β-Myrcene: Implications for Classification* of this Nongenotoxic Carcinogen

* Not limited to GHS classification as CMR

Susan Felter, Ph.D.
Procter & Gamble, Corporate Human Safety

September 8, 2019
ECETOC Workshop (satellite to Eurotox 2019)
Helsinki, Finland
β-Myrcene: Naturally occurring flavor chemical

• Naturally occurring monoterpene found in > 200 plant species

• Given GRAS (Generally Recognized as Safe) status by the Flavor Extract Manufacturers Association (FEMA) in 1965 and approved as a food additive by the US FDA

• Approved for use as an artificial flavor substance by the European Council in 1974
  • Included in the list of approved flavor substances in the European Union (Regulation No. 872/2012)
β-Myrcene: Robust Safety Evaluations

• Assessments by EFSA (2015), JECFA (2015) confirm no safety concerns

• June 21, 2018: FDA conducted updated review of the safety, including the carcinogenicity potential of myrcene as a flavoring substance in food
  • Concluded no safety concerns

• And yet – in October 2018, FDA revoked the allowance for using β-myrcene as a flavor substance in the U.S.
October 2018: FDA Removes 7 Synthetic Flavoring Substances from Food Additives List

• Used to flavor/enhance flavor in baked goods, ice cream, candy, beverages, and chewing gum. Six have tested positive in rodent cancer bioassays:
  • Benzophenone (rose-like, aromatic scent; found in grapes, black tea, papaya)
  • Ethyl acrylate (pineapple, passion fruit; little use as a flavor)
  • Eugenyl methyl ether (clove/cinnamon; found in basil, Kogyoku apples)
  • β-Myrcene (citrus; hops) -- permitted as a food additive in the U.S. since 1965
  • Pulegone (mint)
  • Pyridine (found in cooked bacon, fried chicken. When used in small quantities as an artificial food additive, it imparts a slightly bitter flavor.)
Reason for U.S. FDA’s Action: The Delaney Clause

• FDA action comes in response to legal action brought by the NRDC (Natural Resources Defense Council) and a coalition of health, consumer and environmental groups

• Under the U.S. Food, Drug, and Cosmetic Act's Delaney Clause, enacted in 1958, the FDA cannot allow the legal use of any food additive found to induce cancer in humans or animals at any dose.
  • Human relevance is also not considered
  • Amended in 1996 to accept a practical de minimus excess cancer risk of 1 x 10^{-6} for pesticides
NTP (2010) Cancer Bioassay

• Study Design
  • Gavage administration in corn oil
  • F344/N Rats and B6C3F1 Mice, 50/sex/group
  • Dose levels: 0, 250, 500 and 1000 mg/kg (5 d/wk)

• Results:
  • Male Rats: Clear Evidence (kidney)
  • Female Rats: Equivocal Evidence (kidney)
  • Male Mice: Clear Evidence (liver)
  • Female Mice: Equivocal Evidence (liver)
Genotoxicity Studies: *Uniformly Negative*

Table 1 from US FDA Toxicology Evaluation of Synthetic Myrcene Potential as a Carcinogen
Tumor Dose-Response – a (rodent) carcinogen

**Male Mouse Liver**

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>0</th>
<th>250</th>
<th>500</th>
</tr>
</thead>
<tbody>
<tr>
<td>Renal tubule adenoma or carcinoma*</td>
<td>0/50</td>
<td>14/50</td>
<td>13/50</td>
</tr>
</tbody>
</table>

**Male Rat Kidney**

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>0</th>
<th>250</th>
<th>500</th>
</tr>
</thead>
<tbody>
<tr>
<td>Renal tubule adenoma or carcinoma*</td>
<td>0/50</td>
<td>14/50</td>
<td>13/50</td>
</tr>
</tbody>
</table>

“Clear Evidence” in male mice and male rats

Note:
Highest dose (1000 mg/kg) exceeded the MTD in both species.

All of the male rats and most of the male and female mice receiving 1000 mg/kg β-myrcene died before the end of the study.

* Standard + extended evaluation
Regulatory Classifications of $\beta$-Myrcene

- Listed on California Proposition 65 as a carcinogen (March 2015) based on results of the NTP bioassay
- IARC: Classified in Group 2B, “possible human carcinogen” (June 2017)
  - Based on “Sufficient evidence” in animals
- $\beta$-Myrcene is not listed in the NTP’s Report on Carcinogens, or regulated as a carcinogen by any other agency

**CMR Classifications (GHS)**
- **Category 1A**: Known human carcinogen (H340) based on human evidence
- **Category 1B**: Presumed human carcinogen (H340) based on animal studies;
- **Category 2**: Suspected carcinogen (H341) based on limited evidence from animal studies or/and human.

So... is there a real cancer concern for $\beta$-myrcene used as a flavor chemical?
β-Myrcene: A Naturally Occurring Monoterpene

• Over 200 varieties of plants, including basil, hops, lemongrass, rosemary, tobacco and thyme
• Fruits and vegetables, including mangoes, pomegranates, carrots and citruses, in juice and oil form.
• Lemongrass tea
• Beer (from hops)

* * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * *

• Used as a (“artificial”) flavor chemical in beverages, ice cream, candy and baked goods.
Hops

- > 250 essential oils have been identified in hops.
- Myrcene, humulene and caryophyllene are the main oils found in the highest concentrations.
Dietary Exposure to β-Myrcene

**FDA (2018)**: EDI (estimated daily intake) of myrcene as a synthetic flavoring substance is 74 ug/day (1.23 ug/kg/day for 60 kg person)

**EFSA (2015)**: MSDI (Maximized Survey-Derived Daily Intake)
  - MSDI-EU = 290 ug/d (4.8 ug/kg/d for 60 kg adult)
  - MSDI-USA = 153 ug/d (2.6 ug/kg/d for 60 kg adult)

**FEMA (2011)**: Daily per capita intake (eaters only) for β-myrcene estimated to be 164 ug, corresponding to 3 ug/kg bw (Adams et al., 2011)

- **Overall, range ~ 1.2 – 4.8 ug/kg/d**
- Adams et al (2011) calculated a consumption ratio of 50 for β-myrcene
  - Consumption ratio: (annual consumption via food, kg)/(most recent reported volume as a flavoring substance, kg);

---

**FDA**: US Food and Drug Administration  
**EFSA**: European Food Safety Authority  
**FEMA**: Flavor and Extract Manufacturers Association
Safety Database: Key Studies

• NTP Bioassay
  • 2 Years, rats and mice
  • Gavage administration
  • Increased tumor incidence at all doses
  • *Lowest dose: 250 mg/kg/day (LOAEL)*

• Bastaki et al., 2018 (Bauter et al., 2013 [unpub’d report])
  • 90 day, rats
  • Diet administration
  • Males: 0, 8, 40 or 44 mg/kg/day
  • Females: 0, 9.6, 48 or 53 mg/kg/day

Human Consumption ~ 1 – 5 ug/kg/day
- lowest rodent dose is five orders of magnitude higher than human exposures

Lowest dose is still ~ 3000-fold higher than human exposures
Bastaki et al., 2018
(unpub’d report: Bauter et al., 2013)

- OECD 408 compliant 90-day dietary study with β-myrcene
- Sprague-Dawley rats (10/sex/group)
- Dietary exposure:
  M: 0, 8, 40 or 44 mg/kg/day
  F: 0, 9.6, 48 or 53 mg/kg/day

- No mortalities, clinical pathological findings, changes in macroscopic or microscopic histopathology, or organ weight changes in any treated groups.

Highest dose was a free-standing NOAEL
## Safety Assessments

<table>
<thead>
<tr>
<th>Endpoint</th>
<th>Point of Departure (mg/kg/day)</th>
<th>Human Exposure (ug/kg/day)</th>
<th>MOE*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>US FDA (2017)</strong></td>
<td>Kidney tumors (male rats; NTP bioassay) BMDL&lt;sub&gt;10&lt;/sub&gt; = 90 mg/kg/day Dosing 5 days/week</td>
<td>64 mg/kg/day (BMDL&lt;sub&gt;10&lt;/sub&gt;)</td>
<td>1.23 ug/kg/day</td>
</tr>
<tr>
<td><strong>JECFA (2004)</strong> Joint FAO/WHO Expert Committee on Food Additives</td>
<td>LOAEL for kidney effects (male rats; NTP bioassay) LOAEL = 250 mg/kg/day x 5 d/wk</td>
<td>180 mg/kg/day (LOAEL)</td>
<td>3 ug/kg/day (FEMA)</td>
</tr>
<tr>
<td><strong>JECFA (2015)</strong></td>
<td>NOEL = 115 mg/kg/day (Bauter et al., 2013 [Batista et al., 2018]) <strong>Free standing NOAEL</strong></td>
<td>115 mg/kg/day (NOEL)</td>
<td>3 ug/kg/day (FEMA)</td>
</tr>
<tr>
<td><strong>EFSA (2015)</strong> European Food Safety Authority</td>
<td>NOAEL = 44 mg/kg/day (Bauter et al., 2013 [Batista et al., 2018]) <strong>Free standing NOAEL</strong></td>
<td>44 mg/kg/day (NOEL)</td>
<td>4.8 ug/kg/day</td>
</tr>
</tbody>
</table>

*The term MOS (Margin of Safety) is generally used in Europe*
• “Although we are amending our food additive regulations for these synthetic flavoring substances in accordance with the Delaney Clause, the FDA’s rigorous scientific analysis has determined that they do not pose a risk to public health under the conditions of their intended use.” …

• “Each of these synthetic substances has a natural counterpart in food or in natural substances used to flavor foods. The FDA’s revocation of the listings … does not affect the legal status of foods containing their natural counterparts or of flavoring substances extracted from such food, often labeled as “natural flavors.”
The Big Picture

• Myrcene is not genotoxic, acutely toxic, or toxic to reproduction/development

• Myrcene caused tumors in male rats and mice following gavage administration at very high doses for 2 years
  • Lowest dose (250 mkd) in rodents was ~ 100,000-fold higher than typical human intake

• 90 day dietary study showed no effects at doses up to 44 mkd (free-standing NOAEL)
  • This is still ~ 10,000 fold higher than human intake
Beyond the FDA ban

- Erosion of trust in regulatory agencies (and industry)
- Implications for food manufacturers; cost to society
- Implications for consumer perception of safety (misperception of risk)
  - Contributes to escalating chemophobia in all areas of life

- Where do we go from here?
- How do we get there?
So... is it time to banish the Delaney Clause and other legislation based on classification alone?
And while we’re working toward this...

• How can we as scientists move toxicology and risk assessment out of the 1950-1970s and into the 2020s?

• Can we (and should we) mandate a change to testing protocols and/or study interpretations that requires consideration of human relevance, including consideration of exposure (and potential threshold)?

• Can we (and should we) effect a change to “Hazard ID” and classification of non-genotoxic carcinogens such that it must be in the context of human exposure?

• How do we better inform consumers of what are real vs perceived risks?
What does it mean to be (classified as) a carcinogen?

• For substances classified as carcinogens, many laws state that the substance cannot be used for certain applications, that human exposure must be minimized to the greatest extent possible, etc.

• What does “classified” mean?
  IARC ≠ GHS ≠ EPA ≠ Proposition 65 ≠ German MAK ≠ NTP Rep on Carcinogens...

• What does this mean for:
  • Vitamin A (CARET Trial [Carotene and Retinol Efficacy Trial])
  • Bacon
  • Antioxidants (pro-oxidants at high doses – e.g., BHA)
  • Ethanol
  • β-Myrcene

• How do we explain to the public what constitutes a real risk when the focus is often on classification alone?
Commentary

Classification schemes for carcinogenicity based on hazard-identification have become outmoded and serve neither science nor society

Alan R. Boobis a, Samuel M. Cohen b, Vicki L. Dellarco c, John E. Doe d, *, Penelope A. Fenner-Crisp e, Angelo Moretto f, Timothy P. Pastoor g, Rita S. Schoeny h, Jennifer G. Seed i, Douglas C. Wolf j

a Centre for Pharmacology & Therapeutics, Toxicology Unit, Department of Medicine, Hammersmith Campus, Imperial College London, London, W12 0NN, UK
b Department of Pathology and Microbiology, Hawlik-Wall Professor of Oncology, University of Nebraska Medical Center, Omaha, NE 68198-3135, USA
c Independent Consultant, Silver Spring, MD 20911, USA
d Parker Doe LLP, Carpenter Court, Maple Road, Bramhall, Stockport, Cheshire SK7 2DH, UK
e Independent Consultant, North Garden, VA 22959, USA
f Dipartimento di Scienze Biochimiche e Cliniche (Department of Biomedical and Clinical Sciences), Università degli Studi di Milano, Milan, Italy
g PASCO Science Communication, LLC, Greensboro, NC 27453, USA
h Rita Schoeny LLC, Washington DC, 20002, USA
i Independent Consultant, Alexandria, VA 22301, USA
j Syngenta Crop Protection, LLC, Greensboro, NC 27419, USA
Commentary

Chemical carcinogenicity revisited 2: Current knowledge of carcinogenesis shows that categorization as a carcinogen or non-carcinogen is not scientifically credible

John E. Doe\textsuperscript{a,}\textdagger, Alan R. Boobis\textsuperscript{b}, Vicki Dellarco\textsuperscript{c}, Penelope A. Fenner-Crisp\textsuperscript{d}, Angelo Moretto\textsuperscript{e}, Timothy P. Pastoor\textsuperscript{f}, Rita S. Schoeny\textsuperscript{g}, Jennifer G. Seed\textsuperscript{h}, Douglas C. Wolf\textsuperscript{i}

\textsuperscript{a}Furker Doe LLP, Carpenter Court, Maple Road, Bramhall, Stockport, Cheshire, SK7 2DH, UK
\textsuperscript{b}Centre for Pharmacology & Therapeutics, Toxicology Unit, Department of Medicine, Hammersmith Campus, Imperial College London, London, W12 0NN, UK
\textsuperscript{c}Independent Consultant, Silver Spring, MD, 20901, USA
\textsuperscript{d}Independent Consultant, North Garden, VA, 22959, USA
\textsuperscript{e}Dipartimento di Scienze Biochimiche e Cliniche (Department of Biomedical and Clinical Sciences), Università degli Studi di Milano, Milan, Italy
\textsuperscript{f}Pastoor Science Communication, LLC, Greensboro, NC, 27455, USA
\textsuperscript{g}Rita Schoeny LLC, Washington DC, 20002, USA
\textsuperscript{h}Independent Consultant, Alexandria, VA, 22301, USA
\textsuperscript{i}Syngenta Crop Protection, LLC, Greensboro, NC, 27419, USA