UNIVERSITY OF
FLORIDA

Institute of Food and Agricultural Sciences
Citrus Research and Education Center

700 Experiment Station Road
Lake Alfred FL 33850-2299
Tel. (863) 956-1151
Fax (863) 956-4631

August 18, 2004

Ms Dorothy Shimer, *ab1173@listserv.arb.ca.gov*
Research Division
Air Resources Board
P.O. Box 2815
Sacramento, CA 95812

Re: Comment on Draft Report: **Terpene sections/Indoor Air Pollution in California**

Dear Ms Shimer:

I offer the following discussion concerning terpenes (esp. d-limonene) from citrus oils, related to content in your above report. More specifically, parts of the report deal with the following: 1) suggesting limonene or oxygenated terpenes be considered in the same mammalian toxicity and irritant discussions as formaldehyde 2) discussion of oxidation by O_2 and O_3 3) its status as a VOC and 4) attempts to suggest mitigation by removing terpenes from products. These discussions suggest to me that the CARB has not adequately presented both sides of the issues in their report. In the following comments, I would present some unbiased general comments regarding the previous items.

Health and toxicity of d-limonene

There has been some concern about human exposure and potential hazard at the manufacturing source, in transit or at the use location, as well as environmental harm from d-limonene. However, general biological data of d-limonene describe it as GRAS by the FDA (21 CFR § 182.60) for use in human foods. For rats, mice and rabbits, *it has low toxicity* (acute oral and dermal LD_{50} = 5 g/kg). It irritates human skin on contact, but any dermatitis may be due to contaminants or oxygenated products. The EPA has granted a residue tolerance exemption for d-limonene used as an inert ingredient (e.g. as a solvent or fragrance) in pesticide formulations applied pre- or post-harvest to crops (Federal Register, 1994).

A two-year study of toxic effects of d-limonene reported liver effects at doses of >500 mg/kg body weight in strains of male mice and reduced survival in female rats at >600 mg/kg. In male rat kidney only, there was dose-related evidence of carcinogenic activity (NTP, 1990). However, there were *no observed effects at 250 and 300 mg/kg*, respectively, for the liver studies. Other studies reported that oral doses of d-limonene (100 – 1000 mg/kg) affected kidney weight, but *no observed microscopic effects* in dogs (Webb et al., 1988). Based on the level of *no observed effects*, the EPA concluded that a tolerance was unnecessary to protect the public health for the inert ingredient application mentioned above. Also, in establishing the exemption rule, the EPA considered that the American Industrial Hygiene Association's Workplace Environmental Exposure Level (30 ppm, 8 hr) for d-limonene was also based on the *no observed effect level*.

There is some evidence that *dietary d-limonene may afford protection from chemical tumor formation*. Certain biochemical pathways in tumor tissues may be sensitive to the inhibitory action of d-limonene and certain terpene constituents of essential oils (Elson and Yu, 1994). Mammary tumor development was repressed in rats fed d-limonene during experiments to chemically induce tumors (Crowell et al., 1991). One might conclude that d-limonene is low to moderately toxic to humans, is safe for some applications (food) and requires further study for other applications, because of conflicting or limited data.

Environment and biodegradation

It is probably true that *all green plants in nature produce some limonene through their biochemical metabolism*. The molecular structure and chemical reactivity of d-limonene implies that this chemical should degrade in the environment. Even chlorinated hydrocarbons can be degraded in certain microbial pathways. The specifics of degradation in a natural environment are not well defined in situations of a large d-limonene or terpene spill, or disposal of excessive amounts to a sewage or waste treatment system. A label claim for biodegradability might not be so apparent, without definition of specific conditions.

Inherent biodegradation of organic substances can be tested (40 CFR § 796.334 Ch. 1, 7-1-92). Under this test, a substance with greater than 70% loss of dissolved carbon is evidence of ultimate biodegradability. The time period may be several months for some compounds. A number of limitations applying this test to d-limonene should be considered, e.g. water solubility, vapor pressure and inhibition of certain degradative microorganisms. As described, the test is applicable to water soluble, non-volatile organic chemicals that do not inhibit bacteria at test concentrations. d-Limonene has limited water solubility (13.8 mg/L @ 25 °C), is volatile and can inhibit certain bacteria. For these reasons, conditions of d-limonene biodegradability in the environment need to be established.

VOCs and reactivity

The recent use of d-limonene as a cleaning solvent replacing chlorinated hydrocarbons has expanded its use range and suggested a need for VOC and environmental data. Consequently, there have been experiments to gather data. One study reported the VOCs in maintenance shops using d-limonene as a degreaser. Depending on the location and type of application, VOC concentration of d-limonene *varied widely* from 0.5 to >635 mg/m^3 of air sampled (NIOSH, 1993).

The interest in d-limonene as an industrial VOC stems from the fact that certain chemicals deplete ozone in the stratosphere. *It is not on the EPA hazardous substances list, nor is it a TAC*. d-Limonene will react with O_2, ozone and also nitrous oxide air pollutants. However, unlike chlorinated hydrocarbons, *oxidation ultimately produces CO_2, which is assimilated by plants to make sugars*, etc. (and more limonene). From a manufacturing consideration, green plants (especially citrus and pine trees) produce far more environmental VOCs than man's processes. There is also evidence that singlet oxygen, a component in photochemical air pollution, can react with d-limonene (Rawls and Estes, 1978). Partly because of this reactivity, d-limonene has little potential to reach the stratosphere, where it can deplete ozone, contributing to global warming.

In considering d-limonene and other terpenes as VOCs, there is a problem of determining quantitative limits for setting regulations. It has been reported that the atmosphere contains 720 billion tons of carbon, 100 billion tons of which is in CO_2 pools, which move in and out of plant life. In this scheme, plant terpenes recycle in the carbon pool between 480 – 350 million tons/yr, with an average half-life of an hour (Keyser, 1991). *The average atmospheric concentration is estimated to vary regionally from 0.5 – 42 ppb*. For manufacturing comparison, the US naval stores industry recovers about 400,000 tons of terpenes annually, while annual d-limonene recovery in Florida amounts to only 14,000 tons (Florida Citrus Processors' Assocn, 2004; www.fcplanet.org). Recovery processes, chemical and physical properties and utilization of d-limonene and other essential oils from citrus have been documented (Braddock, 1999).

In considering reactivity of d-limonene and other terpenes with air, ozone and other chemicals, there are many published sources. One source specific to d-limonene, from the flavor and fragrance industry, provides information about most of the chemical reactions of limonene, including potential oxidation reactions (Thomas and Bessiere, 1989). Of particular comment in the CARB report was a misleading comment, suggesting that ozone oxidation of d-limonene produced formaldehyde. While formaldehyde may be a fraction of the oxidation reaction products, *limonene and terpenes react very rapidly with ozone, with ozone not limited, ultimately oxidizing to carbon dioxide*.

One might conclude from your report that no one should live in a house built of pine or cedar, or within a forest, wear perfume indoors, etc. What harm is done to the millions of people who drink limonene and terpenes in their orange juice every day. An 8 oz glass of commercially pasteurized orange juice contains about 0.1 mL limonene (150-250 ppm), and fresh-squeezed juice (reamed in your kitchen) has twice that amount. Even juice for infants has 100 ppm limonene, while many carbonated beverages (colas) contain >150 ppm.

Finally, suggesting that terpene removal from furniture polish, air fresheners, detergents, cleaners, etc. to mitigate the perceived problem is naïve. Pure lemon oil and pine oil have been in use for over a century for polishing fine furniture as well as for perfume ingredients in polishes and cleaners. Consider also banning orange blossoms, lilac or lavender, or roses (at least, don't bring them indoors). To deny consumers these innocuous products, based on limited published studies would be a great disservice, not to mention potential economic harm to manufacturers of the raw materials and products, some who are California businesses.

Sincerely,

Robert J Braddock rjbk@ufl.edu
Professor

References

Braddock, R. J. 1999. Handbook of Citrus By-Products and Processing Technology. John Wiley & Sons, Inc. New York, NY. 247 pp.

Crowell, P.L., Chang, R.R., Ren, Z., Elson, C.E. and Gould, M.N. 1991. Selective inhibition of isoprenylation of 21 – 26 kDa proteins by the anticarcinogen d-limonene and its metabolites. J. Biol. Chem. 266(26): 17679 – 17685.

Elson, C.E. and Yu, S.G. 1994. The chemoprevention of cancer by mevalonate-derived constituents of fruits and vegetables. J. Nutr. 124(5): 607 – 614.

Federal Register. 1994. d-Limonene. Tolerance exemption. 40 CFR Part 180. Vol. 59, No. 89. May 10. P. 24055.

Keyser. 1991. Terpene solvents from forest products. Naval Stores Review. Jan./Feb. pp. 10 – 12.

NIOSH. 1993. Hazard evaluation and technical assistance report no. 92-0101-2341. US Dept. Health & Human Services. Public Health Service. Region IV. Atlanta, GA. August. 29 pp.

NTP. 1990. Toxicology and carcinogenesis studies of d-limonene. US Dept. Health & Human Services. Public Health Service. NIH Pub. No. 90-2802. NTP Technical Report No. 347. Research Triangle Park, NC. 165 pp.

Rawls, H.L. and Estes, F.L. 1978. A preliminary investigation of the use of limonene for detecting singlet oxygen as a component of polluted air. Photochem. and Photobiol. 28: 465 - 468.

Thomas, A.F. and Bessière, Y. 1989. Limonene. Natural Products Reports. 6(3): 291 – 309.

Webb, D.R., Hysell, D.K. and Alden, C.L. 1988. Assessment of the chronic oral toxicity of d-limonene in dogs. Abstr. 356. The Toxicologist. 8(1): 90.