Final Report on the Safety Assessment of Azulene[1]

Azulene is an extract from the volatile oil of several perennial herbs and is detected in tobacco smoke. It functions as a skin conditioning agent in cosmetic formulations, including hair dyes. Azulene is reported to be used in a wide range of cosmetic formulations, but these reported uses are likely to be uses of guaiazulene, a chemically related colorant, because there are currently no suppliers of Azulene to the cosmetics industry. The anti-inflammatory action of Azulene has been demonstrated in several animal studies. Effects at the cellular level are reported to include inhibition of respiration and growth, but no effect on ciliary activity or membrane permeability. Relatively low oral toxicity was seen in acute animal studies. Azulene was not mutagenic in an Ames test, with and without metabolic activation. An allergic response to Azulene was noted in one case report. These data were clearly insufficient to support the safety of Azulene in cosmetics. Additional data needed to make a safety assessment include: methods of manufacture and impurities, especially naphthalenes; current concentration of use; skin penetration, if there is significant skin penetration, then both a 28-day dermal toxicity study to assess general skin and systemic toxicity and a reproductive and developmental toxicity study are needed; one genotoxicity study in a mammalian system, if positive, then a 2-year dermal carcinogenesis study using National Toxicology Program methods is needed; skin irritation and sensitization in animals or humans; and ocular toxicity.

INTRODUCTION

Azulene functions as a skin conditioning agent in cosmetic formulations. Although Azulene has an intense blue or blue-violet color, its use as a colorant in the United States has not been approved by the Food and Drug Administration (FDA). Guaiazulene (1,4-dimethyl-7-isopropylazulene), an azulene derivative, meets U.S. color additive standards and is permitted for use as a colorant in externally applied cosmetics (Code of Federal Regulations §73.2180). Guiazulene is sometimes mistakenly termed Azulene. The true Azulene is an extract from the volatile oil of several perennial herbs such as Yarrow (*Achillea millefolium*), Peppermint (*Mentha X piperita* L.), Elecampane (*Inula helenium* L.), and Calamus (*Acorus calamus*) (Leung 1980). This report reviews the published safety data on Azulene.

Received 24 April 1999; accepted 2 July 1999.

[1]Reviewed by the Cosmetic Ingredient Review Expert Panel. Dawn R. Teufel, former Scientific Analyst and Writer, prepared this report.

Address correspondence to Dr. F. Alan Andersen, Director, CIR, 1101 17th Street, NW, Suite 310, Washington, DC 20036, USA.

International Journal of Toxicology, 18(Suppl. 3):27–32, 1999

1091-5818/99 $12.00 + .00

CHEMISTRY

Definition and Structure

Azulene (CAS No. 275-51-4) conforms to the formula shown in Figure 1 (Gordon 1952; Wenninger and McEwen 1997). Other names for this chemical include: Cyclopentacycloheptene, Bicyclo(5.3.0)decapentaene, Bicyclo(0.3.5)deca-1,3,5,7,9-pentaene, and Bicyclo(5.3.0)deca-2,4,6,8,10-pentaene (Registry of Toxic Effects of Chemical Substances [RTECS] 1994).

Physical and Chemical Properties

The most striking property of azulene is its intense blue or blue-violet color, noticeable even at high dilutions. Azulene and its derivatives are soluble in concentrated mineral acids. They can also be reprecipitated unchanged by dilution with water, allowing for the effective separation from nearly all contaminants (Gordon 1952). The physical and chemical properties of azulene are summarized in Table 1.

Manufacture and Production

Azulene is extracted from plants through distillation followed by chromatography (Rao and Nigam 1974). Azulene can also be prepared from certain volatile oils such as oil of cubebs (Taylor 1988), as well as octahydronaphthalene or indan (Budavari 1989). Small amounts of Azulene have been obtained by the dry distillation of calcium adipate and by the dehydrogenation of caucal oil. A historical method of synthesizing Azulene is to react indan with diazoacetic acid in a ring-expansion procedure. The reagent action on the various Kekulé forms leads to the Azulene end-product (Gordon 1952).

Analytical Methods

Azulene is identified through gas-liquid chromatography, thin layer chromatography, gas chromatography (Adams, Van Engelen, and Thomas 1984), gas chromatography/mass spectroscopy (Hawley-Fedder, Parsons, and Karasek 1984), ultraviolet (UV) absorbance, fluorescence, and column chromatography. Infrared, nuclear magnetic resonance (NMR), and mass spectroscopy data are also available (Grasselli 1973).

Ultraviolet Absorbance

Azulene has absorption maxima at 665, 659, 632, 579, 340, and 274 nm (Grasselli 1973). In other reports, the absorption maxima and minima were at 353, 340, 326, 296, and (the maximum) 272 mμ (Susz, Pfao, and Plattner 1937); and 238, 273

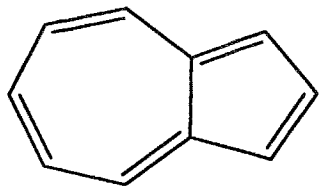

FIGURE 1
Chemical formula for Azulene.

(maximum), 279, 295, 326, 336, 341, 351, and 359 (minimum) (Plattner and Heilbronner 1948).

Reactivity

Azulene is isomeric with naphthalene. One study determined that azulene reacts more like an alkene than a polycyclic aromatic hydrocarbon in gas-phase reactions with O_3 and the OH and NO_3 radicals (Atkinson, Arey, and Aschmann 1992). At 350–430°C the Azulene rearranges to form naphthalene, which was also obtained when azulenes were heated over silica gel at 300°C in a vacuum (Gordon 1952).

Azulene has a significant dipole moment and is prone to electrophilic attack at carbons 1 and 3 (Hanzlik and Bhatia 1981), whereas nucleophilic and radical substitution can occur at position 4 (Gordon 1952).

Reduction of Azulene with sodium in amyl alcohol or sodium amalgam resulted in a hydrocarbon that was isomeric with the sesquiterpene fractions of plant oils from which Azulene was obtained. This hydrocarbon reacted with sulfur at 180°C to give a blue distillate that was identical to the starting compound Gordon 1952).

TABLE 1
Physical and chemical properties of Azulene

Property	Description	Reference
Molecular formula	$C_{10}H_8$	Budavari 1989
Molecular weight	128.16	Budavari 1989
	128.17	
	128.19	Grasselli 1973
Color	Blue leaflets crystallized from alcohol	Budavari 1989
	Violet in solution	Gordon 1952
Odor	Similar to Naphthalene	Budavari 1989
Melting point	98.5–99°C	Budavari 1989
	99–100.5°C	Grasselli 1973
Boiling point	242°C (normal)	Lewis 1993
	115–35°C (10 mm Hg)	Grasselli 1973
Decomposition	270°C	Grasselli 1973
Solubility	Soluble in ethyl alcohol, diethyl ether, acetone, and other common organic solvents	Grasselli 1973
	Soluble in concentrated mineral acids with decomposition	Gordon 1952
	Insoluble in water	Budavari 1989

USE

Cosmetic

Azulene functions as a skin conditioning agent in cosmetic formulations. Product formulation data submitted to the FDA in 1996 indicated that azulene was used in 43 cosmetic product formulations (Table 2) (FDA 1996). There are, however, no suppliers of Azulene to the cosmetics industry. The possibility exists, therefore, that the reports of use actually reflect guiazulene usage. Concentration of use values are no longer submitted to the FDA by the cosmetic industry (FDA 1992). However, product formulation data submitted to the FDA in 1984 stated that azulene was used at concentrations up to 1% (FDA 1984).

TABLE 2
Product formulation data for Azulene (FDA 1996)

Product category	Total no. of formulations in category	Total no. containing ingredient
Bubble baths	211	1
Eye lotion	22	1
Eye makeup remover	95	1
Other eye makeup preparation	136	2
Hair conditioners	715	1
Permanent waves	434	2
Rinses (Noncoloring)	60	1
Tonics, dressings, and other hair grooming aids	604	2
Hair dyes and colors	1612	1
Hair lighteners with color	9	1
Face powders	313	2
Foundations	355	1
Bath soaps and detergents	372	1
Shaving cream	158	1
Other shaving preparation products	63	2
Cleansing	820	3
Depilatories	53	1
Face and neck (excluding shaving)	300	4
Body and hand (excluding shaving)	1012	2
Moisturizing	942	2
Paste masks (mud packs)	300	1
Other skin care preparations	810	7
Suntan gels, creams, and liquids	196	2
Other suntan preparations	68	1
1996 total		**43**

Noncosmetic

Some analytical techniques use Azulene as a real-time performance monitoring agent in column chromatography (Nowicki 1980). Also, experimental evidence suggests that Azulene has antiulcer effects in animals (Leung 1980; Okabe et al. 1975). Azulene is classified as a nonsteroidal anti-inflammatory agent (Chemline 1994). Fractionation studies have also identified Azulene as one of the 3000 compounds in tobacco smoke (Pettersson, Curvall, and Enzell 1980).

GENERAL BIOLOGY

Metabolism and Excretion

In a study using male Sprague-Dawley rats (250–350 g, number not stated), Azulene was administered orally in corn oil (Hanzlik and Bhatia 1981). The rats were given doses ranging from 10 to 200 mg/kg for normal rats and up to 300 mg/kg in rats induced by phenobarbital (60 mg/kg i.p. once a day for 3 days). Azulene metabolism was the same in both normal and induced rats and only data for the normal rats were presented. Urine, collected during the first 24 hours, was dark blue, but at 24–48 hours very little blue color was observed.

Azulene metabolism was studied by orally dosing three rats with [^{3}H]-Azulene in corn oil at 13 mg/kg or 1 μCi/rat. During the first 24 hours 67, 77, and 83% of the administered ^{3}H were detected in the urine. The radioactive compound was isolated and identified as the sulfate conjugate of 1-hydroxyazulene (Hanzlik and Bhatia 1981).

The metabolism of [1,3-$^2H_2$]-Azulene was studied after intraperitoneal administration in corn oil. From the NMR spectrum, the C-3 deuterium was present in the metabolite, but the C-2 deuterium was not detected. This suggested that the NIH shift mechanism retention of deuterium during hydroxylation had not occurred and that if a 1,2-oxide of Azulene formed, then rearrangement with direct loss of the C-1 deuterium could have resulted (Hanzlik and Bhatia 1981).

Anti-Inflammatory Action

Eucalyptus oil Azulene (100 mg/kg) was injected intramuscularly into rabbits (number not stated) which had burns of the external ear. The first injection was within 2 hours after the burn and subsequent injections were made daily for 5 days. A reduction of inflammatory edema and blood coagulation, induced by the burn, was observed. Leukocytosis increased, but hemoglobin and erythrocyte content in the blood were unaffected (Lysenko 1967).

In another study, 1% croton oil was topically applied to the conjunctival sac of rabbits (number not stated) to induce hyperemia and edema. These signs had a peak appearance between 1 and 2 hours after the application and disappeared after 24 hours. The administration of Azulene (1–2%) delayed the onset of the croton oil–induced effects. Side effects were not mentioned in the abstract (Kimura et al. 1985).

Guillot et al. (1983) investigated the potential of 55 substances, including, Azulene, to reduce irritation. An ionic oil in water emulsion of triethanolamine-stearate (TEA-stearate) was made irritating by the addition of 0.25% croton oil and applied to both intact and scarified clipped skin of six male New Zealand white rabbits on 0.5-ml patches for 24 hours. The chemicals were applied to symmetrical areas on the flanks and back of each animal; the irritant base without the test substance served as control. The croton oil–containing emulsion produced a primary cutaneous irritation index (PII) value close to 2. The addition of 0.2% and 0.4% (*w/w*) Azulene decreased the irritancy by 0.21 and 0.42, respectively (Guillot et al. 1983).

Pharmacodynamic Effects

Azulene is one of the 3000 identified components found in tobacco smoke (Pettersson, Curvall, and Enzell 1980). Several studies have tested the effects of individual tobacco smoke constituents on various biological functions. A study by Pettersson, Curvall, and Enzell (1980) measured the effects of 320 tobacco smoke compounds on the noradrenaline-induced oxidative metabolism of brown fat cells isolated from adult hamsters (*Mesocricetus auratus*). Noradrenaline increases metabolic respiration in brown fat cells 15 times the basal rate. The cells were incubated with azulene dissolved in ethanol or dimethyl sulfoxide (controls) for 5 minutes. After this preincubation, 1 μM noradrenaline (approximately twice the dose needed to induce maximal respiratory rate) was added and cellular consumption of oxygen was recorded for 5 minutes. The excess noradrenaline prevented the inactivation of noradrenaline per se by the test substance. Substance toxicity was determined by comparing the inhibition of the noradrenaline-stimulated respiration to that of a control. Azulene (1 mM) inhibited respiration 46% in the noradrenaline-stimulated brown fat cells. Azulene was considered a low to moderate inhibitor. It was noted that in electron micrographs brown fat cells had little development of endoplasmic reticulum, in which case detoxification enzymes could have been lacking.

The ciliotoxicities of 316 compounds, including azulene, present in tobacco smoke were tested in vitro using chicken tracheal organ cultures (Pettersson, Curvall, and Enzell 1982). The tracheas of 16- to 17-day-old embryos were cut transversely into rings and incubated with 5% CO_2 and air at 37°C and 80% relative humidity. (Ciliary activity can be maintained under these conditions for more than 4 weeks.) Azulene, dissolved in ethanol or dimethyl sulfoxide, was tested at a concentration of 5 mM for, at most, 1 hour. The solvent concentration used in the experiment was not inhibitory to the cilia. The cessation of ciliary activity in the presence of the test compound within the 1-hour period was an indication of chemical toxicity. All test compounds, including Azulene, were tested three times using tracheal preparations from different embryos. Azulene ciliostasis did not occur within the 60-minute period. However, precipitation of Azulene in the test mixture was noted. The actual

concentration of dissolved Azulene after precipitation was not determined.

The effects of Azulene and 463 other tobacco smoke compounds on the percent increase in the membrane permeability of cultured human lung fibroblasts were investigated (Thelestam, Curvall, and Enzell 1980). Diploid embryonic lung fibroblasts were exposed to [^{3}H]uridine nucleotides, low-molecular-weight cytoplasmic markers. Cells were incubated for 30 minutes at 37°C at a concentration of 25 mM Azulene or other test compound. The release of the radioactive marker from the cells indicated the degree of membrane damage. To avoid cytotoxic effects and increase sensitivity, a short exposure time was chosen. Maximal radioactive release (positive control) was achieved by treating the cells with sodium borate buffer and scraping. Azulene stimulated the nucleotide release by 2% which was considered a nil effect.

The effects of approximately 250 tobacco smoke compounds were separately tested in vitro using Ascites sarcoma B P8 cells to determine the inhibition of cell multiplication (Pilotti et al. 1975). The compounds were each dissolved in ethanol (10 μl) or dimethyl sulfoxide (10 μl), added to the cell suspension, and incubated at 37°C for 48 hours. The controls were cultures treated with only solvent or cultures inoculated with chemicals producing known inhibitory effects. Azulene, tested in the cell cultures at final concentrations of 1 mM and 0.1 mM, inhibited the growth rate by 85% and 7%, respectively.

ANIMAL TOXICOLOGY

Acute Toxicity

The acute oral LD_{50}s in the rat and mouse were 4 g/kg and 3 g/kg, respectively. Experimental details were not reported. The intravenous LD_{50} in the mouse was 56 mg/kg. The subcutaneous LD_{50}s in the rat and mouse were 520 mg/kg and 145 mg/kg, respectively. The intraperitoneal LD_{50}s in the rat and mouse were 180 mg/kg and 108 mg/kg, respectively (RTECS 1994).

Chronic Toxicity

Gershbein and Benuck (1975) implanted crystallized fragments or saline suspensions of hydrocarbons, including Azulene (as the control chemical), into the brains of 70-day-old Holtzmann rats and adult BDF_1 mice in order to discern early brain changes. Crystals of each of the hydrocarbons (1,2,5,6-dibenzanthracene, 9,10-dimethyl-1,2-benzanthracene [DMBA], 3-methylcholanthrene, and Azulene) were crushed together to form pellets of uniform size, which were then implanted cortically into groups of 14 male rats. Fine suspensions (1.0 mg/ml) of each of the hydrocarbons in saline (total volume of 0.05 ml) were introduced intracerebrally into groups of 12 male mice. Control rats and mice received saline cortically at, respectively, 0.1 and 0.05 ml. Rats were killed after 56 days and mice were killed at 140 days by intracardial perfusion with saline followed by formalin. The brains were removed, examined, sectioned, and stained with hematoxylin and eosin. Any detected lesions were mapped. Of the 14 brains of rats implanted with DMBA, six had inflammatory, space-occupying lesions at day 56, and the rats were lethargic. The lesions were focal areas of necrosis and surrounded by an edematous border. Compression of the contralateral hemisphere was noted. Although a neoplasm was not detected, the lesion contained crystals of hydrocarbon in its interior. Outcropping and infiltration into the corpus callosum and hippocampus were observed. The remainder of the brains of rats treated with DMBA, as well as those treated with Azulene and the other hydrocarbons, had non–space-occupying lesions without edema or atypical cells encountered. The lesions due to Azulene implantation were somewhat larger and deeper than those arising from 3-methylcholanthrene treatment (1.55 mm in length; greatest diameter of 2.5 mm with superficial damage to the hemispheres), but the dimensions were not given. Rats injected with saline had negligible changes.

In a second series of rats in the same study, five males and four females (32- and 68-day-old, respectively) were similarly injected with DMBA and killed on day 128. Azulene was implanted into 18 males: 10 were 32 days old and the remainder were 74 days old. All rats treated with Azulene were killed on days 128 and 146, respectively.

Five male and two female rats injected with DMBA had hemorrhagic, cortical space–occupying lesions of foreign-body granulomatous inflammation with multinucleated cells. The remaining rats (DMBA- and Azulene-treated) all had non–space-occupying lesions. No other details were given. Treated mice underwent little change in brain anatomy up to 140 days after treatment (Gershbein and Benuck 1975).

MUTAGENICITY

The mutagenic potential of Azulene was evaluated in an Ames test that was performed using *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537 (Florin et al. 1980). Without metabolic activation, the positive control was *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine. With metabolic activation, 2-aminoanthracene served as the positive control. Azulene was spot-tested at 3 μmol/plate with and without S-9_A (arochlor-induced rats) and S-9_M (3-methylcholanthrene–induced rats). Azulene was not mutagenic. In the same study, Azulene was tested quantitatively for toxicity on strains TA98 and TA100 with and without metabolic activation using S-9_M. Azulene was plated at concentrations of 0.03, 0.3, 3, and 30 μmol/plate. Azulene was toxic to the bacterial strain at 3 μmol/plate and higher.

CLINICAL ASSESSMENT OF SAFETY

In one case report, a 26-year-old woman who had been using a toothpaste containing Azulene for several years developed redness, dryness, scaling, and cracking of the skin and vermillion surrounding her lips. Patch tests produced allergic cheilitis at 48 and 72 hours when both the toothpaste and 1% Azulene were administered. All signs and clinical manifestations cleared

when use of the toothpaste was discontinued and the patient underwent topical steroid treatment (Balato et al. 1985).

SUMMARY

Azulene functions as a skin conditioning agent in cosmetic formulations. Although Azulene exhibits an intense blue or blue-violet color, use of Azulene as a colorant in the United States has not been approved by the FDA. Data submitted to the FDA in 1996 stated that Azulene is found in 43 cosmetic formulations, although there are no suppliers to the cosmetics industry. Another cosmetic ingredient, Guaiazulene, is sometimes mistakenly called Azulene, which could explain the reported uses.

Azulene is an extract from the volatile oil of several perennial herbs and is found in tobacco smoke. Azulene is obtained from plants by extraction and distillation. Azulene is classified as a nonsteroidal anti-inflammatory agent. It is also isomeric with napthalene. Azulene has a significant dipole moment and is prone to electrophilic attack at carbons 1 and 3; and nucleophilic and radical substitution occur at carbon 4. Maximum UV absorbance occurs at 272–277 nm; the minimum occurs at 359 nm.

In labeling studies using rats, Azulene was metabolized to the sulfate conjugate of 1-hydroxyazulene. Between 67–83% of the administered radioactivity was detected in the urine during the first 24 hours after dosing.

The anti-inflammatory action of Azulene has been demonstrated in several animal studies. Azulene injected into burned rabbits reduced inflammatory edema and blood coagulation induced by the burns. Leukocytosis increased, but hemoglobin content and erythrocyte counts in the blood did not change. In another study, the onset of hyperemia and edema induced by topical application of 1% croton oil into the conjunctival sac of rabbits was delayed by treatment with Azulene. In a third study using rabbits, the dermal irritancy caused by 0.25% croton oil was decreased by the application of Azulene.

Azulene was a low to moderate inhibitor of respiration in noradrenaline-stimulated brown fat cells. Azulene did not induce the cessation of ciliary activity in cultures of chicken tracheal organs, and negligible effects were observed in membrane permeability of cultured human lung fibroblasts after treatment with Azulene. Azulene tested at 1 mM and 0.1 mM inhibited the growth of Ascites sarcoma B P8 cells by 85% and 7%, respectively.

The oral LD_{50}s of Azulene in the rat and mouse were 4 g/kg and 3 g/kg, respectively. The intravenous LD_{50} in the mouse was 56 mg/kg. The subcutaneous LD_{50}s in the rat and mouse were, respectively, 520 mg/kg and 145 mg/kg. The intraperitoneal LD_{50}s were 180 mg/kg in the rat and 108 mg/kg in the mouse.

When implanted into the brains of rats, Azulene caused non–space-occupying lesions to form, but little change in brain anatomy occurred up to 140 days after treatment.

In an Ames test using *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537, Azulene was nonmutagenic both with an without metabolic activation.

An allergic reaction to Azulene was noted in one case report. Patch tests produced allergic cheilitis when 1% Azulene and a product formulation were administered.

DISCUSSION

It was brought to the attention of the Cosmetic Ingredient Review (CIR) Expert Panel that a structurally similar compound, Guaiazulene, is generally known in the cosmetics industry as Azulene. Guaiazulene is an FDA-regulated color additive that is exempt from CIR review. Because Azulene has no suppliers, FDA frequency of use data for Azulene should be attributed to Guaiazulene. Other data in this review apply only to Azulene.

Few data on Azulene were available. For example, although it is clear that Azulene is extracted from plant materials, sufficient detail is lacking to determine if naphthalenes or other compounds will be present in the extraction. Section 1, paragraph (p) of the CIR Procedures states that "a lack of information about an ingredient shall not be sufficient to justify a determination of safety." In accordance with Section 30(j)(2)(A) of the Procedures, the Expert Panel informed the public of its decision that the data on Azulene were not sufficient for determining whether this ingredient, under relevant conditions of use, was either safe or unsafe. The Panel released an Insufficient Data Announcement on March 5, 1996, outlining the data needed to assess the safety of Azulene. No comments were received during the 90-day public comment period. Additional data needed to make a safety assessment are: (1) methods of manufacture and impurities, especially naphthalenes; (2) concentration of use; (3) skin penetration; if there is significant skin penetration, then both a 28-day dermal toxicity study to assess general skin and systemic toxicity and a reproductive and developmental toxicity study are needed; (4) one genotoxicity study in a mammalian system; if positive, then a 2-year dermal carcinogenesis study using National Toxicology program (NTP) methods is needed; (5) because of UV absorption, phototoxicity and photosensitization data are needed; (6) skin irritation and sensitization in animals or humans; and (7) ocular toxicity.

CONCLUSION

The CIR Expert Panel concludes that the available data are insufficient to support the safety of Azulene for use in cosmetic products.

REFERENCES

Adams, A. K., D. L. Van Engelen, and L. C. Thomas. 1984. Detection of gas chromatography eluates by simultaneous absorbance and fluorescence measurements. *J. Chromatogr.* 303:341–350.

Atkinson, R., J. Arey, and S. M. Aschmann. 1992. Gas-phase reactions of azulene with hydroxyl and nitrate radicals and ozone at 298 plus/minus 2 K. *Int. J. Chem. Kinet.* 24:467–480.

Balato, N., G. Lembo, P. Nappa, and F. Ayala. 1985. Allergic cheilitis to azulene. *Contact Dermatitis* 13:39–40.

Budavari, S., ed. 1989. *The Merck Index. An encyclopedia of chemicals, drugs, and biologicals.* 11th ed., 148. Rahway, NJ: Merck & Co.

Chemline. 1994. *National Library of Medicine database.* Bethesda, MD: National Library of Medicine.

Florin, I., L. Rutberg, M. Curval, and C. R. Enzell. 1980. Screening of tobacco smoke constituents for mutagenicity using the Ames test. *Toxicology* 15:219–232.

Food and Drug Administration (FDA). 1984. Cosmetic product formulation and frequency of use data. *FDA database.* Washington, DC: author.

FDA. 1992. Modification in voluntary filing of cosmetic product ingredient and cosmetic raw material composition statements. *Fed. Register* 57:3128–3130.

FDA. 1996. Frequency of use of cosmetic ingredients. *FDA database.* Washington, DC: author.

Gershbein, L. L., and I. Benuck. 1975. Cortical implantation of hydrocarbons in rats and mice. *Oncology (Basel)* 31:76–82.

Gordon, M. 1952. The Azulenes. *Chem. Rev.* 50:127–200.

Grasselli, J. G., ed. 1973. *Atlas of spectral data and physical constants for organic compounds*, B-189. Cleveland: CRC Press.

Guillot, J. P., M. C. Martini, J. Y. Giauffret, J. F. Gonnet, and J. Y. Guyot. 1983. Anti-irritant potential of cosmetic raw materials and formulations. *Int. J. Cosmet. Sci.* 5:255–265.

Hanzlik, R. P., and P. Bhatia. 1981. Metabolism of azulene in rats. *Xenobiotica* 11:779–783.

Hawley-Fedder, R. A., M. L. Parsons, and F. W. Karasek. 1984. Products obtained during combustion of polymers under simulated incinerator conditions. III. Polyvinyl chloride. *J. Chromatogr.* 315:211–221.

Kimura, H., J. Niimi, Y. Kajiwara, and S. Yoshida. 1985. Trial of screening method for antiinflammatory preparations on mucous membrane using croton oil-induced rabbit conjunctivitis. *J. Med. Soc. Toho Univ.* 32:249–254.

Leung, L. Y. 1980. *Encyclopedia of Common Natural Ingredients Used in Food, Drugs, and Cosmetics*, 8, 83, 162, 231–234, 326–328. New York: John Wiley.

Lewis, R. J., Sr., ed. 1993. *Hawley's condensed chemical dictionary.* 12th ed., 110. New York: Van Nostrand Reinhold.

Lysenko, L. V. 1967. Antiphlogistic action of eucalyptus oil azulene. *Farmakol. Toksikol.* 30:341–343.

Nowicki, H. G. 1980. Application of azulene as visual aid to monitor column chromatographic fractionation of samples for pesticide and polychlorinated biphenyl determination. *J. Assoc. Of. Anal. Chem.* 64:16–18.

Okabe, S., K. Takeuchi, K. Honda, M. Ishikawa, and K. Takagi. 1975. Effects of azulene and L-glutamine on the development or healing of experimental gastric or duodenal ulcer and gastric secretion in the rat. *Oyo Yakuri* 9:31–37.

Pettersson, B., M. Curvall, and C. R. Enzell. 1980. Effects of tobacco smoke compounds on the noradrenaline-induced oxidative metabolism in isolated brown fat cells. *Toxicology* 18:1–16.

Pettersson, B., M. Curvall, and C. R. Enzell. 1982. Effects of tobacco smoke compounds on the ciliary activity of the embryo chicken trachea in vitro. *Toxicology* 23:41–55.

Pilotti, A., K. Ancker, E. Arrhenius, and C. Enzell. 1975. Effects of tobacco smoke constituents on cell multiplication in vitro. *Toxicology* 5:49–62.

Plattner, P. A., and E. Heilbronner. 1948. Zur Kenntnis der Sesquiterpene und Azulene). Die Ultraviolett-Absorptionsspektren der fünf Monomethyl und einiger mehrfach substituierter Azulene. *Helv. Chim. Acta* 31:804–813.

Rao, J. T., and S. S. Nigam. 1974. Essential oil from the rhizomes of Cucuma aromatica. Salisb. *Flavour Industry* 5:234–236.

Registry of Toxic Effects of Chemical Substances (RTECS). 1994. *National Library of Medicine (NLM) database.* Bethedsa, MD: NLM.

Susz, B., A. S. Pfao, and P. A. Plattner. 1937. Etudes sur les mattières végétales volatiles VI). Sur les spectres d'absorption de l'azulène, du gaïazulène et du vétivazulène. *Helv. Chim. Acta* 20:469–477.

Taylor E. J., ed. 1988. *Dorland's medical dictionary.* 27th ed., 178. Philadelphia: WB Saunders.

Thelestam, M., M. Curvall, and C. Enzell. 1980. Effect of tobacco smoke compounds on the plasma membrane of cultured human lung fibroblasts. *Toxicology* 15:203–217.

Wenninger, J. A., and G. N. McEwen, Jr., eds. 1997. *International Cosmetic Ingredient Dictionary and Handbook.* 7th ed. Vol. 1, 99. Washington, DC: CTFA.