)	39 (78%)	16 (32%)	11 (22%)
Hyperplasia, lymphoid	6 (12%)	4 (8%)	4 (8%)	1 (2%)
Infiltration cellular, plasma cell	1 (2%)	1 (2%)		
Pigmentation, hemosiderin	9 (18%)	16 (32%)	11 (22%)	14 (28%)
Lymphoid follicle, atrophy	4 (8%)	11 (22%)	11 (22%)	32 (64%)
Lymphoid follicle, hyperplasia			1 (2%)	
Thymus	(49)	(48)	(47)	(48)
Amyloid deposition	1 (2%)			
Atrophy	22 (45%)	14 (29%)	19 (40%)	38 (79%)
Hyperplasia, atypical			1 (2%)	
Infiltration cellular, plasma cell	1 (2%)	1 (2%)		
Inflammation		1 (2%)		
Integumentary System				
Mammary gland	(50)	(50)	(50)	(50)
Hyperplasia			1 (2%)	
Duct, dilatation			1 (2%)	
Skin	(50)	(50)	(50)	(50)
Ulcer			1 (2%)	
Hair follicle, inflammation, diffuse		1 (2%)		
Sebaceous gland, hyperplasia, focal			1 (2%)	
Subcutaneous tissue, fibrosis	1 (2%)	1 (2%)		
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Joint, degeneration	1 (2%)			
Tibia, osteosclerosis	1 (2%)			
Skeletal muscle	(0)	(3)	(0)	(0)
Inflammation		1 (33%)		
Nervous System				
Brain	(50)	(50)	(50)	(50)
Cerebellum, necrosis			1 (2%)	
Cerebrum, necrosis			1 (2%)	
Hippocampus, necrosis			1 (2%)	
Medulla, demyelination, focal		1 (2%)		
Vein, infiltration cellular, lymphocyte	1 (2%)		1 (2%)	
Peripheral nerve	(0)	(1)	(0)	(0)

Table D4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Gavage Study of β-Myrcene

	Vehicle Control	0.25 g/kg	0.5 g/kg	1 g/kg
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Hyperplasia, lymphoid				1 (2%)
Inflammation, suppurative		1 (2%)		
Inflammation, chronic active	4 (8%)	1 (2%)	1 (2%)	3 (6%)
Pigmentation, hemosiderin			1 (2%)	
Alveolar epithelium, hyperplasia	2 (4%)	3 (6%)	2 (4%)	5 (10%)
Alveolus, infiltration cellular, histiocyte	5 (10%)		3 (6%)	1 (2%)
Bronchiole, hyperplasia	1 (2%)	1 (2%)		1 (2%)
Serosa, fibrosis, focal	1 (2%)			
Serosa, inflammation, suppurative				1 (2%)
Nose	(50)	(50)	(50)	(50)
Olfactory epithelium, degeneration	2 (4%)	5 (10%)	5 (10%)	12 (24%)
Olfactory epithelium, metaplasia, respiratory		1 (2%)		
Respiratory epithelium, hyperplasia	1 (2%)			
Trachea	(50)	(50)	(50)	(50)
Epithelium, glands, cytoplasmic alteration			1 (2%)	
Special Senses System				
Ear	(0)	(0)	(1)	(0)
Necrosis			1 (100%)	
Eye	(50)	(50)	(50)	(49)
Atrophy			1 (2%)	
Synechia	1 (2%)			
Cornea, inflammation	1 (2%)	1 (2%)	1 (2%)	
Lens, cataract			2 (4%)	
Retina, retinal detachment			1 (2%)	
Harderian gland	(50)	(50)	(50)	(49)
Fibrosis	1 (2%)			
Hyperplasia	1 (2%)	2 (4%)	1 (2%)	
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Accumulation, hyaline droplet				1 (2%)
Amyloid deposition		1 (2%)	1 (2%)	
Infarct	4 (8%)	4 (8%)	3 (6%)	1 (2%)
Infiltration cellular, lymphocyte	1 (2%)	3 (6%)	4 (8%)	
Mineralization	10 (20%)	13 (26%)	8 (16%)	8 (16%)
Nephropathy	20 (40%)	21 (42%)	16 (32%)	16 (32%)
Cortex, cyst	1 (2%)			
Cortex, metaplasia, osseous		2 (4%)		
Glomerulus, amyloid deposition	1 (2%)			
Papilla, inflammation	1 (2%)	2 (4%)		2 (4%)
Papilla, necrosis	2 (4%)		1 (2%)	
Pelvis, dilatation			1 (2%)	
Renal tubule, hyperplasia		1 (2%)	1 (2%)	
Renal tubule, necrosis	2 (4%)	2 (4%)	2 (4%)	17 (34%)
Ureter	(0)	(0)	(1)	(0)
Urinary bladder	(50)	(50)	(50)	(49)
Infiltration cellular, lymphocyte	1 (2%)	2 (4%)		1 (2%)
Arteriole, inflammation		1 (2%)		

APPENDIX E
GENETIC TOXICOLOGY

Salmonella typhimurium Mutagenicity Test Protocol E-2
Mouse Peripheral Blood Micronucleus Test Protocol E-2
Evaluation Protocol E-3
Results E-3
Table E1 Mutagenicity of β-Myrcene in *Salmonella typhimurium* E-4
Table E2 Frequency of Micronuclei in Peripheral Blood Erythrocytes of Mice Following Administration of β-Myrcene by Gavage for 3 Months E-6

GENETIC TOXICOLOGY

SALMONELLA TYPHIMURIUM MUTAGENICITY TEST PROTOCOL

Tests performed at SRI International followed protocols reported by Zeiger *et al.* (1992); in the tests conducted at SITEK Research Laboratories, a modified protocol was used. β-Myrcene was sent to both laboratories as a coded aliquot. In the tests conducted at SRI International, β-myrcene was incubated with the *Salmonella typhimurium* tester strains TA97, TA98, TA100, and TA1535 either in buffer or S9 mix (metabolic activation enzymes and cofactors from Aroclor 1254-induced male Sprague-Dawley rat or Syrian hamster liver) for 20 minutes at 37° C. Top agar supplemented with L-histidine and d-biotin was added, and the contents of the tubes were mixed and poured onto the surfaces of minimal glucose agar plates. Histidine-independent mutant colonies arising on these plates were counted following incubation for 2 days at 37° C.

The test conducted by SITEK Research Laboratories used only 10% rat liver S9 for exogenous metabolic activation and employed *Escherichia coli* strain WP2 *uvrA* pKM101 as a bacterial tester strain in addition to *S. typhimurium* strains TA98 and TA100. The same lot of β-myrcene that was used in the 2-year bioassay was tested for mutagenicity testing under this protocol. Incubation of bacterial strains with β-myrcene and subsequent plating were carried out as reported by Zeiger *et al.* (1992).

Each trial consisted of triplicate plates of concurrent positive and negative controls and at least five doses of β-myrcene. The high dose was limited by toxicity in a majority of trials and by the limit dose of 10,000 µg/plate in those trials where only slight toxicity was observed. All trials were repeated, and those that were conducted with S9 activation enzymes were repeated using the same or higher concentrations of S9.

In this assay, a positive response is defined as a reproducible, dose-related increase in histidine-independent (revertant) colonies in any one strain/activation combination. An equivocal response is defined as an increase in revertants that is not dose related, is not reproducible, or is not of sufficient magnitude to support a determination of mutagenicity. A negative response is obtained when no increase in revertant colonies is observed following chemical treatment. There is no minimum percentage or fold increase required for a chemical to be judged positive or weakly positive.

MOUSE PERIPHERAL BLOOD MICRONUCLEUS TEST PROTOCOL

A detailed discussion of this assay is presented by MacGregor *et al.* (1990). At the end of the 3-month toxicity study, peripheral blood samples were obtained from male and female mice. Smears were immediately prepared and fixed in absolute methanol. The methanol-fixed slides were stained with acridine orange and coded. Slides were scanned to determine the frequency of micronuclei in 2,000 normochromatic erythrocytes (NCEs) per animal. In addition, the percentage of polychromatic erythrocytes (PCEs) in a population of 1,000 erythrocytes was determined as a measure of bone marrow toxicity.

The results were tabulated as the mean of the pooled results from all animals within a treatment group plus or minus the standard error of the mean. The frequency of micronucleated cells among NCEs was analyzed by a statistical software package that tested for increasing trend over dose groups with a one-tailed Cochran-Armitage trend test, followed by pairwise comparisons between each dosed group and the control group (ILS, 1990). In the presence of excess binomial variation, as detected by a binomial dispersion test, the binomial variance of the Cochran-Armitage test was adjusted upward in proportion to the excess variation. In the micronucleus test, an individual trial is considered positive if the trend test P value is less than or equal to 0.025 or if the P value for any single dosed group is less than or equal to 0.025 divided by the number of dosed groups. A final call of positive for micronucleus induction is preferably based on reproducibly positive trials (as noted above). Results of the 3-month studies were accepted without repeat tests, because additional test data could not be obtained.

Ultimately, the final call is determined by the scientific staff after considering the results of statistical analyses, the reproducibility of any effects observed, and the magnitudes of those effects.

Evaluation Protocol

These are the basic guidelines for arriving at an overall assay result for assays performed by the National Toxicology Program. Statistical as well as biological factors are considered. For an individual assay, the statistical procedures for data analysis have been described in the preceding protocols. There have been instances, however, in which multiple aliquots of a chemical were tested in the same assay, and different results were obtained among aliquots and/or among laboratories. Results from more than one aliquot or from more than one laboratory are not simply combined into an overall result. Rather, all the data are critically evaluated, particularly with regard to pertinent protocol variations, in determining the weight of evidence for an overall conclusion of chemical activity in an assay. In addition to multiple aliquots, the *in vitro* assays have another variable that must be considered in arriving at an overall test result. *In vitro* assays are conducted with and without exogenous metabolic activation. Results obtained in the absence of activation are not combined with results obtained in the presence of activation; each testing condition is evaluated separately. The summary table in the Abstract of this Technical Report presents a result that represents a scientific judgement of the overall evidence for activity of the chemical in an assay.

Results

β-Myrcene did not show evidence of genotoxicity in assays conducted by the NTP. No mutagenic activity was observed in any of several strains of *S. typhimurium* (TA97, TA98, TA100, and TA1535) or *E. coli* strain WP2 *uvrA* pKM101 exposed to β-myrcene concentrations ranging up to 10,000 µg/plate in two independent Ames assays conducted with and without exogenous metabolic activation provided by Aroclor 1254-induced rat or hamster liver enzymes (Table E1). In addition, no significant increases in the frequencies of micronucleated NCEs, biomarkers of chromosomal damage, were observed in male or female B6C3F1 mice administered β-myrcene (0.25 to 2 g/kg) for 3 months by gavage (Table E2). The percentage of reticulocytes among total erythrocytes (% PCEs) increased slightly with dose, but remained within the normal range, suggesting an absence of β-myrcene-induced bone marrow toxicity over this dose range (Table E2).

Table E1
Mutagenicity of β-Myrcene in *Salmonella typhimurium*[a]

Strain	Dose (μg/plate)	Revertants/Plate[b] –S9 Trial 1	–S9 Trial 2	+hamster S9 10%	+hamster S9 30%	+rat S9 10%	+rat S9 30%
Study performed at SRI International							
TA100	0	103 ± 2.0	114 ± 6.0	104 ± 8.0	108 ± 6.0	107 ± 8.0	110 ± 5.0
	33	113 ± 6.0	96 ± 11.0	98 ± 6.0		126 ± 22.0	
	100	100 ± 6.0	116 ± 0.0	103 ± 7.0	94 ± 6.0	116 ± 10.0	119 ± 4.0
	333	96 ± 8.0	107 ± 10.0	101 ± 10.0	101 ± 4.0	115 ± 21.0	113 ± 4.0
	1,000	100 ± 6.0	117 ± 3.0	81 ± 4.0	111 ± 4.0	90 ± 14.0	111 ± 10.0
	3,333	66 ± 6.0[c]	70 ± 9.0[c]	59 ± 4.0[c]	107 ± 10.0	55 ± 8.0[c]	103 ± 4.0
	10,000				75 ± 6.0[c]		77 ± 2.0[c]
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control[d]		781 ± 17.0	765 ± 39.0	597 ± 8.0	663 ± 31.0	488 ± 23.0	553 ± 52.0
TA1535	0	9 ± 2.0	13 ± 2.0	10 ± 2.0	9 ± 0.0	9 ± 1.0	12 ± 1.0
	33	9 ± 2.0	8 ± 2.0	12 ± 3.0		10 ± 1.0	
	100	6 ± 1.0	10 ± 2.0	9 ± 1.0	11 ± 1.0	12 ± 1.0	11 ± 1.0
	333	10 ± 1.0	9 ± 1.0	8 ± 3.0	9 ± 1.0	11 ± 1.0	10 ± 1.0
	1,000	9 ± 1.0	9 ± 1.0	9 ± 2.0	10 ± 2.0	10 ± 1.0	9 ± 0.0
	3,333	6 ± 0.0[c]	7 ± 1.0[c]	4 ± 1.0[c]	5 ± 1.0	5 ± 0.0[c]	10 ± 1.0
	10,000				5 ± 2.0[c]		8 ± 0.0[c]
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		700 ± 13.0	819 ± 48.0	92 ± 11.0	363 ± 5.0	89 ± 12.0	196 ± 8.0
TA97	0	106 ± 0.0	126 ± 16.0	162 ± 9.0	144 ± 4.0	172 ± 7.0	145 ± 7.0
	33	104 ± 7.0	111 ± 9.0	167 ± 5.0		157 ± 6.0	
	100	100 ± 8.0	127 ± 10.0	159 ± 3.0	149 ± 4.0	154 ± 10.0	128 ± 11.0
	333	96 ± 4.0	142 ± 13.0	151 ± 16.0	112 ± 2.0	161 ± 7.0	106 ± 5.0
	1,000	106 ± 6.0	144 ± 14.0	142 ± 18.0	117 ± 11.0	153 ± 8.0	121 ± 10.0
	3,333	63 ± 29.0[c]	77 ± 6.0[c]	106 ± 3.0[c]	125 ± 7.0	89 ± 21.0[c]	132 ± 2.0
	10,000				117 ± 9.0[c]		124 ± 2.0[c]
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		293 ± 22.0	575 ± 53.0	689 ± 1.0	609 ± 28.0	648 ± 15.0	431 ± 35.0
TA98	0	22 ± 4.0	16 ± 2.0	19 ± 4.0	16 ± 1.0	19 ± 3.0	17 ± 2.0
	33	12 ± 2.0	9 ± 1.0	23 ± 2.0		26 ± 1.0	
	100	16 ± 2.0	11 ± 1.0	19 ± 1.0	18 ± 2.0	20 ± 3.0	13 ± 2.0
	333	19 ± 5.0	11 ± 2.0	22 ± 4.0	18 ± 4.0	19 ± 1.0	16 ± 1.0
	1,000	24 ± 5.0	11 ± 1.0	20 ± 1.0	23 ± 4.0	18 ± 0.0	13 ± 2.0
	3,333	8 ± 2.0[c]	7 ± 1.0[c]	8 ± 1.0[c]	22 ± 2.0	12 ± 3.0[c]	18 ± 3.0
	10,000				9 ± 1.0[c]		11 ± 3.0[c]
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		294 ± 8.0	269 ± 5.0	278 ± 21.0	237 ± 16.0	206 ± 26.0	251 ± 21.0

TABLE E1
Mutagenicity of β-Myrcene in *Salmonella typhimurium*

Strain	Dose (µg/plate)	Revertants/Plate –S9 Trial 1	–S9 Trial 2	–S9 Trial 3	+10% rat S9 Trial 1	+10% rat S9 Trial 2
Study performed at SITEK Research Laboratories						
TA100	0	57 ± 7.0	36 ± 3.0	45 ± 3.0	83 ± 12.0	56 ± 2.0
	10	66 ± 6.0	47 ± 5.0	54 ± 1.0	78 ± 11.0	
	25	35 ± 6.0	48 ± 5.0	28 ± 1.0		
	50	7 ± 1.0	42 ± 3.0	24 ± 2.0	65 ± 6.0	61 ± 3.0
	75	8 ± 1.0	42 ± 5.0	27 ± 2.0		
	100	Toxic	Toxic	28 ± 4.0	69 ± 8.0	56 ± 6.0
	250				62 ± 3.0	42 ± 4.0
	500				48 ± 4.0	20 ± 2.0
	750					12 ± 1.0
Trial summary		Negative	Negative	Negative	Negative	Negative
Positive control		387 ± 19.0	403 ± 12.0	653 ± 32.0	738 ± 31.0	665 ± 29.0
TA98	0	21 ± 2.0	35 ± 3.0		30 ± 4.0	22 ± 2.0
	10	16 ± 1.0	15 ± 4.0			27 ± 3.0
	25	6 ± 0.0				
	50	6 ± 0.0	14 ± 3.0		26 ± 2.0	27 ± 3.0
	75	4 ± 1.0				
	100	5 ± 1.0	10 ± 0.0		25 ± 2.0	28 ± 5.0
	250		7 ± 3.0		23 ± 1.0	25 ± 4.0
	500		5 ± 1.0		16 ± 1.0	23 ± 2.0
	750				11 ± 2.0	
Trial summary		Negative	Negative		Negative	Negative
Positive control		295 ± 14.0	417 ± 31.0		418 ± 22.0	372 ± 38.0
***Escherichia coli* WP2 *uvrA*/pKM101**						
	0	181 ± 46.0	144 ± 6.0	158 ± 4.0	168 ± 2.0	200 ± 7.0
	50		111 ± 5.0			
	100		86 ± 4.0			
	500	57 ± 6.0	84 ± 5.0	149 ± 4.0	174 ± 1.0	204 ± 6.0
	1,000	74 ± 6.0	74 ± 4.0	149 ± 2.0	156 ± 5.0	218 ± 15.0
	2,000	70 ± 5.0		147 ± 6.0	164 ± 4.0	190 ± 12.0
	5,000	99 ± 7.0	76 ± 1.0	134 ± 6.0	166 ± 11.0	190 ± 3.0
	10,000	82 ± 3.0	84 ± 5.0	149 ± 7.0	177 ± 14.0	186 ± 18.0
Trial summary		Negative	Negative	Negative	Negative	Negative
Positive control		1,183 ± 140.0	807 ± 71.0	806 ± 76.0	814 ± 45.0	823 ± 3.0

[a] The detailed protocol for the SRI International study is presented by Zeiger *et al.* (1992); the study performed at SITEK Research Laboratories used a modification of that protocol. 0 µg/plate was the solvent control.
[b] Revertants are presented as mean ± standard error from three plates.
[c] Slight toxicity
[d] The positive controls in the absence of metabolic activation were sodium azide (TA100 and TA1535), 9-aminoacridine (TA97), 4-nitro-*o*-phenylenediamine (TA98), and methyl methanesulfonate (pKM101). The positive control for metabolic activation with all strains was 2-aminoanthracene.

TABLE E2
Frequency of Micronuclei in Peripheral Blood Erythrocytes of Mice Following Administration of β-Myrcene by Gavage for 3 Months[a]

Compound	Exposure Concentration (g/kg)	Number of Mice with Erythrocytes Scored	Micronucleated NCEs/ 1,000 NCEs[b]	P Value[c]	PCEs[b] (%)
Male					
Corn oil[d]	0	5	1.00 ± 0.22		1.720 ± 0.27
β-myrcene	0.25	5	0.40 ± 0.19	0.9457	2.420 ± 0.35
	0.5	5	1.00 ± 0.27	0.5000	2.820 ± 0.38
	1	5	1.40 ± 0.24	0.2070	2.480 ± 0.21
	2[e]	1			
			P=0.069[f]		
Female					
Corn oil	0	5	1.30 ± 0.34		2.060 ± 0.27
β-myrcene	0.25	5	0.40 ± 0.19	0.9855	2.500 ± 0.23
	0.5	5	1.50 ± 0.35	0.3526	2.640 ± 0.37
	1	5	1.20 ± 0.34	0.5793	2.180 ± 0.27
	2[e]	2			
			P=0.299		

[a] Study was performed at SITEK Research Laboratories. The detailed protocol is presented by MacGregor *et al.* (1990).
[b] PCE=polychromatic erythrocyte; NCE=normochromatic erythrocyte.
[c] Mean ± standard error
[d] Pairwise comparison with the vehicle control group; significant at P≤0.008 (ILS, 1990)
[e] Vehicle control
[f] Insufficient number of animals available for statistical analysis
Significance of micronucleated NCEs/1,000 NCEs tested by the one-tailed trend test, significant at P≤0.025 (ILS, 1990)

APPENDIX F
CLINICAL PATHOLOGY RESULTS

TABLE F1 Hematology and Clinical Chemistry Data for Rats in the 3-Month Gavage Study of β-Myrcene F-2
TABLE F2 Hematology Data for Mice in the 3-Month Gavage Study of β-Myrcene F-6

TABLE F1
Hematology and Clinical Chemistry Data for Rats in the 3-Month Gavage Study of β-Myrcene[a]

	Vehicle Control	0.25 g/kg	0.5 g/kg	1 g/kg	2 g/kg
Male					
Hematology					
n					
Day 23	10	10	10	9	5
Week 14	9	10	9	9	8
Hematocrit (auto) (%)					
Day 23	41.7 ± 0.5	42.4 ± 0.5	41.6 ± 0.4	42.2 ± 0.6	43.2 ± 0.8
Week 14	42.1 ± 0.3	43.2 ± 0.3	42.4 ± 0.3	42.7 ± 0.4	43.0 ± 0.3
Hemoglobin (g/dL)					
Day 23	14.8 ± 0.2	14.9 ± 0.2	14.8 ± 0.1	14.9 ± 0.2	14.9 ± 0.2
Week 14	14.5 ± 0.1	15.0 ± 0.1*	14.7 ± 0.1	14.7 ± 0.1	14.9 ± 0.1
Erythrocytes (10^6/μL)					
Day 23	7.72 ± 0.10	7.80 ± 0.12	7.70 ± 0.07	7.83 ± 0.14	8.04 ± 0.16
Week 14	8.48 ± 0.07	8.70 ± 0.05	8.70 ± 0.07	8.73 ± 0.09	8.74 ± 0.06
Reticulocytes (10^6/μL)					
Day 23	0.17 ± 0.01	0.18 ± 0.02	0.19 ± 0.01	0.19 ± 0.01	0.21 ± 0.03
Week 14	0.06 ± 0.01	0.07 ± 0.01	0.08 ± 0.01	0.10 ± 0.01*	0.09 ± 0.01*
Nucleated erythrocytes (10^3/μL)					
Day 23	0.03 ± 0.02	0.02 ± 0.01	0.02 ± 0.01	0.03 ± 0.02	0.06 ± 0.03
Week 14	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Mean cell volume (fL)					
Day 23	54.0 ± 0.2	54.4 ± 0.3	54.0 ± 0.3	53.9 ± 0.2	53.7 ± 0.2
Week 14	49.6 ± 0.1	49.7 ± 0.2	48.7 ± 0.2**	48.9 ± 0.1**	49.2 ± 0.1*
Mean cell hemoglobin (pg)					
Day 23	19.2 ± 0.2	19.1 ± 0.1	19.2 ± 0.1	19.0 ± 0.1	18.6 ± 0.2
Week 14	17.1 ± 0.1	17.2 ± 0.1	16.9 ± 0.1	16.9 ± 0.0	17.0 ± 0.1
Mean cell hemoglobin concentration (g/dL)					
Day 23	35.5 ± 0.4	35.1 ± 0.2	35.5 ± 0.3	35.2 ± 0.1	34.6 ± 0.2
Week 14	34.5 ± 0.1	34.7 ± 0.1	34.7 ± 0.1	34.6 ± 0.1	34.6 ± 0.1
Platelets (10^3/μL)					
Day 23	832.9 ± 27.8	797.3 ± 30.2	875.8 ± 14.5	892.6 ± 29.1	842.0 ± 50.9
Week 14	670.7 ± 9.4	671.6 ± 10.8	714.9 ± 23.4	712.7 ± 10.4	705.9 ± 27.6
Leukocytes (10^3/μL)					
Day 23	10.54 ± 0.55	10.24 ± 0.40	9.65 ± 0.37	10.09 ± 0.64	7.70 ± 0.46**
Week 14	7.32 ± 0.28	8.49 ± 0.50	7.86 ± 0.55	7.52 ± 0.61	7.33 ± 0.39
Segmented neutrophils (10^3/μL)					
Day 23	1.10 ± 0.08	1.21 ± 0.16	1.04 ± 0.13	1.16 ± 0.12	1.51 ± 0.22
Week 14	0.99 ± 0.12	0.99 ± 0.15	1.03 ± 0.09	0.89 ± 0.08	1.49 ± 0.28
Bands (10^3/μL)					
Day 23	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Week 14	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Lymphocytes (10^3/μL)					
Day 23	9.22 ± 0.53	8.78 ± 0.42	8.43 ± 0.33	8.65 ± 0.54	6.00 ± 0.41**
Week 14	6.15 ± 0.29	7.31 ± 0.50	6.70 ± 0.56	6.45 ± 0.59	5.66 ± 0.19
Monocytes (10^3/μL)					
Day 23	0.18 ± 0.05	0.19 ± 0.04	0.17 ± 0.03	0.26 ± 0.06	0.19 ± 0.04
Week 14	0.12 ± 0.04	0.12 ± 0.03	0.09 ± 0.03	0.12 ± 0.03	0.13 ± 0.07
Basophils (10^3/μL)					
Day 23	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Week 14	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Eosinophils (10^3/μL)					
Day 23	0.03 ± 0.02	0.06 ± 0.02	0.01 ± 0.01	0.02 ± 0.01	0.00 ± 0.00
Week 14	0.07 ± 0.03	0.06 ± 0.02	0.04 ± 0.02	0.06 ± 0.02	0.05 ± 0.01

Table F1
Hematology and Clinical Chemistry Data for Rats in the 3-Month Gavage Study of β-Myrcene

	Vehicle Control	0.25 g/kg	0.5 g/kg	1 g/kg	2 g/kg
Male					
Clinical Chemistry					
n					
Day 23	10	10	10	9	6
Week 14	10	10	9	9	8
Urea nitrogen (mg/dL)					
Day 23	12.0 ± 0.4	12.0 ± 0.6	10.4 ± 0.5	9.8 ± 0.5*	13.8 ± 1.0
Week 14	12.2 ± 0.6	11.8 ± 0.3	11.4 ± 0.4	9.7 ± 0.5**	10.9 ± 0.6*
Creatinine (mg/dL)					
Day 23	0.44 ± 0.02	0.49 ± 0.01	0.47 ± 0.02	0.49 ± 0.01*	0.52 ± 0.02**
Week 14	0.56 ± 0.02	0.58 ± 0.01	0.50 ± 0.00*	0.47 ± 0.02**	0.40 ± 0.00**
Total protein (g/dL)					
Day 23	6.3 ± 0.1	6.6 ± 0.1*	6.5 ± 0.1	6.6 ± 0.1	6.6 ± 0.1*
Week 14	6.4 ± 0.1	6.8 ± 0.1**	6.8 ± 0.1**	6.8 ± 0.1**	6.6 ± 0.0
Albumin (g/dL)					
Day 23	4.2 ± 0.0	4.3 ± 0.0**	4.3 ± 0.1*	4.4 ± 0.1**	4.5 ± 0.0**
Week 14	4.3 ± 0.0	4.5 ± 0.0**	4.6 ± 0.0**	4.6 ± 0.0**	4.6 ± 0.1**
Alanine aminotransferase (IU/L)					
Day 23	63 ± 7	59 ± 5	58 ± 5	54 ± 2	64 ± 4
Week 14	86 ± 17	71 ± 9	56 ± 3*	57 ± 3	67 ± 5
Alkaline phosphatase (IU/L)					
Day 23	607 ± 19	605 ± 15	577 ± 15	550 ± 15	553 ± 18
Week 14	258 ± 10	240 ± 3	226 ± 5*	233 ± 5	273 ± 4
Creatine kinase (IU/L)					
Day 23	244 ± 40	240 ± 23	240 ± 22	222 ± 25	207 ± 17
Week 14	342 ± 43	328 ± 46	301 ± 40	311 ± 28	530 ± 160
Sorbitol dehydrogenase (IU/L)					
Day 23	33 ± 9	31 ± 7	31 ± 6	18 ± 2*	15 ± 1**
Week 14	49 ± 17	46 ± 11	22 ± 2*	21 ± 2*	21 ± 2*
Bile acids (μmol/L)					
Day 23	24.7 ± 1.8	27.0 ± 1.8	29.9 ± 2.7	32.3 ± 2.3*	37.0 ± 2.8**
Week 14	28.1 ± 3.9	27.2 ± 2.5	30.8 ± 1.5	27.9 ± 2.0	30.9 ± 1.7
Female					
Hematology					
n					
Day 23	9	7	8	8	5
Week 14	10	9	10	9	6
Hematocrit (auto) (%)					
Day 23	43.8 ± 0.5	45.3 ± 0.4	44.1 ± 0.4	44.2 ± 0.5	43.8 ± 0.8
Week 14	41.8 ± 0.4	43.0 ± 0.4	43.7 ± 0.3**	43.9 ± 0.3**	43.9 ± 0.5**
Hemoglobin (g/dL)					
Day 23	15.3 ± 0.2	15.9 ± 0.2	15.2 ± 0.1	15.2 ± 0.1	15.0 ± 0.2
Week 14	14.6 ± 0.1	14.9 ± 0.1	15.2 ± 0.1**	15.2 ± 0.1**	15.2 ± 0.2*
Erythrocytes (10^6/μL)					
Day 23	8.09 ± 0.10	8.36 ± 0.08	8.14 ± 0.07	8.09 ± 0.09	8.08 ± 0.13
Week 14	8.01 ± 0.10	8.19 ± 0.08	8.34 ± 0.07*	8.33 ± 0.07*	8.40 ± 0.10*

Table F1
Hematology and Clinical Chemistry Data for Rats in the 3-Month Gavage Study of β-Myrcene

	Vehicle Control	0.25 g/kg	0.5 g/kg	1 g/kg	2 g/kg
Female					
Hematology					
n					
Day 23	9	7	8	8	5
Week 14	10	9	10	9	6
Reticulocytes (10^6/μL)					
Day 23	0.09 ± 0.01	0.10 ± 0.02	0.09 ± 0.01	0.11 ± 0.01	0.17 ± 0.05
Week 14	0.06 ± 0.01	0.06 ± 0.01	0.08 ± 0.01	0.10 ± 0.01	0.09 ± 0.02
Nucleated erythrocytes (10^3/μL)					
Day 23	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Week 14	0.00 ± 0.00	0.01 ± 0.01	0.01 ± 0.01	0.00 ± 0.00	0.00 ± 0.00
Mean cell volume (fL)					
Day 23	54.2 ± 0.2	54.2 ± 0.2	54.2 ± 0.2	54.7 ± 0.2	54.2 ± 0.3
Week 14	52.2 ± 0.2	52.5 ± 0.1	52.4 ± 0.1	52.7 ± 0.1**	52.3 ± 0.2
Mean cell hemoglobin (pg)					
Day 23	18.9 ± 0.1	19.0 ± 0.1	18.7 ± 0.1	18.9 ± 0.1	18.5 ± 0.1
Week 14	18.2 ± 0.1	18.2 ± 0.1	18.2 ± 0.1	18.2 ± 0.0	18.0 ± 0.0
Mean cell hemoglobin concentration (g/dL)					
Day 23	34.9 ± 0.2	35.2 ± 0.1	34.6 ± 0.1	34.5 ± 0.2	34.2 ± 0.1*
Week 14	34.9 ± 0.1	34.6 ± 0.2	34.7 ± 0.1	34.5 ± 0.1**	34.5 ± 0.1*
Platelets (10^3/μL)					
Day 23	786.0 ± 37.6	755.3 ± 33.0	820.3 ± 19.9	786.5 ± 31.6	851.4 ± 20.7
Week 14	679.1 ± 12.5	680.0 ± 19.6	669.3 ± 15.9	671.8 ± 12.3	633.7 ± 23.4
Leukocytes (10^3/μL)					
Day 23	11.08 ± 0.37	12.14 ± 0.67	10.45 ± 0.36	9.60 ± 0.58	8.36 ± 0.48**
Week 14	7.83 ± 0.66	7.53 ± 0.29	8.45 ± 0.62	8.92 ± 0.66	7.72 ± 0.52
Segmented neutrophils (10^3/μL)					
Day 23	1.06 ± 0.11	1.02 ± 0.25	0.97 ± 0.13	0.95 ± 0.08	0.91 ± 0.16
Week 14	0.90 ± 0.13	1.13 ± 0.12	1.14 ± 0.17	0.93 ± 0.12	1.15 ± 0.10
Bands (10^3/μL)					
Day 23	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Week 14	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Lymphocytes (10^3/μL)					
Day 23	9.74 ± 0.34	10.76 ± 0.63	9.26 ± 0.34	8.46 ± 0.51	7.27 ± 0.63**
Week 14	6.76 ± 0.52	6.23 ± 0.24	7.14 ± 0.47	7.83 ± 0.58	6.42 ± 0.43
Monocytes (10^3/μL)					
Day 23	0.26 ± 0.04	0.28 ± 0.02	0.21 ± 0.04	0.17 ± 0.04	0.17 ± 0.04
Week 14	0.14 ± 0.04	0.12 ± 0.05	0.13 ± 0.02	0.08 ± 0.02	0.11 ± 0.04
Basophils (10^3/μL)					
Day 23	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Week 14	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Eosinophils (10^3/μL)					
Day 23	0.03 ± 0.02	0.09 ± 0.02	0.01 ± 0.01	0.03 ± 0.02	0.02 ± 0.02
Week 14	0.03 ± 0.03	0.06 ± 0.04	0.05 ± 0.02	0.09 ± 0.03	0.04 ± 0.02

Table F1
Hematology and Clinical Chemistry Data for Rats in the 3-Month Gavage Study of β-Myrcene

	Vehicle Control	0.25 g/kg	0.5 g/kg	1 g/kg	2 g/kg
Female					
Clinical Chemistry					
n	10	10	10	9	6
Urea nitrogen (mg/dL)					
Day 23	13.0 ± 0.5	13.2 ± 0.6	10.3 ± 0.4**	9.2 ± 0.4**	10.2 ± 0.5**
Week 14	10.9 ± 0.2	10.3 ± 0.4	9.5 ± 0.4**	8.7 ± 0.6**	8.8 ± 0.6**
Creatinine (mg/dL)					
Day 23	0.49 ± 0.01	0.48 ± 0.01	0.48 ± 0.01	0.48 ± 0.02	0.48 ± 0.02
Week 14	0.57 ± 0.02	0.50 ± 0.02**	0.48 ± 0.01**	0.49 ± 0.01**	0.43 ± 0.02**
Total protein (g/dL)					
Day 23	6.3 ± 0.1	6.5 ± 0.1	6.3 ± 0.1	6.3 ± 0.1	6.2 ± 0.1
Week 14	6.3 ± 0.1	6.4 ± 0.1	6.3 ± 0.1	6.4 ± 0.1	6.6 ± 0.1
Albumin (g/dL)					
Day 23	4.4 ± 0.0	4.4 ± 0.0	4.3 ± 0.0	4.3 ± 0.1	4.3 ± 0.0
Week 14	4.5 ± 0.0	4.5 ± 0.0	4.4 ± 0.0	4.6 ± 0.1	4.7 ± 0.1
Alanine aminotransferase (IU/L)					
Day 23	43 ± 1	43 ± 2	47 ± 2	55 ± 5*	70 ± 9**
Week 14	58 ± 5	49 ± 3	46 ± 2	49 ± 4	60 ± 12
Alkaline phosphatase (IU/L)					
Day 23	438 ± 18	431 ± 12	412 ± 16	430 ± 16	442 ± 30
Week 14	233 ± 9	203 ± 8	221 ± 5	209 ± 6	233 ± 12
Creatine kinase (IU/L)					
Day 23	283 ± 54	274 ± 42	321 ± 71	241 ± 36	295 ± 65
Week 14	342 ± 55	293 ± 69	338 ± 39	283 ± 50	383 ± 76
Sorbitol dehydrogenase (IU/L)					
Day 23	20 ± 2	22 ± 1	22 ± 3	26 ± 5	22 ± 4
Week 14	25 ± 5	23 ± 3	19 ± 1	19 ± 3	26 ± 9
Bile acids (µmol/L)					
Day 23	23.6 ± 3.8	22.3 ± 1.0	23.3 ± 2.5	27.9 ± 3.7	33.8 ± 3.3**
Week 14	25.5 ± 3.4	23.3 ± 3.8	19.3 ± 1.2	21.8 ± 1.1	24.0 ± 2.8

* Significantly different ($P \leq 0.05$) from the vehicle control group by Dunn's or Shirley's test
** $P \leq 0.01$
[a] Mean ± standard error. Statistical tests were performed on unrounded data. No data available for 4 g/kg males or females due to 100% mortality.

TABLE F2
Hematology Data for Mice in the 3-Month Gavage Study of β-Myrcene[a]

	Vehicle Control	0.25 g/kg	0.5 g/kg	1 g/kg	2 g/kg
Male					
n	10	10	9	10	1[b]
Hematocrit (auto) (%)	48.7 ± 0.3	48.7 ± 0.5	48.4 ± 0.4	47.0 ± 0.4**	45.5
Hemoglobin (g/dL)	16.4 ± 0.1	16.4 ± 0.1	16.3 ± 0.1	15.8 ± 0.2**	15.4
Erythrocytes (10^6/μL)	10.78 ± 0.10	10.65 ± 0.11	10.50 ± 0.07	10.11 ± 0.10**	9.59
Reticulocytes (10^6/μL)	0.09 ± 0.01	0.07 ± 0.01	0.08 ± 0.01	0.07 ± 0.01	0.13
Nucleated erythrocytes (10^3/μL)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00
Mean cell volume (fL)	45.2 ± 0.3	45.7 ± 0.1	46.0 ± 0.1*	46.4 ± 0.1**	47.5
Mean cell hemoglobin (pg)	15.3 ± 0.1	15.4 ± 0.1	15.5 ± 0.1	15.7 ± 0.1**	16.1
Mean cell hemoglobin concentration (g/dL)	33.8 ± 0.2	33.6 ± 0.2	33.6 ± 0.1	33.7 ± 0.1	33.9
Platelets (10^3/μL)	592.1 ± 37.2	759.7 ± 32.6**	629.1 ± 26.7	703.6 ± 19.9	823.0
Leukocytes (10^3/μL)	3.76 ± 0.31	3.35 ± 0.31	2.93 ± 0.33	3.57 ± 0.22	2.30
Segmented neutrophils (10^3/μL)	0.49 ± 0.06	0.47 ± 0.06	0.29 ± 0.08	0.42 ± 0.05	0.41
Bands (10^3/μL)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00
Lymphocytes (10^3/μL)	3.14 ± 0.27	2.75 ± 0.26	2.55 ± 0.27	3.01 ± 0.17	1.79
Monocytes (10^3/μL)	0.06 ± 0.02	0.04 ± 0.02	0.03 ± 0.01	0.04 ± 0.02	0.00
Basophils (10^3/μL)	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000
Eosinophils (10^3/μL)	0.07 ± 0.02	0.09 ± 0.02	0.06 ± 0.02	0.10 ± 0.02	0.09
Female					
n	10	10	10	10	2
Hematocrit (auto) (%)	47.5 ± 0.5	48.0 ± 0.6	47.4 ± 0.4	46.8 ± 0.2	40.8 ± 3.3
Hemoglobin (g/dL)	16.1 ± 0.2	16.3 ± 0.2	16.1 ± 0.1	15.7 ± 0.1	13.6 ± 1.1*
Erythrocytes (10^6/μL)	10.37 ± 0.11	10.50 ± 0.14	10.29 ± 0.10	9.92 ± 0.07*	8.19 ± 0.76*
Reticulocytes (10^6/μL)	0.11 ± 0.01	0.08 ± 0.01	0.07 ± 0.00**	0.08 ± 0.01	0.13 ± 0.00
Nucleated erythrocytes (10^3/μL)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Mean cell volume (fL)	45.8 ± 0.1	45.8 ± 0.1	46.1 ± 0.1	47.2 ± 0.2**	49.9 ± 0.6**
Mean cell hemoglobin (pg)	15.5 ± 0.0	15.6 ± 0.0*	15.7 ± 0.1**	15.9 ± 0.1**	16.6 ± 0.2**
Mean cell hemoglobin concentration (g/dL)	33.8 ± 0.1	34.0 ± 0.1	34.0 ± 0.1	33.6 ± 0.1	33.3 ± 0.1
Platelets (10^3/μL)	605.9 ± 36.0	584.2 ± 43.2	642.3 ± 13.9	612.6 ± 26.8	963.0 ± 99.0
Leukocytes (10^3/μL)	2.60 ± 0.11	3.28 ± 0.23*	3.22 ± 0.18*	3.02 ± 0.14	2.25 ± 0.25
Segmented neutrophils (10^3/μL)	0.44 ± 0.08	0.34 ± 0.08	0.38 ± 0.05	0.35 ± 0.05	0.58 ± 0.13
Bands (10^3/μL)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Lymphocytes (10^3/μL)	2.08 ± 0.09	2.81 ± 0.20*	2.73 ± 0.17*	2.60 ± 0.13	1.61 ± 0.39
Monocytes (10^3/μL)	0.04 ± 0.01	0.04 ± 0.02	0.03 ± 0.01	0.02 ± 0.01	0.05 ± 0.01
Basophils (10^3/μL)	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Eosinophils (10^3/μL)	0.04 ± 0.01	0.09 ± 0.02	0.08 ± 0.02	0.06 ± 0.02	0.02 ± 0.02

* Significantly different ($P \leq 0.05$) from the vehicle control group by Dunn's or Shirley's test
** $P \leq 0.01$
[a] Mean ± standard error. Statistical tests were performed on unrounded data. No data available for 4 g/kg males or females due to 100% mortality.
[b] No standard error calculated; less than two measurements available.

APPENDIX G
ORGAN WEIGHTS
AND ORGAN-WEIGHT-TO-BODY-WEIGHT RATIOS

TABLE G1 Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 3-Month Gavage Study of β-Myrcene G-2
TABLE G2 Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 3-Month Gavage Study of β-Myrcene G-3

TABLE G1
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 3-Month Gavage Study of β-Myrcene[a]

	Vehicle Control	0.25 g/kg	0.5 g/kg	1 g/kg	2 g/kg
Male					
n	10	10	9	9	8
Necropsy body wt	341 ± 7	335 ± 7	318 ± 5*	300 ± 8**	255 ± 8**
Heart					
Absolute	0.943 ± 0.014	0.910 ± 0.028	0.870 ± 0.014*	0.857 ± 0.018**	0.791 ± 0.033**
Relative	2.767 ± 0.033	2.716 ± 0.052	2.739 ± 0.037	2.865 ± 0.036	3.092 ± 0.063**
R. Kidney					
Absolute	0.964 ± 0.020	1.186 ± 0.021**	1.306 ± 0.028**	1.524 ± 0.033**	1.792 ± 0.064**
Relative	2.826 ± 0.049	3.545 ± 0.033**	4.109 ± 0.045**	5.099 ± 0.092**	7.014 ± 0.121**
Liver					
Absolute	11.47 ± 0.21	12.76 ± 0.35*	12.78 ± 0.29*	13.44 ± 0.32**	12.55 ± 0.43
Relative	33.688 ± 0.742	38.084 ± 0.398**	40.205 ± 0.563**	44.930 ± 0.653**	49.121 ± 0.647**
Lung					
Absolute	1.545 ± 0.088	1.708 ± 0.099	1.492 ± 0.067	1.385 ± 0.054	1.321 ± 0.122
Relative	4.522 ± 0.230	5.123 ± 0.315	4.684 ± 0.163	4.640 ± 0.183	5.256 ± 0.622
R. Testis					
Absolute	1.385 ± 0.026	1.411 ± 0.020	1.403 ± 0.021	1.344 ± 0.027	1.279 ± 0.025**
Relative	4.059 ± 0.047	4.226 ± 0.073	4.421 ± 0.076**	4.499 ± 0.072**	5.019 ± 0.071**
Thymus					
Absolute	0.350 ± 0.016	0.340 ± 0.018	0.285 ± 0.009**	0.272 ± 0.015**	0.205 ± 0.017**
Relative	1.024 ± 0.040	1.013 ± 0.048	0.899 ± 0.038	0.913 ± 0.052	0.795 ± 0.052**
Female					
n	10	10	10	9	6
Necropsy body wt	196 ± 3	196 ± 3	187 ± 3	188 ± 3	185 ± 5
Heart					
Absolute	0.622 ± 0.012	0.648 ± 0.017	0.620 ± 0.011	0.617 ± 0.013	0.630 ± 0.017
Relative	3.171 ± 0.058	3.318 ± 0.085	3.312 ± 0.043	3.275 ± 0.042	3.415 ± 0.085
R. Kidney					
Absolute	0.633 ± 0.012	0.799 ± 0.012**	0.828 ± 0.019**	0.953 ± 0.027**	1.197 ± 0.043**
Relative	3.229 ± 0.056	4.091 ± 0.062**	4.418 ± 0.056**	5.055 ± 0.085**	6.483 ± 0.143**
Liver					
Absolute	5.990 ± 0.162	6.717 ± 0.109**	7.022 ± 0.164**	7.819 ± 0.219**	9.421 ± 0.326**
Relative	30.533 ± 0.641	34.407 ± 0.622**	37.463 ± 0.463**	41.499 ± 0.831**	51.003 ± 0.867**
Lung					
Absolute	1.089 ± 0.049	1.203 ± 0.046	1.074 ± 0.045	1.082 ± 0.039	1.009 ± 0.030
Relative	5.540 ± 0.204	6.163 ± 0.248	5.747 ± 0.263	5.741 ± 0.172	5.483 ± 0.224
Thymus					
Absolute	0.265 ± 0.009	0.256 ± 0.009	0.248 ± 0.012	0.266 ± 0.009	0.224 ± 0.008*
Relative	1.353 ± 0.046	1.313 ± 0.050	1.321 ± 0.057	1.410 ± 0.036	1.213 ± 0.042

* Significantly different ($P \leq 0.05$) from the vehicle control group by Williams' or Dunnett's test
** ($P \leq 0.01$)
[a] Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error). No data available for 4 g/kg males or females due to 100% mortality.

TABLE G2
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 3-Month Gavage Study of β-Myrcene[a]

	Vehicle Control	0.25 g/kg	0.5 g/kg	1 g/kg	2 g/kg
Male					
n	10	10	10	10	1[b]
Necropsy body wt	35.8 ± 1.0	36.6 ± 0.7	35.8 ± 0.9	32.7 ± 0.8*	28.6
Heart					
Absolute	0.150 ± 0.004	0.160 ± 0.004	0.156 ± 0.003	0.150 ± 0.003	0.131
Relative	4.221 ± 0.097	4.387 ± 0.104	4.377 ± 0.153	4.618 ± 0.126	4.580
Liver					
Absolute	1.453 ± 0.043	1.593 ± 0.036*	1.537 ± 0.042	1.557 ± 0.038	1.714
Relative	40.689 ± 0.735	43.580 ± 0.850*	43.104 ± 1.201*	47.687 ± 0.516**	59.930
Lung					
Absolute	0.306 ± 0.008	0.288 ± 0.014	0.290 ± 0.016	0.289 ± 0.009	0.324
Relative	8.635 ± 0.387	7.915 ± 0.464	8.106 ± 0.380	8.897 ± 0.383	11.329
R. Kidney					
Absolute	0.289 ± 0.006	0.293 ± 0.007	0.273 ± 0.008	0.274 ± 0.006	0.300
Relative	8.125 ± 0.200	8.010 ± 0.163	7.659 ± 0.190	8.409 ± 0.209	10.490
R. Testis					
Absolute	0.122 ± 0.003	0.124 ± 0.002	0.117 ± 0.003	0.118 ± 0.001	0.099
Relative	3.431 ± 0.106	3.396 ± 0.091	3.287 ± 0.093	3.625 ± 0.083	3.462
Thymus					
Absolute	0.050 ± 0.002	0.050 ± 0.003	0.052 ± 0.004	0.042 ± 0.001*	0.032
Relative	1.405 ± 0.049	1.376 ± 0.078	1.445 ± 0.082	1.299 ± 0.046	1.119
Female					
n	10	10	10	10	2
Necropsy body wt	27.9 ± 0.7	26.1 ± 0.5	26.1 ± 0.5	26.9 ± 0.3	27.0 ± 0.2
Heart					
Absolute	0.132 ± 0.004	0.128 ± 0.002[c]	0.133 ± 0.005	0.138 ± 0.004	0.135 ± 0.007
Relative	4.764 ± 0.230	4.896 ± 0.127[c]	5.089 ± 0.199	5.134 ± 0.149	4.980 ± 0.204
Liver					
Absolute	1.127 ± 0.024	1.143 ± 0.036	1.236 ± 0.025*	1.369 ± 0.019**	1.889 ± 0.199**
Relative	40.604 ± 1.024	43.862 ± 1.481	47.458 ± 1.076**	50.985 ± 0.531**	70.021 ± 7.889**
Lung					
Absolute	0.288 ± 0.013	0.290 ± 0.012	0.295 ± 0.009	0.301 ± 0.014	0.301 ± 0.035
Relative	10.403 ± 0.556	11.123 ± 0.498	11.348 ± 0.397	11.207 ± 0.526	11.158 ± 1.379
R. Kidney					
Absolute	0.164 ± 0.004	0.175 ± 0.004	0.173 ± 0.003	0.193 ± 0.004**	0.250 ± 0.004**
Relative	5.901 ± 0.150	6.719 ± 0.178**	6.621 ± 0.084**	7.197 ± 0.116**	9.242 ± 0.198**
Thymus					
Absolute	0.047 ± 0.003	0.050 ± 0.002	0.051 ± 0.003	0.051 ± 0.002	0.039 ± 0.012
Relative	1.688 ± 0.071	1.910 ± 0.095	1.947 ± 0.097	1.908 ± 0.081	1.441 ± 0.434

* Significantly different (P≤0.05) from the vehicle control group by Williams' or Dunnett's test
** (P≤0.01)
[a] Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error). No data available for 4 g/kg males or females due to 100% mortality.
[b] No standard error calculated; less than two measurements available.
[c] n=9

APPENDIX H
REPRODUCTIVE TISSUE EVALUATIONS AND ESTROUS CYCLE CHARACTERIZATION

TABLE H1 Summary of Reproductive Tissue Evaluations for Male Rats in the 3-Month Gavage Study of β-Myrcene H-2
TABLE H2 Estrous Cycle Characterization for Female Rats in the 3-Month Gavage Study of β-Myrcene H-2
TABLE H3 Summary of Reproductive Tissue Evaluations for Male Mice in the 3-Month Gavage Study of β-Myrcene H-3
TABLE H4 Estrous Cycle Characterization for Female Mice in the 3-Month Gavage Study of β-Myrcene H-3

TABLE H1
Summary of Reproductive Tissue Evaluations for Male Rats in the 3-Month Gavage Study of β-Myrcene[a]

	Vehicle Control	0.25 g/kg	0.5 g/kg	1 g/kg
n	10	10	9	9
Weights (g)				
Necropsy body wt	341 ± 7	335 ± 7	318 ± 5*	300 ± 8**
L. Cauda epididymis	0.1281 ± 0.0041	0.1279 ± 0.0050	0.1174 ± 0.0058	0.1191 ± 0.0032
L. Epididymis	0.3982 ± 0.0076	0.4000 ± 0.0058	0.3725 ± 0.0151	0.3825 ± 0.0108
L. Testis	1.4638 ± 0.0276	1.4590 ± 0.0163	1.4433 ± 0.0218	1.4249 ± 0.0231
Spermatid measurement				
Spermatid heads (10^6/g testis)	111.4 ± 2.5	107.7 ± 3.8	106.3 ± 4.3	107.0 ± 3.1
Spermatid heads (10^6/testis)	153.8 ± 3.8	147.9 ± 6.2	144.9 ± 5.2	142.6 ± 5.0
Epididymal spermatozoal measurements				
Sperm motility (%)	79.99 ± 0.91	79.44 ± 0.95	70.96 ± 8.90	79.42 ± 0.59
Sperm (10^6/g cauda epididymis)	742.2 ± 36.1	729.4 ± 46.0	716.6 ± 86.4	761.8 ± 35.6
Sperm (10^6/cauda epididymis)	94.27 ± 3.68	91.77 ± 3.91	85.72 ± 10.37	90.84 ± 5.37

* Significantly different ($P \leq 0.05$) from the vehicle control group by Williams' test
** $P \leq 0.01$
[a] Data are presented as mean ± standard error. Differences from the vehicle control group are not significant by Dunnett's test (tissue weights) or Dunn's test (spermatid and epididymal spermatozoal measurements).

TABLE H2
Estrous Cycle Characterization for Female Rats in the 3-Month Gavage Study of β-Myrcene[a]

	Vehicle Control	0.25 g/kg	0.5 g/kg	1 g/kg
Number weighed at necropsy	10	10	10	9
Necropsy body wt (g)	196 ± 3	196 ± 3	187 ± 3	188 ± 3
Proportion of regular cycling females[b]	9/10	10/10	9/10	8/9
Estrous cycle length (days)	4.70 ± 0.13	5.00 ± 0.15	5.40 ± 0.48	5.56 ± 0.35
Estrous stages (% of cycle)				
Diestrus	35.9	43.1	46.2	46.6
Proestrus	12.0	12.9	5.0	8.7
Estrus	34.2	26.7	30.3	29.1
Metestrus	17.9	17.2	18.5	15.5

[a] Necropsy body weights and estrous cycle length data are presented as mean ± standard error. Differences from the vehicle control group are not significant by Dunnett's test (body weight) or Dunn's test (estrous cycle length). By multivariate analysis of variance, dosed females do not differ significantly from the vehicle control females in the relative length of time spent in the estrous stages.
[b] Number of females with a regular cycle/number of females cycling

TABLE H3
Summary of Reproductive Tissue Evaluations for Male Mice in the 3-Month Gavage Study of β-Myrcene[a]

	Vehicle Control	0.25 g/kg	0.5 g/kg	1 g/kg
n	10	10	10	10
Weights (g)				
Necropsy body wt	35.8 ± 1.0	36.6 ± 0.7	35.8 ± 0.9	32.7 ± 0.8*
L. Cauda epididymis	0.0122 ± 0.0006	0.0124 ± 0.0005	0.0115 ± 0.0012	0.0128 ± 0.0008
L. Epididymis	0.0407 ± 0.0006	0.0414 ± 0.0010	0.0401 ± 0.0007	0.0390 ± 0.0009
L. Testis	0.1171 ± 0.0020	0.1178 ± 0.0017	0.1129 ± 0.0026	0.1140 ± 0.0018
Spermatid measurements				
Spermatid heads (10^6/g testis)	192.2 ± 6.3	185.8 ± 10.0	198.4 ± 8.3	192.6 ± 4.6
Spermatid heads (10^6/testis)	21.35 ± 1.00	20.39 ± 1.10	20.84 ± 1.15	20.30 ± 0.47
Epididymal spermatozoal measurements				
Sperm motility (%)	86.56 ± 0.86	85.37 ± 1.00	87.52 ± 1.30	86.44 ± 1.23
Sperm heads (10^6/g cauda epididymis)	1,283 ± 86	1,409 ± 93	1,822 ± 567	1,388 ± 95
Sperm heads (10^6/cauda epididymis)	15.50 ± 1.06	17.47 ± 1.45	15.82 ± 1.04	17.47 ± 1.07

* Significantly different ($P \leq 0.05$) from the vehicle control group by Dunnett's test
[a] Data are presented as mean ± standard error. Differences from the vehicle control group are not significant by Dunnett's test (tissue weights) or Dunn's test (spermatid and epididymal spermatozoal measurements).

TABLE H4
Estrous Cycle Characterization for Female Mice in the 3-Month Gavage Study of β-Myrcene[a]

	Vehicle Control	0.25 g/kg	0.5 g/kg	1 g/kg
Number weighed at necropsy	10	10	10	10
Necropsy body wt (g)	27.9 ± 0.7	26.1 ± 0.5	26.1 ± 0.5	26.9 ± 0.3
Proportion of regular cycling females[b]	8/10	6/10	8/10	5/10
Estrous cycle length (days)	4.05 ± 0.16	4.47 ± 0.31	4.24 ± 0.13	4.17 ± 0.17
Estrous stages (% of cycle)				
Diestrus	35.4	33.3	38.5	33.3
Proestrus	0.9	0.0	0.0	0.0
Estrus	43.4	44.4	42.7	47.9
Metestrus	20.4	22.2	18.8	18.8

[a] Necropsy body weights and estrous cycle length data are presented as mean ± standard error. Differences from the vehicle control group are not significant by Dunnett's test (body weight) or Dunn's test (estrous cycle length). By multivariate analysis of variance, dosed females do not differ significantly from the vehicle control females in the relative length of time spent in the estrous stages.
[b] Number of females with a regular cycle/number of females cycling

APPENDIX I
CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

Procurement and Characterization I-2
Preparation and Analysis of Dose Formulations. I-3
Figure I1 Infrared Absorption Spectrum of β-Myrcene I-4
Figure I2 Proton Nuclear Magnetic Resonance Spectrum of β-Myrcene I-5
Figure I3 Carbon-13 Nuclear Magnetic Resonance Spectrum of β-Myrcene. I-6
Table I1 Preparation and Storage of Dose Formulations in the Gavage Studies of β-Myrcene I-7
Table I2 Results of Analyses of Dose Formulations Administered to Rats and Mice in the 3-Month Gavage Studies of β-Myrcene. I-8
Table I3 Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Gavage Studies of β-Myrcene I-9

CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

PROCUREMENT AND CHARACTERIZATION

β-*Myrcene*

β-Myrcene was obtained from Millennium Specialty Chemicals (Jacksonville, FL) in two lots (0LB410 and 1WB503). Lot 0LB410 was used in the 3-month studies and lot 1WB503 was used in the 2-year studies. Identity and purity studies were performed by the analytical chemistry laboratory at Battelle Columbus Operations (Chemistry Support Services, Columbus, OH), Galbraith Laboratories, Inc. (Knoxville, TN), and the study laboratory at Battelle Columbus Operations (Columbus, OH). Karl Fischer titration was performed by Galbraith Laboratories, Inc. Reports on analyses performed in support of the β-myrcene studies are on file at the National Institute of Environmental Health Sciences.

Both lots of the chemical, a clear to slightly yellow liquid, were identified as β-myrcene by the analytical chemistry laboratory using infrared (IR), proton, and carbon-13 nuclear magnetic resonance (NMR) spectroscopy and by Galbraith Laboratories, Inc., using boiling point determinations. In addition, the identity of both lots was confirmed by the study laboratory using IR spectroscopy. All spectra were consistent with literature spectra (*Aldrich*, 1992, 1997), spectra of frozen reference samples from each lot, computer-calculated spectra, and the structure of β-myrcene. Boiling point determinations were consistent with literature values of 167° C (*CRC Handbook*, 1981; *Hawley's*, 1993). Representative IR, proton, and carbon-13 NMR spectra are presented in Figures I1, I2, and I3, respectively.

The purities of both lots were determined by the analytical chemistry laboratory using gas chromatography with flame ionization detection (GC-FID) and high-performance liquid chromatography (HPLC). GC-FID was performed on a Hewlett-Packard (Palo Alto, CA) system that included a Stabilwax®-DA or Stabilwax® Crossbond® 30 m × 0.25 mm, 0.25-µm film thickness column (Restek, Bellefonte, PA), helium carrier gas at flow rates varying from 2 to 4 mL/minute, and an oven temperature program starting at 35° C for 5 minutes, then increased to 55° C at 2° C/minute, held for 1 minute, then increased to 230° C at 10° C/minute, and held for 1 to 3 minutes. HPLC was performed on a Waters (Milford, MA) system that included a 5-µm Prodigy ODS-3 150 mm × 4.6 mm column (Phenomenex, Torrance, CA), an isocratic mobile phase of 85:15 or 90:10 methanol:water, ultraviolet light detection at 225 nm, and a flow rate of 0.8 mL/minute.

For lot 0LB410, Karl Fischer titration indicated a water content of less than 0.016%. GC-FID by the analytical chemistry laboratory indicated one major peak and nine impurity peaks with areas of 0.1% or greater, with a total peak area for all impurities of 9.55%. HPLC analysis indicated one major peak and one impurity with a peak area of 0.36%. Elemental analyses of carbon and hydrogen were generally consistent with the theoretical values for β-myrcene. The largest peak (4.4% of total peak area) was identified as *psi*-limonene. The second largest impurity (1.4% of total peak area) was tentatively identified as *dl*-limonene. The other seven impurity peaks, small by comparison, were chiefly terpene hydrocarbons and were tentatively identified as isomers and dimers of β-myrcene. The overall purity of lot 0LB410 was determined to be greater than 90%.

For lot 1WB503, Karl Fischer titration indicated a water content of less than 0.06%. GC-FID by the analytical chemistry laboratory indicated one major peak and 12 impurities with areas of 0.1% or greater, with a total peak area for all impurities of 6.5%. HPLC analysis indicated one major peak and one impurity with a peak area of 0.4%. Elemental analyses of carbon and hydrogen were generally consistent with the theoretical values for β-myrcene. The largest impurity in β-myrcene was identified as *psi*-limonene (approximately 5%). The other 11 impurity peaks, small by comparison, were chiefly the same as in the earlier lot, tentatively identified as isomers and dimers of β-myrcene. The overall purity of lot 1WB503 was determined to be greater than 93%.

To ensure stability, the bulk chemical was stored in amber glass bottles sealed with Teflon®-lined lids at less than or equal to –20° C. Periodic reanalyses of the bulk chemical were performed during the 3-month and 2-year studies by the study laboratory using GC-FID by the method described above; no degradation of the bulk chemical was detected.

Corn Oil

USP-grade corn oil was obtained in multiple lots from Spectrum Chemicals and Laboratory Products (Gardena, CA) and was used as the vehicle during the 3-month and 2-year studies. Periodic analyses of the corn oil vehicle performed by the study laboratory using potentiometric titration demonstrated peroxide concentrations below the acceptable limit of 3 mEq/kg.

Preparation and Analysis of Dose Formulations

The dose formulations were prepared by mixing β-myrcene with corn oil to give the required concentrations (Table I1). Because all dose formulations in these studies were determined to be solutions, no homogeneity studies were required. The dose formulations were stored at room temperature in amber glass bottles sealed with Teflon®-lined lids for up to 37 days.

Stability studies of a 50 mg/mL β-myrcene dose formulation were performed by the analytical chemistry laboratory on lot 09116TQ obtained from Aldrich Chemical Company (Milwaukee, WI). Analyses were performed with a GC-FID Hewlett-Packard (Palo Alto, CA) system that included a Stabilwax® Crossbond® 30 m × 0.25 mm, 0.25-µm film thickness column (Restek), helium carrier gas at a flow rate of 1.5 mL/minute, and an oven temperature program starting at 40° C for 3 minutes and then increased to 220° C at 15° C/minute. Stability was confirmed for at least 37 days for dose formulations stored in amber glass bottles sealed with Teflon®-lined lids at temperatures up to room temperature, and for up to 3 hours under simulated animal room conditions.

Periodic analyses of the dose formulations of β-myrcene were conducted by the study laboratory using GC-FID by the system used for the purity analyses. During the 3-month studies, the dose formulations were analyzed at the beginning, midpoint, and end of the studies; all 13 dose formulations analyzed for rats and all 15 for mice were within 10% of the target concentrations (Table I2). Animal room samples of these dose formulations were also analyzed; all 13 animal room samples for rats and mice were within 10% of the target concentrations. During the 2-year studies, the dose formulations were analyzed approximately every 3 months; animal room samples were also analyzed (Table I3). All 27 dose formulations for rats and mice were within 10% of the target concentrations; all nine animal room samples for rats and mice were within 10% of the target concentrations.

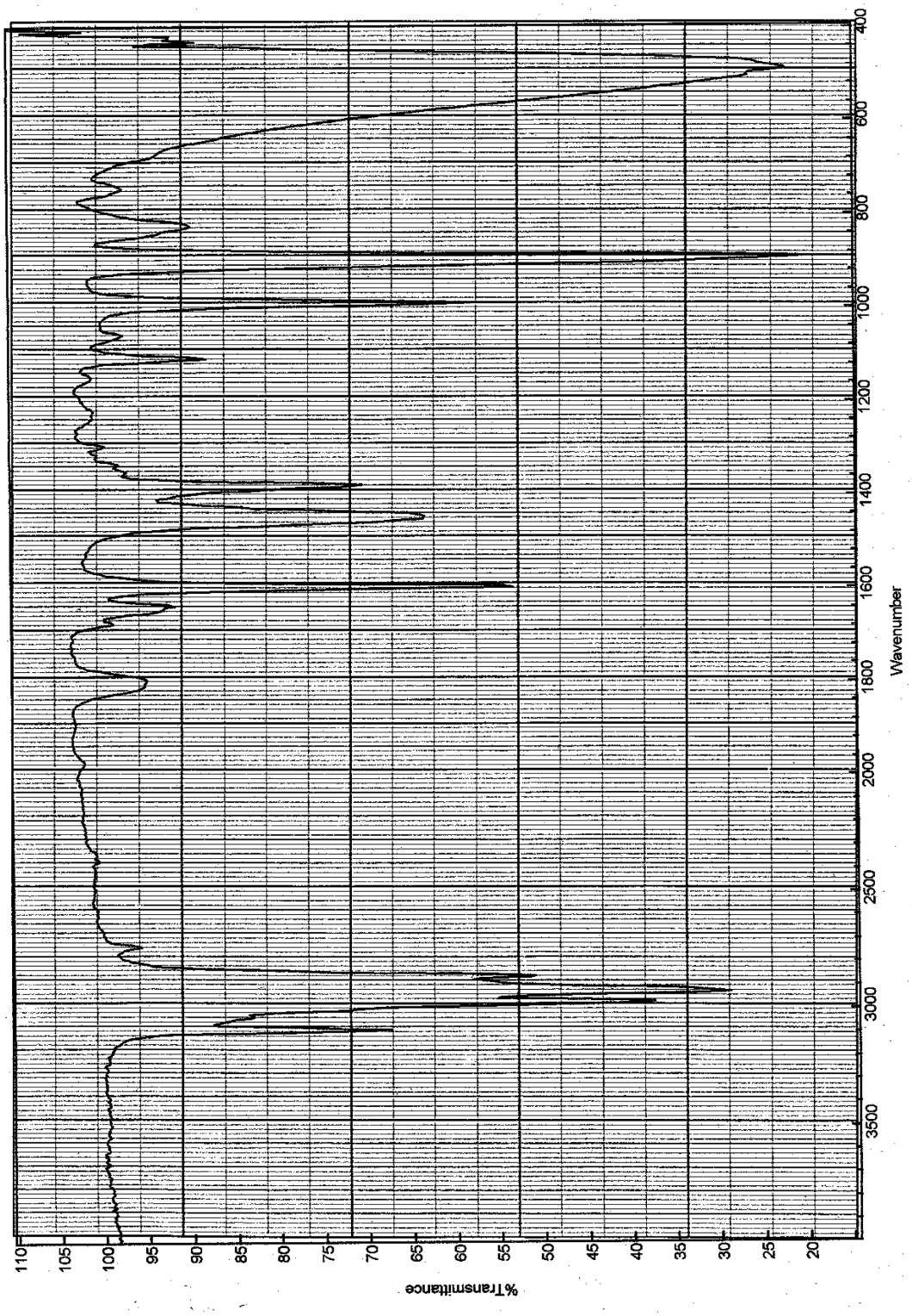

FIGURE I1
Infrared Absorption Spectrum of β-Myrcene

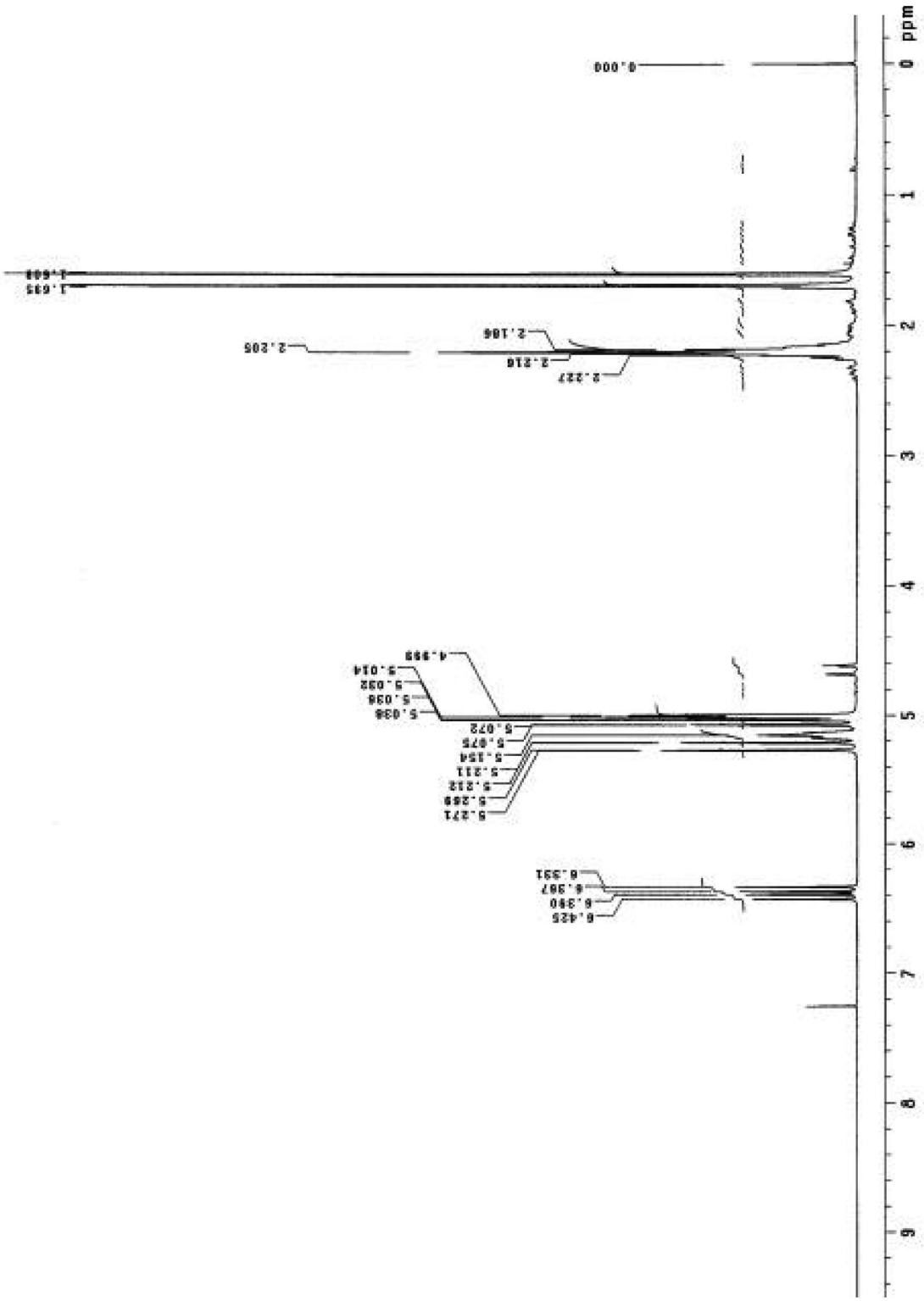

Figure I2
Proton Nuclear Magnetic Resonance Spectrum of β-Myrcene

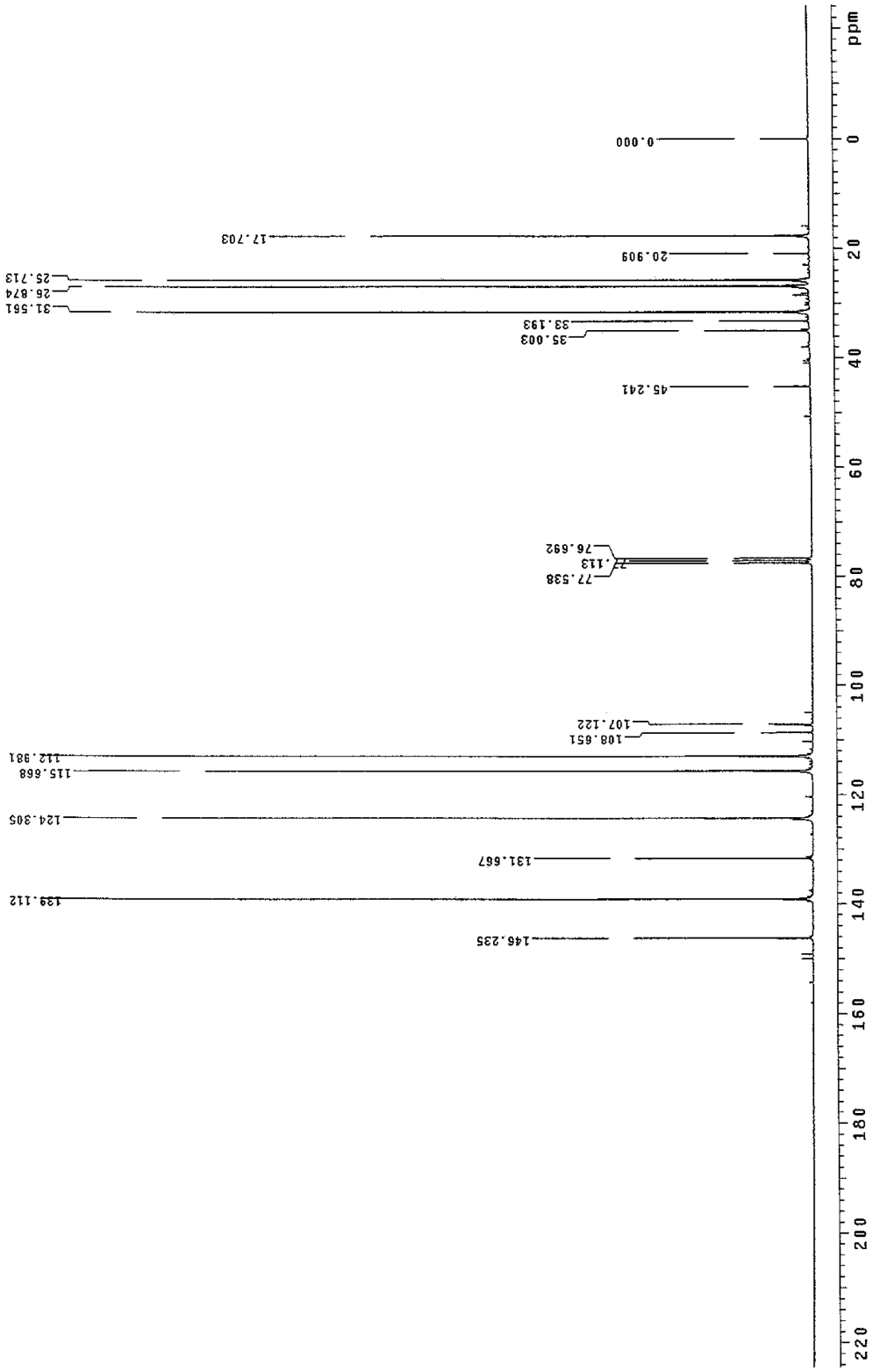

FIGURE I3
Carbon-13 Nuclear Magnetic Resonance Spectrum of β-Myrcene

Table I1
Preparation and Storage of Dose Formulations in the Gavage Studies of β-Myrcene

3-Month Studies	2-Year Studies
Preparation	
The appropriate volumes of β-myrcene and corn oil were combined in a calibrated glass mixing container and mixed on a paint shaker for 5 minutes. The 800 mg/mL dose formulation for rats consisted of neat β-myrcene. The dose formulations were prepared approximately monthly.	The appropriate volumes of β-myrcene and corn oil were combined in a calibrated glass mixing container and stirred with a vigorous vortex for 10 minutes using an overhead stirrer. Water droplets, if noticed to have separated out in the container of bulk test article, were avoided. The dose formulations were prepared approximately monthly.
Chemical Lot Number	
0LB410	1WB503
Maximum Storage Time	
37 days	37 days
Storage Conditions	
Stored in amber glass bottles sealed with Teflon®-lined lids at room temperature	Stored in amber glass bottles sealed with Teflon®-lined lids at room temperature
Study Laboratory	
Battelle Columbus Operations (Columbus, OH)	Battelle Columbus Operations, (Columbus, OH)

TABLE I2
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 3-Month Gavage Studies of β-Myrcene

Date Prepared	Date Analyzed	Target Concentration[a] (mg/mL)	Determined Concentration[b] (mg/mL)	Difference from Target (%)
January 17, 2001	January 18-19, 2001	25	24.70	–1
		50	49.88	0
		100	97.79	–2
		200	197.8	–1
		400	405.8	+1
		800	815.1[c]	+2
	February 22-26, 2001[d]	50	49.79	0
		100	97.58	–2
		200	197.1	–1
		400	417.0	+4
		800	836.0[c]	+5
	February 22-26, 2001[e]	25	24.72	–1
		50	52.32	+5
		100	98.54	–1
		200	202.2	+1
		400	404.9	+1
February 12, 2001	February 14-15, 2001	25	25.55	+2
		50	53.98	+8
		100	102.3	+2
		200	206.0	+3
		400	431.3	+8
	March 19-20, 2001[d]	50	50.03	0
		100	107.4	+7
		200	211.8	+6
		400	433.3	+8
	March 19-20, 2001[e]	25	24.53	–2
		50	49.76	0
		100	98.59	–1
		200	201.7	+1
April 4, 2001	April 7-8, 2001	25	23.98	–4
		50	47.19	–6
		100	92.25	–8
		200	187.7	–6
		400	380.3	–5
	May 10-12, 2001[d]	50	48.72	–3
		100	95.40	–5
		200	193.4	–3
		400	388.7	–3
	May 10-12, 2001[e]	25	24.61	–2
		50	48.48	–3
		100	94.25	–6
		200	191.8	–4

[a] 25 and 800 mg/mL dose formulations were used for mice and rats only, respectively
[b] Results of duplicate analyses. For rats, dosing volume=5 mL/kg; 50 mg/mL=0.25 g/kg, 100 mg/mL=0.5 g/kg, 200 mg/mL=1 g/kg, 400 mg/mL=2 g/kg, 800 mg/mL=4 g/kg; for mice, dosing volume=10 mL/kg; 25 mg/mL=0.25 g/kg, 50 mg/mL=0.5 g/kg, 100 mg/mL=1 g/kg, 200 mg/mL=2 g/kg, 400 mg/mL=4 g/kg
[c] Extrapolated value; concentration outside calibration curve. Confirmed by analysis of archive sample.
[d] Rat animal room samples
[e] Mouse animal room samples

TABLE I3
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Gavage Studies of β-Myrcene

Date Prepared	Date Analyzed	Target Concentration[a] (mg/mL)	Determined Concentration[b] (mg/mL)	Difference from Target (%)
March 12, 2002	March 15-16, 2002	25	24.39	–2
		50	49.48	–1
		100	93.16	–7
		200	195.7	–2
	April 22-23, 2002[c]	50	52.07	+4
		100	99.46	–1
		200	205.8	+3
	April 22-23, 2002[d]	25	25.20	+1
		50	51.40	+3
		100	98.76	–1
June 3, 2002	June 5-6, 2002	25	24.56	–2
		50	51.65	+3
		100	100.2	0
		200	197.5	–1
August 26, 2002	August 28-29, 2002	25	24.66	–1
		50	49.91	0
		100	99.23	–1
		200	204.0	+2
November 18, 2002	November 21-22, 2002	25	24.53	–2
		50	48.63	–3
		100	97.67	–2
		200	198.4	–1
	December 26-28, 2002[c]	50	48.50	–3
		100	95.44	–5
		200	200.4	0
	December 26-28, 2002[d]	25	22.57	–10
		50	48.77	–3
		100	96.20	–4
February 11, 2003	February 11-13, 2003	25	24.13	–4
		50	49.93	0
		100	95.49	–5
		200	193.0	–4
May 5, 2003	May 8-9, 2003	25	25.11	0
		50	50.40	+1
		100	96.76	–3
		200	197.0	–2

TABLE I3
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Gavage Studies of β-Myrcene

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration (mg/mL)	Difference from Target (%)
July 28, 2003	July 30-31, 2003	25	23.61	–6
		50	48.70	–3
		100	102.1	+2
		200	197.7	–1
	September 10-11, 2003[c]	50	50.11	0
		100	103.1	+3
		200	203.1	+2
	September 10-11, 2003[d]	25	24.40	–2
		50	49.55	–1
		100	102.4	+2
October 20, 2003	October 20-21, 2003	25	23.83	–5
		50	49.16	–2
	November 3-4, 2003	100	92.96[e]	–7
		200	183.0[e]	–9
January 12, 2004	January 12-13, 2004	25	24.21	–3
		50	49.49	–1
		100	99.42	–1
		200	198.9	–1

[a] 25 and 200 mg/mL dose formulations were used for mice and rats only, respectively
[b] Results of duplicate analyses. For rats, dosing volume=5 mL/kg; 50 mg/mL=0.25 g/kg, 100 mg/mL=0.5 g/kg, 200 mg/mL=1 g/kg; for mice, dosing volume=10 mL/kg; 25 mg/mL=0.25 g/kg, 50 mg/mL=0.5 g/kg, 100 mg/mL=1 g/kg
[c] Rat animal room samples
[d] Mouse animal room samples
[e] Extrapolated value; concentration outside calibration curve. Confirmed by analysis of archive sample.

APPENDIX J
INGREDIENTS, NUTRIENT COMPOSITION, AND CONTAMINANT LEVELS IN NTP-2000 RAT AND MOUSE RATION

TABLE J1 Ingredients of NTP-2000 Rat and Mouse Ration J-2
TABLE J2 Vitamins and Minerals in NTP-2000 Rat and Mouse Ration J-2
TABLE J3 Nutrient Composition of NTP-2000 Rat and Mouse Ration J-3
TABLE J4 Contaminant Levels in NTP-2000 Rat and Mouse Ration J-4

TABLE J1
Ingredients of NTP-2000 Rat and Mouse Ration

Ingredients	Percent by Weight
Ground hard winter wheat	22.26
Ground #2 yellow shelled corn	22.18
Wheat middlings	15.0
Oat hulls	8.5
Alfalfa meal (dehydrated, 17% protein)	7.5
Purified cellulose	5.5
Soybean meal (49% protein)	5.0
Fish meal (60% protein)	4.0
Corn oil (without preservatives)	3.0
Soy oil (without preservatives)	3.0
Dried brewer's yeast	1.0
Calcium carbonate (USP)	0.9
Vitamin premix[a]	0.5
Mineral premix[b]	0.5
Calcium phosphate, dibasic (USP)	0.4
Sodium chloride	0.3
Choline chloride (70% choline)	0.26
Methionine	0.2

[a] Wheat middlings as carrier
[b] Calcium carbonate as carrier

TABLE J2
Vitamins and Minerals in NTP-2000 Rat and Mouse Ration[a]

	Amount	Source
Vitamins		
A	4,000 IU	Stabilized vitamin A palmitate or acetate
D	1,000 IU	D-activated animal sterol
K	1.0 mg	Menadione sodium bisulfite complex
α-Tocopheryl acetate	100 IU	
Niacin	23 mg	
Folic acid	1.1 mg	
d-Pantothenic acid	10 mg	*d*-Calcium pantothenate
Riboflavin	3.3 mg	
Thiamine	4 mg	Thiamine mononitrate
B_{12}	52 µg	
Pyridoxine	6.3 mg	Pyridoxine hydrochloride
Biotin	0.2 mg	*d*-Biotin
Minerals		
Magnesium	514 mg	Magnesium oxide
Iron	35 mg	Iron sulfate
Zinc	12 mg	Zinc oxide
Manganese	10 mg	Manganese oxide
Copper	2.0 mg	Copper sulfate
Iodine	0.2 mg	Calcium iodate
Chromium	0.2 mg	Chromium acetate

[a] Per kg of finished product

TABLE J3
Nutrient Composition of NTP-2000 Rat and Mouse Ration

Nutrient	Mean ± Standard Deviation	Range	Number of Samples
Protein (% by weight)	14.8 ± 0.46	13.3 – 15.7	24
Crude fat (% by weight)	8.0 ± 0.27	7.4 – 8.6	24
Crude fiber (% by weight)	9.0 ± 0.39	8.4 – 9.9	24
Ash (% by weight)	5.0 ± 0.25	4.4 – 5.6	24
Amino Acids (% of total diet)			
Arginine	0.750 ± 0.048	0.670 – 0.850	15
Cystine	0.225 ± 0.025	0.150 – 0.250	15
Glycine	0.701 ± 0.039	0.620 – 0.750	15
Histidine	0.365 ± 0.090	0.310 – 0.680	15
Isoleucine	0.533 ± 0.038	0.430 – 0.590	15
Leucine	1.077 ± 0.059	0.960 – 1.150	15
Lysine	0.703 ± 0.125	0.310 – 0.830	15
Methionine	0.402 ± 0.049	0.260 – 0.460	15
Phenylalanine	0.615 ± 0.035	0.540 – 0.660	15
Threonine	0.492 ± 0.040	0.430 – 0.590	15
Tryptophan	0.135 ± 0.018	0.110 – 0.160	15
Tyrosine	0.378 ± 0.048	0.280 – 0.460	15
Valine	0.658 ± 0.043	0.550 – 0.710	15
Essential Fatty Acids (% of total diet)			
Linoleic	3.90 ± 0.256	3.49 – 4.54	15
Linolenic	0.30 ± 0.035	0.21 – 0.35	15
Vitamins			
Vitamin A (IU/kg)	4,956 ± 116	3,400 – 8,900	24
Vitamin D (IU/kg)	1,000[a]		
α-Tocopherol (ppm)	84.2 ± 16.60	52.0 – 110.0	15
Thiamine (ppm)[b]	8.7 ± 3.73	5.9 – 25.2	24
Riboflavin (ppm)	6.8 ± 2.11	4.20 – 11.20	15
Niacin (ppm)	79.0 ± 10.50	66.4 – 98.2	15
Pantothenic acid (ppm)	23.9 ± 3.73	17.4 – 29.8	15
Pyridoxine (ppm)[b]	9.21 ± 2.20	6.4 – 13.7	15
Folic acid (ppm)	1.75 ± 0.54	1.20 – 3.27	15
Biotin (ppm)	0.332 ± 0.12	0.225 – 0.704	15
Vitamin B_{12} (ppb)	60.5 ± 46.5	18.3 – 174.0	15
Choline (ppm)[b]	3,064 ± 270	2,700 – 3,790	15
Minerals			
Calcium (%)	0.978 ± 0.049	0.873 – 1.050	24
Phosphorus (%)	0.594 ± 0.026	0.549 – 0.641	24
Potassium (%)	0.665 ± 0.023	0.626 – 0.694	15
Chloride (%)	0.376 ± 0.041	0.300 – 0.474	15
Sodium (%)	0.191 ± 0.017	0.160 – 0.222	15
Magnesium (%)	0.201 ± 0.009	0.185 – 0.217	15
Sulfur (%)	0.170 ± 0.029	0.116 – 0.209	15
Iron (ppm)	182 ± 46.7	135 – 311	15
Manganese (ppm)	54.1 ± 7.89	42.1 – 73.1	15
Zinc (ppm)	55.0 ± 9.55	43.3 – 78.5	15
Copper (ppm)	6.65 ± 1.790	3.21 – 10.50	15
Iodine (ppm)	0.512 ± 0.221	0.233 – 0.972	15
Chromium (ppm)	0.604 ± 0.253	0.330 – 1.380	14
Cobalt (ppm)	0.25 ± 0.074	0.20 – 0.47	14

[a] From formulation
[b] As hydrochloride (thiamine and pyridoxine) or chloride (choline)

TABLE J4
Contaminant Levels in NTP-2000 Rat and Mouse Ration[a]

	Mean ± Standard Deviation[b]	Range	Number of Samples
Contaminants			
Arsenic (ppm)	0.40 ± 0.150	0.14 – 0.50	24
Cadmium (ppm)	0.06 ± 0.022	0.04 – 0.10	24
Lead (ppm)	0.07 ± 0.029	0.05 – 0.17	24
Mercury (ppm)	<0.02		24
Selenium (ppm)	0.19 ± 0.029	0.14 – 0.23	24
Aflatoxins (ppb)	<5.00		24
Nitrate nitrogen (ppm)[c]	14.8 ± 3.54	10.0 – 23.2	24
Nitrite nitrogen (ppm)[c]	<0.61		24
BHA (ppm)[d]	<1.0		24
BHT (ppm)[d]	<1.0		24
Aerobic plate count (CFU/g)	27 ± 71	10 – 360	24
Coliform (MPN/g)	3.0 ± 0.1	3.0 – 3.0	24
Escherichia coli (MPN/g)	<10		24
Salmonella (MPN/g)	Negative		24
Total nitrosoamines (ppb)[e]	4.0 ± 1.68	2.3 – 8.4	24
N-Nitrosodimethylamine (ppb)[e]	2.5 ± 1.52	1.1 – 6.9	24
N-Nitrosopyrrolidine (ppb)[e]	1.5 ± 0.55	0.9 – 4.1	24
Pesticides (ppm)			
α-BHC	<0.01		24
β-BHC	<0.02		24
γ-BHC	<0.01		24
δ-BHC	<0.01		24
Heptachlor	<0.01		24
Aldrin	<0.01		24
Heptachlor epoxide	<0.01		24
DDE	<0.01		24
DDD	<0.01		24
DDT	<0.01		24
HCB	<0.01		24
Mirex	<0.01		24
Methoxychlor	<0.05		24
Dieldrin	<0.01		24
Endrin	<0.01		24
Telodrin	<0.01		24
Chlordane	<0.05		24
Toxaphene	<0.10		24
Estimated PCBs	<0.20		24
Ronnel	<0.01		24
Ethion	<0.02		24
Trithion	<0.05		24
Diazinon	<0.10		24
Methyl chlorpyrifos	0.096 ± 0.066	0.020 – 0.259	24
Methyl parathion	<0.02		24
Ethyl parathion	<0.02		24
Malathion	0.316 ± 0.483	0.020 – 1.850	24
Endosulfan I	<0.01		24
Endosulfan II	<0.01		24
Endosulfan sulfate	<0.03		24

[a] All samples were irradiated. CFU=colony-forming units; MPN=most probable number; BHC=hexachlorocyclohexane or benzene hexachloride
[b] For values less than the limit of detection, the detection limit is given as the mean.
[c] Sources of contamination: alfalfa, grains, and fish meal
[d] Sources of contamination: soy oil and fish meal
[e] All values were corrected for percent recovery.

APPENDIX K
SENTINEL ANIMAL PROGRAM

METHODS K-2
RESULTS K-3

SENTINEL ANIMAL PROGRAM

METHODS

Rodents used in the Carcinogenesis Program of the National Toxicology Program are produced in optimally clean facilities to eliminate potential pathogens that may affect study results. The Sentinel Animal Program is part of the periodic monitoring of animal health that occurs during the toxicologic evaluation of chemical compounds. Under this program, the disease state of the rodents is monitored via serology on sera from extra (sentinel) animals in the study rooms. These animals and the study animals are subject to identical environmental conditions. The sentinel animals come from the same production source and weanling groups as the animals used for the studies of chemical compounds.

In the 3-month studies, serum samples were collected from five male and five female sentinel rats and mice at approximately 4 weeks after start of dosing and at study termination. In the 2-year studies, serum samples were collected from up to five male and five female sentinel rats and mice at approximately 1, 6, 12, and 18 months and from 0.5 g/kg male rats and 1 g/kg female rats and male and female mice at the end of the 2-year studies. Blood from each animal was collected and allowed to clot, and the serum was separated. The samples were processed appropriately and sent to BioReliance (Rockville, MD) for determination of antibody titers. At 18 months, fecal samples were collected from sentinel mice and tested for for *Helicobacter hepaticus*. The laboratory methods and viral agents for which testing was performed are tabulated below along with the times at which the samples were collected during the studies.

Method and Test	Time of Analysis
RATS	
3-Month Study	
ELISA	
Mycoplasma arthritidis	Study termination
Mycoplasma pulmonis	Study termination
PVM (pneumonia virus of mice)	1 month, study termination
RCV/SDA (rat coronavirus/sialodacryoadenitis virus)	1 month, study termination
Sendai	1 month, study termination
Immunofluorescence Assay	
Parvovirus	1 month, study termination
2-Year Study	
ELISA	
M. arthritidis	Study termination
M. pulmonis	Study termination
PVM	1, 6, 12, and 18 months, study termination
RCV/SDA	1, 6, 12, and 18 months, study termination
Sendai	1, 6, 12, and 18 months, study termination
Immunofluorescence Assay	
Parvovirus	1, 6, 12, and 18 months, study termination

Mice

3-Month Study

ELISA

Ectromelia virus	1 month, study termination
EDIM (epizootic diarrhea of infant mice)	1 month, study termination
GDVII (mouse encephalomyelitis virus)	1 month, study termination
LCM (lymphocytic choriomeningitis virus)	1 month, study termination
Mouse adenovirus-FL	1 month, study termination
MCMV (mouse cytomegalovirus)	Study termination
MHV (mouse hepatitis virus)	1 month, study termination
M. arthritidis	Study termination
M. pulmonis	Study termination
PVM (pneumonia virus of mice)	1 month, study termination
Reovirus 3	1 month, study termination
Sendai	1 month, study termination

Immunofluorescence Assay

Parvovirus	1 month, study termination

2-Year Study

ELISA

Ectromelia virus	1, 6, 12, and 18 months, study termination
EDIM	1, 6, 12, and 18 months, study termination
GDVII	1, 6, 12, and 18 months, study termination
LCM	1, 6, 12, and 18 months, study termination
Mouse adenovirus-FL	1, 6, 12, and 18 months, study termination
MCMV	Study termination
MHV	1, 6, 12, and 18 months, study termination
M. arthritidis	Study termination
M. pulmonis	Study termination
PVM	1, 6, 12, and 18 months, study termination
Reovirus 3	1, 6, 12, and 18 months, study termination
Sendai	1, 6, 12, and 18 months, study termination

Immunofluorescence Assay

Parvovirus	1, 6, 12, and 18 months, study termination

Polymerase Chain Reaction

Helicobacter spp.	18 months

Results

All test results were negative.