Final Report on the Safety Assessment of Bisabolol[1]

Bisabolol is a naturally occuring unsaturated monocyclic terpene alcohol, the alpha form of which is used in a wide range of cosmetic formulations as a skin conditioning agent at low concentrations ranging from 0.001% in lipstick to 1% in underarm deodorants. Animal studies demonstrate that Bisabolol is well absorbed following dermal exposure and one study using cadaver skin demonstrated that Bisabolol can enhance the penetration of 5-fluorouracil. Bisabolol was relatively nontoxic in acute oral studies in rats, dogs, and monkeys. Short-term oral exposure using rats did produce inflammatory changes in several organs, and reduced body weight and increased liver weights relative to body weight in dogs. The no-observable-adverse-effect level in a 28-day dermal toxicity study using rats was 200 mg/kg/day. No evidence of sensitization or photosensitization was found. Bisabolol was negative in bacterial and mammalian genotoxicity tests, and it did not produce reproductive or developmental toxicity in rats. The results of oral and dermal toxicity, genotoxicity, reproductive/developmental toxicity, sensitization, and photosensitization studies show little toxicity at levels expected in cosmetic formulations. Formulators should be alert to the possibility that use of Bisabolol may increase the penetration of other components of a cosmetic formulation. Based on the available data it was concluded that Bisabolol is safe as used in cosmetic formulations.

INTRODUCTION

Bisabolol is a naturally occurring sesquiterpenoid. It exists in both a beta and alpha configuration. Beta-Bisabolol (CAS No. 15352-77-9) is found in the *Zea mays L.* plant (corn) and is a major volatile component of cotton (Thompson et al. 1974; Dickens 1986). However, Bisabolol as it appears in the *International Cosmetic Ingredient Dictionary* (Wenninger and McEwen 1997) refers to the alpha-formation identified by the CAS No. 515-69-5. This α-Bisabolol itself possesses four stereoisomers which are as follows: (*l*)-α-Bisabolol (CAS No. 23089-26-1); (*d*)-α-Bisabolol (CAS No. 23178-88-3); (*l*)-epi-α-Bisabolol (CAS No. 78148-59-1); and (*d*)-epi-α-Bisabolol (CAS No. 76738-75-5). Although the (+), (−)-epi, and (+)-epi isomers have been isolated as naturally occurring in various plants, their rarity (especially that of (+)-α-Bisabolol) is noted (Brunke and Hammerschmidt 1985; Thappa and Agarwal 1989). (−)-α-Bisabolol is the main active principle of the herb chamomile, *Matricaria chamomilla* (Habersang et al. 1979). Isaac (1979) reported that up to 50% of the essential oil of chamomile is comprised of (−)-α-Bisabolol. Literature available regarding the safety of Bisabolol concerns the (−)-α-Bisabolol configuration and it is this stereoisomer that is discussed in this review unless otherwise noted.

CHEMISTRY

Definition and Structure

Bisabolol (CAS No. 515-69-5) is an unsaturated monocyclic sesquiterpene alcohol that conforms to the formula shown in Figure 1 (Isaac 1979; Wenninger and McEwen 1997). Synonyms for Bisabolol include: α, 4-Dimethyl-α-(4-Methyl-3-Pentenyl)-3-Cyclohexene-1-Methanol; alpha-Bisabolol; and 6-Methyl-2-(4-Methyl-3-Cyclohexen-1-YL)-5-Hepten-2-ol (Wenninger and McEwen 1997; RTECS 1993; Chemline 1993). In Japan it is listed as 1-Methyl-4-(1-hydroxy-1,5,5-trimethyl-4-pentenyl)-cyclohex-1-ene (Rempe and Santucci 1997).

Method of Manufacture

Alpha-Bisabolol can be synthesized by stirring ketodiene in ether into a solution of methyl magnesium iodide at room temperature for 2 hours as shown in Figure 1. The mixture is worked up by adding saturated aqueous ammonium acetate solution and separating the ether and aqueous layers. Washing the aqueous phase with ether and evaporation of the combined ether washes extracts the α-Bisabolol as a colorless oil (Forrester and Money 1972).

Analytical Methods

Bisabolol can be detected by gas chromatography (Andre et al. 1991; Guenther et al. 1993).

Impurities

A supplier of Bisabolol reports that it offers a 95% optically pure [(−)-α-Bisabolol, natural] isolated from a natural source, as well as an 85% pure synthetic racemic mixture [(±)-α-Bisabolol, racemic]. Other components identified are bisabolene, bisabolol oxide, farnesol, chemazulene, and nerolidol at concentrations of <0.1% and <0.5% in the natural and synthetic material, respectively (BASF 1995).

USE

Cosmetic

Bisabolol is used in cosmetic formulations as a skin conditioning agent—miscellaneous (Wenninger and McEwen 1997).

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[1]Reviewed by the Cosmetic Ingredient Review Expert Panel. Bindu Nair Madhavan, 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.

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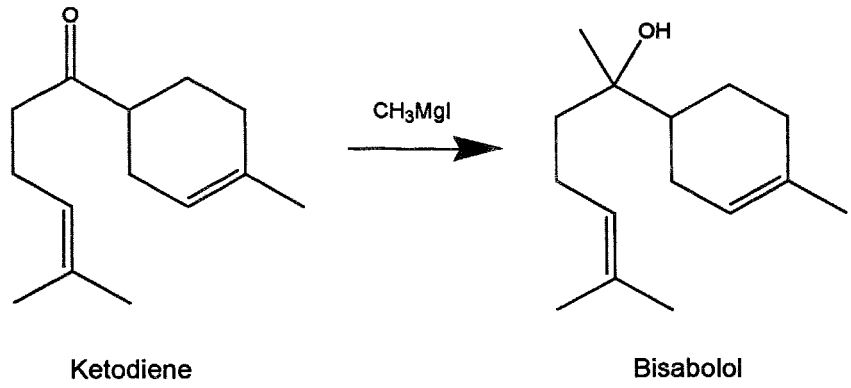

FIGURE 1
Synthesis of Bisabolol from Ketodiene (Forrester and Money 1972).

Data submitted to the Food and Drug Administration (FDA) in 1997 by cosmetic firms participating in the voluntary cosmetic registration program indicated that Bisabolol was used in 184 formulations (Table 1) (FDA 1997).

Concentration of use values are no longer reported to the FDA by the cosmetic industry (FDA 1992). One supplier notes that it recommends a use level of 0.1–2% and that typical concentrations of use are 0.1–0.2% (BASF 1995). Data submitted to CIR indicated use at concentrations of <0.1% up to 1% (see Table 1) (CTFA 1995).

International

Bisabolol is listed (under its Japanese name) in the *Comprehensive Licensing Standards of Cosmetics by Category* (CLS). Bisabolol which conforms to the specifications of the *Japanese Cosmetic Ingredient Codex* has precedent for use without restriction in "nail makeup preparations." Bisabolol has precedent for use at concentrations of 0.1, 0.3, and 0.5% in the various other CLS categories (Rempe and Santucci 1997).

GENERAL BIOLOGY

Absorption, Distribution, Metabolism, and Excretion

In Vitro

Pretreatment of epidermis obtained from abdominal human cadaver skin with a 1:1 α-Bisabolol:propylene glycol mixture increased the permeability of 5-fluorouracil (5-FU) and triamcinolone acetonide by 17- and 73-fold, respectively. A 5.4-fold increase in the permeability of 5-FU was noted after pretreatment with α-Bisabolol alone. It was determined that α-Bisabolol did not affect the stratum corneum–vehicle partition coefficient of 5-FU. Rather the penetration enhancement was suggested to result from an increase in the diffusion coefficient of 5-FU. Bisabolol altered the transition enthalpy of skin lipids (Kadir and Barry 1991).

In Vivo

^{14}C-Levomenol [(−)-6-methyl-2-(4-methyl-3-cyclohexen-1-yl)-5-hepten-2-ol and (−)-α-Bisabolol] was applied to the shaved skin of mice (number and strain not specified) (Hahn and Hölzl 1987). Each mouse received a radioactive dose of 40.6 kBq. The levomenol solution was delivered with either arlatone or acetone as a solubilizer. Animals were killed after 1, 3, and 5 hours of exposure. Samples of skin, fat, and muscle tissues were obtained from various sites and analyzed. Total radioactivity was measured as [^{14}C]CO_2 from combusted samples of neck and leg skin. Thin layer chromatography (TLC) fractionations of skin samples were analyzed for metabolism by autoradiography.

After 1 hour, 80% of the applied radioactivity remained in the arlatone solution on and in the skin at the site of application. By 3 hours, the radioactivity at the exposure site decreased to 57%; by 5 hours the activity was 50%. Similar results were observed with the acetone solution; at 3 hours, approximately 60% of the administered radioactivity was detected at the application site. At 5 hours, 50% of the applied radioactivity was detected at the application site; 90% of it was detected as intact levomenol. Densitometric measurements of skin section autoradiograms indicated that the radioactive levomenol was detected in both fatty and muscular tissues of the neck. About 75% of the absorbed radioactivity penetrated to the 120-μm layer after 1 hour by 3 hours, radioactivity was detected at 160 μm. By 5 hour, a concentration maximum was recorded between layers 90 and 180 μm (corium region), and some radioactivity was detected in the lower corium regions (Hahn and Hölzl 1987).

ANIMAL TOXICOLOGY

Acute Toxicity

Oral

The LD_{50} of (−)-α-Bisabolol (98% pure, oily liquid) in mice was 15.1 ml/kg body weight; sedation in males and ataxia in females was observed at a dose of 6.35 ml/kg. The values were 14.9 and 15.6 ml/kg for male and female rats, respectively. Again, sedation and ataxia were observed, initially at 6.35 ml/kg. The LD_{50} value could not be determined for dogs (5–10 kg) due to vomiting which began at a dose of 12.6 ml/kg. The value could not be determined in rhesus monkeys (3–5 kg) as the gag

TABLE 1
Product formulation data for Bisabolol

Product category	Number of formulations in category[1]	Number containing Bisabolol[1]	Concentration of use[2]
Baby lotions, oils, powders, creams	51	3	
Other bath preparations	141	1	0.25%
Eye lotion	18	2	
Eye makeup remover	80	3	
Mascara	158	2	
Other eye makeup preparations	116	4	<0.1%
Other fragrance preparations	137	3	0.01%
Hair conditioners	596	1	
Hair bleaches	104	1	
Foundations	283	3	
Lipstick	758	2	0.001% (lip balm 0.2%)
Makeup bases	125	2	
Cuticle softeners	19	1	0.05%
Other manicuring preparations	59	1	
Bath soaps and detergents	341	2	up to 0.02% (shower gel)
Deodorants (underarm)	241	4	1%
Other personal cleanliness	262	3	
Aftershave lotions	212	20	0.01%
Preshave lotions	14	1	
Shaving cream	138	2	0.01%
Other shaving preparation products	60	1	
Cleansing	630	14	0.01%
Depilatories	27	1	
Face and neck skin care	251	14	0.05%
Body and hand skin care	776	18	0.01%
Foot powders and sprays	32	1	
Moisturizing	743	21	0.01%
Night	185	6	0.02%
Paste masks (mud packs)	247	12	
Skin fresheners	181	4	
Other skin care preparations (creams/lotions/powders/sprays)	683	25	0.1–1%
Suntan gels, creams, and liquids	134	3	
Indoor tanning preparations	50	2	
Other suntan preparations	43	1	
1997 totals		**184**	

[1]FDA 1997.
[2]CTFA 1995.

reflex and salivation were observed upon dosing with 0.5 ml/kg (Habersang et al. 1979).

BASF (1980a) reported an oral LD_{50} for (±)-α-Bisabolol of >5 g/kg in rats. Dyspnea, apathy, ruffled fur, and "poor general state" were noted at that dose.

Inhalation Toxicity

Twelve rats were exposed for 7 hours to air containing (±)-α-Bisabolol. Bisabolol was aerosolized by passing 200 L air/h through 5 cm of the test substance. There were no deaths and no lesions at necropsy. Details were not reported (BASF 1980b).

Parenteral Toxicity

BASF (1980c) reported an intraperitoneal LD_{50} value for (±)-α-Bisabolol (in emulsion) of 633 mg/kg in mice. Dyspnea, apathy, trembling, staggering gait, rolling fits, ruffled fur, and "poor general state" were noted at the lowest dose, 200 mg/kg. At necroposy of mice that died during the study, no intra-abdominal

deposits of the test substance or adhesions were found. Sacrificed animals had intra-abdominal adhesions of the liver, blunt hepatic margins, and astringed serosa.

Short-Term Toxicity

Oral

Habersang et al. (1979) performed a short-term oral toxicity study. An orientation study was first conducted in which groups of 20 Wistar Br 46-II rats (10 of each sex) received 1 ml/kg Bisabolol (oily liquid, 85% pure) by stomach tube, 7 days a week, for 6 weeks. A control group received aqueous tylosis mucus (resin). No intolerance reactions were detected. In the toxicity assay, two groups of 40 Sprague-Dawley rats (20 of each sex, average body weight 100 g) received Bisabolol (85%) via a stomach tube, 7 days a week, for 4 weeks. One group received 2 ml/kg, the other received 3 ml/kg. A control group received 4 ml of aqueous tylosis mucus. Slight motor agitation was noted in animals of the 2 ml/kg treatment group. A positive ketone body reaction was detected in the urine. Mortality rate was 20% in the 3 ml/kg group. Increased motor agitation and decreased body weight gain were noted. A significant increased in activities of serum glutamic-oxaloacetic transaminase (SGOT) and alkali phosphatase was noted in females; these activities were slightly increased in males. The positive ketone reaction in the urine was intensified in the 3-ml/kg group. Animals of this group were emaciated and had a shaggy haircoat. Postmortem findings for the 3 ml/kg group were different from those of the control group, but the researchers noted a lack of reliable indications of substance-dependent intolerance reactions (details not reported). Inflammatory changes were observed in the liver, trachea, spleen, thymus, and stomach; these changes were more severe in animals of the high-dose group. The researchers characterized the inflammations as an "infection defense weakness triggered by the emaciation."

Habersang et al. (1979) conducted a similarly designed study on dogs. In the orientation study two mixed breed dogs (average weight 8 kg) received 1 ml/kg body weight Bisabolol (oily liquid, 85% pure) via a stomach tube, 7 days a week, for 2 weeks. Another two dogs received aqueous tylosis mucus and served as controls. No intolerance reactions were observed. In the toxicity assay groups of six dogs (three of each sex) received either 2.0 or 3.0 ml Bisabolol/kg, 7 days a week, for 4 weeks. Two weeks into the study, the 3.0 ml/kg dose was increased to 4.0 ml/kg. A control group received 4 ml of aqueous tylosis mucus/kg body weight. A loss of appetite, reduced feed intake, and vomiting were observed in two of the six dogs receiving 2 ml Bisabolol/kg. No other toxic reactions were noted in this treatment group. The reactions were more severe in animals of the 4.0-ml/kg group. Body weight gain was reduced. By the 4th week, creatine concentrations and serum glutamate pyruvate transaminase (SGPT) activity had significantly increased as did liver function (details not reported). At necropsy it was noted that the liver weight relative to body weight was increased significantly. No other changes were noted as compared to controls.

Dermal Toxicity

In a study by BASF (1996a), α-Bisabolol in an olive oil vehicle was applied in a semiocclusive dressing at doses of 50, 200, and 1000 mg/kg body weight/day to the clipped skin of 10 Wistar rats (five each sex). The concentrations of α-Bisabolol (87.5% pure) used to achieve the doses were 1%, 4%, and 20%, respectively. Rats were exposed for 6 h/day, 7 days a week, for 4 weeks. Male rats weighed between 274–297 g and female rats weighed between 209–232 g. Daily observations were made and feed consumption and body weight were measured weekly. Urine and blood samples were obtained towards the end of the study. Rats were examined for gross lesions followed by histopathological examination of fixed tissues. No treatment-related effects were noted in rats of the low- and mid-dose groups. A slight decrease in body weight gain and feed efficiency was noted in all rats of the high-dose group on day 7 only. Transient moderate erythema and diffuse scale formation were noted in some female rats of the high-dose group. Various changes in clinical pathology values were noted in high-dose male rats but none were considered treatment-related. Leukopenia coupled with lymphopenia was considered questionable as concurrent controls had "extremely high" leukocytes and lymphocyte values. Values for both parameters for the control and high-dose group were respectively higher and lower than the range of historical data and were thus considered "fortuitous." A significant increase in serum glucose was within historical range, and a significant increase in serum calcium concentrations was discounted because similar changes were not noted in high-dose females. A significant decrease in mean absolute liver weight was noted in high-dose females and an increase in mean relative testes weight was noted in high-dose males. The changes were considered to result from the decreased mean terminal body weight of female (5.4% lower than controls) and male (3.7% lower than controls) high-dose rats. The no-observable-adverse-effect level (NOAEL) was 200 mg/kg/day (BASF 1996a).

Dermal Irritation

Semiocclusive patches containing undiluted (−)-α-Bisabolol were applied for 4 hours contact to the clipped back or flank of three white Vienna rabbits. Very slight erythema was noted in all rabbits at the 4-hour reading. By 24 hours, the reaction increased to well-defined erythema in two rabbits, one of which also developed very slight edema. By 48 hours, the erythema was no longer observed in rabbit 1, had decreased to very slight in rabbit 2, and remained well-defined in rabbit 3. The edema which had been noted at 24 hours in rabbit 3 cleared. By 72 hours, very slight erythema was noted only in rabbit 3. However, scaling was noted in rabbits 2 and 3. At 7 days scaling was noted in all three rabbits (BASF 1989a).

Ocular Irritation

Undiluted (−)-α-Bisabolol was instilled into one conjunctival sac of each of three rabbits. Eyes were not rinsed. No changes were noted in the cornea or iris at any observation. Well-defined conjunctival redness was noted in all rabbits at the 1, 24, and 48 hours readings. None was noted at the 72 hours reading. Increased discharge was noted in all animals at the 1 hour reading; the reaction cleared in two rabbits and was reduced to a "slight increase" in one rabbit at the 24 hours reading, and thereafter was not noted in any rabbit at subsequent observations (BASF 1989b).

Photosensitization

In a photosensitization assay, Bisabolol (3 and 15% *v/v*) was applied to the shaved neck skin of groups of five male white Pirbright guinea pigs (291–365 g). The test material was dissolved in absolute alcohol. Following application the guinea pigs were irradiated for 15 minutes with 7.9 kilolumen of 240–540 nm light emitted from a quartz lamp at a distance of 65 cm between the light source and the experimental animal. The protocol of dermal treatment followed by radiation was followed for 5 days. A group which was treated with 15% *v/v* Bisabolol but not irradiated was maintained to monitor the effect of the high dose. The vehicle control was treated with alcohol followed by irradiation, and the positive control was treated with tetrachlorosalicylanilide followed by irradiaton. Following a 9-day nontreatment period the protocol was repeated on 2 successive days. In this phase, the protocol was modified in that the test material and the positive control material were now dissolved in olive oil and the vehicle control was olive oil. After a 12-day nontreatment period the hind legs were shaved and a commercial soap solution was applied to the right leg of all animals. The 3 and 15% Bisabolol solutions were dissolved in the commercial soap solution and applied to the left leg of Bisabolol-treated animals. The left leg of positive-control animals was treated with tetrachlorosalicylanilide dissolved in the soap solution. Irradiation followed and the procedure was repeated for 3 days. Animals continued to be observed during a 3-day follow-up period. No indication of photosensitization was noted in Bisabolol-treated animals (BASF 1981).

Anti-Inflammatory Effects

There are numerous articles concerning the anti-inflammatory action of Bisabolol (Fernandes, Periera, and Paulo 1992; Jakovlev et al. 1979; Thiele et al. 1969; Yakovlev and Von-Schlichtegroll 1969). Jakovlev et al. (1979) reported the following three studies and results.

Testing on Carrageenin Edema of Rat Paws

Groups of 30–50 male SIV-50 rats (95–130 g) were orally dosed with one of the following test substances (mixed with methylcarboxyl cellulose):

1. (−)-α-Bisabolol (naturally occurring, 99.5% pure, no isopropylene polymers)
2. (+)-α-Bisabolol (naturally occurring)
3. (±)-α-Bisabolol (racemate produced by mixing the above two naturally occurring isomers)
4. (±)-α-Bisabolol (synthetic, contains 77% isopropylidene and 23% isopropenyl isomers)
5. Commercial Bisabolol preparation (78.7% (±)-α-Bisabolol in isopropylidene form and 5.7% in the isoprenyl form)
6. Bisabolol Oxide A
7. Bisabolol Oxide B

The test substances were administered at doses of 500, 1000, and 2000 mg/kg. The commercial Bisabolol preparation (5) was tested at a high dose of 3000 mg/kg. Olive Oil was tested as a control. One hour after dosing the animals received an injection of 1% carrageenin suspension (0.1 ml) into the right rear paw. Two hours later the animals were killed and the rear paws removed and weighed. The weight difference yielded the amount of edema in milligram.

Naturally occurring (−)-α-Bisabolol at a dose of 1465 mg/kg inhibited edema development by 50% (ED_{50}). In comparison, a similar effect was not produced by even the highest dose tested of the other Bisabolol isomers and oxides and olive oil. However an ED_{50} of 3164 mg/kg was determined for Bisabolol Oxide A. Using (−)-α-Bisabolol as the standard, the commercial preparation had about 25% the antiphlogistic effect, Bisabolol Oxide A was 33% as effective, and the other forms of Bisabolol were half as effective.

Test on Adjuvant Arthritis in Rats

Arthritis was induced in female SIV-50 albino rats (90–100 g) by injecting dead *Mycobacterium butyricum* suspended in paraffin oil into the plantar side of the right rear paw. (−)-α-Bisabolol was administered daily perorally in 1.5% aqueous tragacanth gum solution. A control group received the gum solution alone. Feed and water were provided ad libitum. The volume of the injected paw was measured daily by plethysmography a few hours after the test substance was administered. A clear inhibition in the increase of paw volume was noted with a dose of 250 mg (−)-α-Bisabolol/kg. A dose of 500 mg/kg corresponded in action to about 1.5 mg/kg of the steroid prednisolone (which was used as the standard).

Testing of Ultraviolet Erythema in the Guinea Pig

(−)-α-Bisabolol (in a 1% aqueous tylose mucous) was administered either orally or applied to the back of groups of nine shaved white male Pirbright guinea pigs (200–260 g). The dose range was 125–4000 mg/kg. Salicylamide was used as the comparison substance. Thirty minutes post application, the animals were irradiated for 15 seconds at a distance of 40 cm from the light source (PQ 600, Hanauer Quarzlampen GmbH, Hanau). A plastic sleeve containing six (1-cm) holes was used to direct exposure. Erythema was assessed 2 hours after irradiation and erythema inhibition was rated when at least three fields had no reddening.

A dose-dependent inhibition of erythema development was observed following oral administration. The dose of 2000 mg/kg was toxic. The ED_{50} was 650 mg/kg. Bisabolol administered orally was 1/3 as effective as salicylamide.

In contrast, although percutaneous administration also inhibited erythema, an ED_{50} value was not reached even at the highest dose tested. The highest dose of 400 mg Bisabolol/kg protected 44% of the guinea pigs.

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

Pregnant rats (number not specified; weight 200–262 g) received Bisabolol (98%) daily by stomach tube on days 6–15 of gestation. The doses used were 0.250, 0.500, 1.0, and 3.0 ml/kg body weight. (The 3.0 ml/kg dose was used to test maternal toxicity.) There were two control groups; one group received 1% tylosis mucus and the second group received 1% carboxyethyl cellulose gel and was used for the maternal range-finding aspect of the study. Fetuses were removed on day 20 and examined. No effect on prenatal development was observed at doses of Bisabolol up to 1.0 ml/kg. A significant reduction in fetal number and subsequent increase in resorption rate was observed in the 3.0 ml/kg group (details not reported). No deformities were noted. Slight sedation, ataxia, reduced feed intake, and reduction of body weight gain were observed in females of this dose group. The researchers considered the lowest toxic dose for both fetuses and dams was between 1.0 and 3.0 ml Bisabolol/kg body weight perorally (Habersang et al. 1979).

A similarly designed study was conducted using New Zealand rabbits weighing between 3.6 and 4.4 kg. Pregnant rabbits received either 0.3, 1.0, or 3.0 ml Bisabolol/kg body weight by stomach tube on days 6–15 of gestation. A control group received, by way of replacement, 3 ml of 1% tylosis mucus. Fetuses were removed on day 30 and examined. No adverse effects on either prenatal development or on the dams were noted using Bisabolol at doses up to 1.0 ml/kg. A reduction in the number of living fetuses was noted in the 3.0 ml/kg group; no dead fetuses or deformities were noted. Dams of this treatment group were slightly sedated and had reduced body weight gains (details not provided). Again, the lowest toxic dose for both fetuses and dams was between 1.0 and 3.0 ml Bisabolol/kg body weight perorally (Habersang et al. 1979).

MUTAGENICITY

Bisabolol (86.8% pure) was tested in the Ames assay using *Salmonella typhimurium* strains TA 1535, TA 100, TA 1537, and TA 98. The standard plate protocol (Ames et al. 1973; Ames, McCann, and Yamasaki 1975) was used to test Bisabolol (in DMSO) at doses of 20.0–5000 μg/plate and the preincubation protocol (Yahagi et al. 1977; Matsushima et al. 1980) was used to test Bisabolol (in DMSO) at doses of 1.5–1500 μg/plate. Both test protocols were conducted with and without metabolic activation (S9 from aroclor induced rat liver). A bacteriotoxic effect was noted at $\geq$100–500 μg/plate in the standard plate test and at $\geq$5–50 μg/plate in the preincubation test. Bisabolol was negative in the assay (BASF 1996b).

Bisabolol (86.8% pure) was tested in the chromosome aberration assay using Chinese hamster V79 cells. In the first experiment, test cells were incubated for 4 hours with 7.81, 15.63, or 31.25 μg Bisabolol/ml in the presence of metabolic activation (S9 from aroclor induced rat liver), or 0.78, 1.56, or 3.13 μg Bisabolol/ml without activation. In the second experiment, test cells were incubated with 10.0, 20.0, 30.0, or 40.0 μg Bisabolol/ml with activation, or 2.0, 3.0, or 4.0 μg Bisabolol/ml without metabolic activation. Positive control cells were treated with ethyl methane sulfonate (EMS) (without S9) and cyclophosphamide (with S9), and negative control cells were treated with vehicle (DMSO) or left untreated. Duplicate cultures were made at all doses. Colcemid was added prior to harvesting and chromosomes were prepared 18 hours after treatment. In addition, chromosomes from high-dose cells of the second experiment (both with and without activation) were also prepared at 28 hours. After staining with Giemsa, 100 metaphases of each culture (50 cells of the positive control) were analyzed for chromosomal aberrations. A significant increase in gap aberrations was noted in the first experiment in cells treated with 31.35 μg Bisabolol/ml with S9 and $\geq$1.56 μg Bisabolol/ml without S9. However the finding was not considered relevant because (i) the values were within the range for historical controls (the investigators noted the low spontaneous rate in the concurrent negative control), (ii) the results were not duplicated, and, (iii) "gaps alone are generally not a suitable criterion for assessing clastogenicity." α-Bisabolol was negative in the assay (BASF 1996c).

CLINICAL ASSESSMENT OF SAFETY

Dermal Irritation and Sensitization

A patch testing reference book by DeGroot (1994) noted that the published literature does not contain data concerning Bisabolol. To serve as a guide to the reader, DeGroot reported that an unpublished (and at the time, ongoing) study found no irritant reaction in 1 to 20 patients suffering from or suspected to suffer from cosmetic product contact allergy who had been patch-tested with 5% Bisabolol in petrolatum.

The Kligman maximization protocol was used in a clinical sensitization assay using 25 panelists (Ivey Laboratories 1992). The test substance was a commercial product containing 0.1% Bisabolol. A 24-hour occlusive patch containing aqueous sodium lauryl sulfate (SLS) was applied (and removed) prior to application of a 48-hour patch containing 0.1 g of the test substance. This protocol of a 24-hour SLS patch followed by a 48-hour exposure to the test substance was continued for five induction exposures. The patch containing the test substance was left in place for 72 hours if testing occurred over a weekend. No irritation was noted at any time during the induction phase in the 25 panelists. Induction was followed by a 10-day nontreatment period. Prior to challenge panelists were pretreated with

10.0% aqueous SLS which was kept in contact with the skin for 1 hour. This pretreatment was followed by a 48-hour occlusive patch containing the test substance. Pretreatment and challenge occurred on an unexposed site on the opposite arm. The challenge sites were graded at the time of patch removal and again 24 hours later. No reactions were noted.

SUMMARY

(−)-α-Bisabolol is an unsaturated monocyclic terpene alcohol used in cosmetic formulations as a skin conditioning agent. In January 1997 it was reportedly used in 184 cosmetic formulations, typically at concentrations of 0.1–0.2% (1995 data).

Animal studies indicated that Bisabolol is well absorbed following dermal exposure. Bisabolol had anti-inflammatory properties and an in vitro study demonstrated its penetration enhancement activity.

Acute oral LD_{50} values include 15.1 ml/kg in mice, and 14.9 and 15.6 ml/kg in male and female rats, respectively. The values could not be determined in dogs or rhesus monkeys.

Short-term oral exposure produced inflammatory changes in the liver, trachea, spleen, thymus, and stomach in rats. A similar study conducted using dogs noted significantly increased liver weights relative to body weight and reduced body weight.

A 28-day dermal toxicity study using rats determined the no-observable-adverse-effect level (NOAEL) to be 200 mg/kg/day (applied solution contained 4% α-Bisabolol, 87.5% pure).

Bisabolol was negative in a dermal photosensitization study with guinea pigs and was not teratogenic in an oral dose study using rats.

Bisabolol was negative in the Ames and the chromosome aberration assays using Chinese hamster cells.

In a clinical study, a commercial product containing 0.1% Bisabolol was negative for sensitization.

DISCUSSION

In reviewing the safety of Bisabolol, the Cosmetic Ingredient Review (CIR) Expert Panel was satisfied that the results of oral and dermal toxicity, mutagenicity, reproductive/developmental toxicity, photosensitization, and clinical sensitization studies cited in this report show little toxicity at levels expected in cosmetic formulations. In particular, a 28-day dermal toxicity study determined a NOAEL of 200 mg/kg/day which corresponded to a 4% Bisabolol solution (87.5% pure). As reported use concentrations were generally $\leq 1\%$, the Panel was of the opinion that the 28-day dermal toxicity study, in conjunction with findings of the other studies, supported the assertion that Bisabolol is safe as used.

The Panel acknowledged that the ingredient is well-absorbed following dermal application. Further, an in vitro study demonstrated that Bisabolol is a penetration enhancer and appeared to have synergistic action with propylene glycol. Noting that Bisabolol is used in baby lotions, the Panel cautioned formulators to the possibility of increased absorption of other ingredients also contained in the formulation, especially those ingredients whose safety is based on their lack of dermal absorption.

CONCLUSION

Based on the available data, the CIR Expert Panel concludes that Bisabolol is safe as used in cosmetic formulations.

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