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Final Report of the Safety Assessment of Alcohol Denat., Including SD Alcohol 3-A, SD Alcohol 30, SD Alcohol 39, SD Alcohol 39-B, SD Alcohol 39-C, SD Alcohol 40, SD Alcohol 40-B, and SD Alcohol 40-C, and the Denaturants, Quassin, Brucine Sulfate/Brucine, and Denatonium Benzoate[1]

Alcohol Denat. is the generic term used by the cosmetics industry to describe denatured alcohol. Alcohol Denat. and various specially denatured (SD) alcohols are used as cosmetic ingredients in a wide variety of products. Many denaturants have been previously considered, on an individual basis, as cosmetic ingredients by the Cosmetic Ingredient Review (CIR) Expert Panel, whereas others, including Brucine and Brucine Sulfate, Denatonium Benzoate, and Quassin, have not previously been evaluated. *Quassin* is a bitter alkaloid obtained from the wood of *Quassia amara*. Quassin has been used as an insect antifeedant and insecticide and several studies demonstrate its effectiveness. At oral doses up to 1000 mg/kg using rats, Quassin was not toxic in acute and short-term tests, but some reversible piloerection, decrease in motor activity, and a partial loss of righting reflex were found in mice at 500 mg/kg. At 1000 mg/kg given intraperitoneally (i.p.), all mice died within 24 h of receiving treatment. In a cytotoxicity test with brine shrimp, 1 mg/ml of Quassin did not possess any cytotoxic or antiplasmodial activity. Quassin administered to rat Leydig cells in vitro at concentrations of 5–25 ng/ml inhibited both the basal and luteinizing hormone (LH)-stimulated testosterone secretion in a dose-related fashion. Quassin at doses up to 2.0 g/kg in drinking water using rats produced no significant effect on the body weights, but the mean weights of the testes, seminal vesicles, and epididymides were significantly reduced, and the weights of the anterior pituitary glands were significantly increased. The sperm counts and levels of LH, follicle-stimulating hormone (FSH), and testosterone were significantly lower in groups treated with Quassin. *Brucine* is a derivative of 2-hydroxystrychnine. Swiss-Webster mice given Brucine base, 30 ml/kg, had an acute oral LD_{50} of 150 mg/kg, with central nervous system depression followed by convulsions and seizures in some cases. In those animals that died, respiratory arrest was the cause. The acute i.p. LD_{50} for 15 ml/kg of Brucine base was 62.0 mg/kg, with central nervous system depression prior to the onset of convulsions, just as with oral Brucine. The acute intravenous (i.v.) LD_{50} was 12.0 mg/kg. Brucine was nonmutagenic in an Ames assay at levels up to 6666 μg/plate, with and without metabolic activation. In a repeat-insult patch test, for a hair care product containing 47% SD Alcohol 40 (95%), it was reported that Brucine Sulfate may be considered a nonprimary irritant and a nonprimary sensitizer. Three different sunscreen products (35% SD Alcohol 40-B, 72.4% SD Alcohol 40, and 74.5% SD Alcohol 40) did not show any signs of photoallergy in human subjects. Also, these three formulas did not exhibit any evidence of phototoxicity in humans. *Denatonium Benzoate* is a bitter substance detectable at a concentration of 10 ppb, discernibly bitter at 50 ppb, and unpleasantly bitter at 10 ppm. The distribution of topically applied lidocaine, a topical anesthetic chemically related to Denatonium Benzoate demonstrated that virtually no lidocaine appears in the plasma, suggesting that the larger Denatonium Benzoate molecule also would have little or no systemic exposure. Denatonium Benzoate (0.1%) did not show adverse effects in 10 rats in an acute inhalation toxicity test and 0.005% to 0.05% was nonirritating to ocular mucosa in 6 albino rabbits. The acute oral LD_{50} for the male rats was 640 mg/kg and for females, 584 mg/kg. The LD_{50} for the male rabbits was 508 mg/kg and for the female rabbits, 640 mg/kg. In two chronic toxicity studies, Denatonium Benzoate was administered (by gavage) at 1.6, 8, and 16 mg/kg/day, one using cynomologus monkeys and the other rats, resulted in no compound-related toxicity. The toxicity of SD Alcohols has also been tested, with implications for the particular denaturant used. An irritation test of 55.65% SD Alcohol 40-B denatured with Denatonium Benzoate using rabbits produced minimal effects. A spray formula containing 12% SD Alcohol 40-B was found to be nonirritating when evaluated for vaginal mucosal irritation in New Zealand white rabbits. Cosmetic formulations containing SD Alcohol 40-B (denatured with Denatonium Benzoate) were not sensitizers in repeated insult patch tests. A gel formula containing 29% SD Alcohol 40-B and a spray liquid containing 12% SD Alcohol 40-B did not induce photoallergy, dermal sensitization, or phototoxic response in human subjects. Although the absorption of ethanol (aka Alcohol for purposes of cosmetic ingredient labeling) occurs through skin, ethanol does not appear to affect the integrity of the skin barrier nor reach a very high systemic concentration following dermal exposure. Ethanol may be found in the bloodstream as a result of inhalation exposure and ingestion. Topically applied, ethanol can act as a penetration enhancer. Most of the systemic toxicity of ethanol appears to be

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[1]Reviewed by the Cosmetic Ingredient Review Expert Panel. Address correspondence to F. Alan Andersen, Cosmetic Ingredient Review, 1101 17th Street, NW, Suite 412, Washington, DC 20036, USA.

associated with chronic abuse of alcohol. Although ethanol is denatured to make it unfit for consumption, there have been reports of intentional and unintentional consumption of products containing denatured alcohol. Ethanol is a reproductive and developmental toxicant. Ethanol is genotoxic in some test systems and it has been proposed that the genotoxic effects of ethanol are mediated via its metabolite, acetaldehyde. A brief summary is provided of the effects of chronic ingestion of alcohol including intoxication, liver damage, brain damage, and possible carcinogenicity. The CIR Expert Panel recognizes that certain ingredients in this group are reportedly used in a given product category, but the concentration of use is not available. Because dermal application or inhalation of cosmetic products containing these ingredients will not produce significant systemic exposure to ethanol, the CIR Expert Panel concluded that safety of the ingredients should be predicated on the safety of the denaturants used. The Panel considered that the adverse effects known to be associated with Alcohol ingestion included in this safety assessment do not suggest a concern for Alcohol Denat. or SD Alcohols because of the presence of the denaturants, which are added for the express purpose of making the Alcohol unpotable. The CIR Expert Panel has previously conducted safety assessments of t-Butyl Alcohol, Diethyl Phthalate, Methyl Alcohol, Salicylic Acid, Sodium Salicylate, and Methyl Salicylate, in which each was affirmed safe or safe with qualifications. Given their use as denaturants are at low concentrations of use in Alcohol, the CIR Expert Panel determined that Alcohol Denat. denatured with t-Butyl Alcohol, Diethyl Phthalate, Methyl Alcohol, Salicylic Acid, Sodium Salicylate, and Methyl Salicylate is safe as used in cosmetic formulations with no qualifications. Likewise, because they are denatured with either t-Butyl Alcohol, Diethyl Phthalate, or Methyl Alcohol, SD Alcohols 3-A, 30, 39-B, 39-C, and 40-C all are considered safe as used. The Panel considered the available data for Denatonium Benzoate and SD Alcohol 40-B to be sufficient to support the safety of these ingredients in cosmetics. Denatonium Benzoate is sufficiently bitter that it is an effective denaturant at only 0.0006%. The Panel recognized that data on dermal penetration of Denatonium Benzoate were not available, but considered that the available data on lidocaine, a smaller structurally related chemical, indicates that dermal exposure does not result in measurable systemic exposure. The available data, however, were not sufficient to support the safety of Quassin, Brucine, and Brucine Sulfate, Alcohol Denat. denatured with those denaturants, or SD Alcohol 39 and SD Alcohol 40 (SD Alcohols denatured with Quassin, Brucine, and/or Brucine Sulfate), and in order for the Expert Panel to reach a conclusion for these denaturants, additional data are needed.

INTRODUCTION

According to the *International Cosmetic Ingredient Dictionary and Handbook* (Gottschalck and McEwen 2004), Alcohol is the name given by convention to ethanol as used in cosmetics, and Alcohol Denat. is the name given to denatured Alcohol for use in cosmetics. Alcohol Denat. is prepared by adding one or more denaturing agents to potable Alcohol, making it unfit for beverage or internal human medicinal use. Alcohol may be completely denatured (CD Alcohol) or specially denatured (SD Alcohol) depending on the type(s) of denaturant(s) used.

This report addresses Alcohol Denat. and the following specific denatured Alcohols: SD Alcohol 3-A, SD Alcohol 30, SD Alcohol 39, SD Alcohol 39-B, SD Alcohol 39-C, SD Alcohol 40, SD Alcohol 40-B, and SD Alcohol 40-C. These are the most widely used Alcohol Denat. ingredients in cosmetics.

Denaturants in Alcohol Denat. and the above SD Alcohols include many ingredients previously reviewed by the Cosmetic Ingredient Review (CIR) Expert Panel and will not be extensively discussed. In addition to the many denaturants already considered, Quassin, Brucine Sulfate, Brucine, and Denatonium Benzoate are used and are a focus of this review.

Part I of this safety assessment addresses the available safety test data relevant to these denaturants in Alcohol Denat. and the widely used SD Alcohols.

Because ethanol is the primary component of Alcohol Denat. and the SD Alcohols, Part II is an overview of safety test data on ethanol itself. Other terms commonly used include undenatured ethyl alcohol or just ethyl alcohol.

In concept, the entire purpose of denaturing Alcohol is to make it unfit to drink, so the safety assessment does not include the vast literature on alcoholism. On the other hand, Alcohol Denat. may be ingested, so a limited number of references to the effects of Alcohol ingestion are included.

PART I: DENATURANTS

Denatured alcohols, in the United States, must conform to the Code of Federal Regulations (CFR) specifications for completely and specially denatured alcohol found in 27CFR§21. Table 1 lists the specifications for those denatured alcohols in this safety assessment—those denaturants in bold type have been previously considered by the CIR Expert Panel, with the following conclusions:

t-Butyl Alcohol—safe as used in cosmetic products (Andersen 2005a);

Diethyl Phthalate—safe for topical application in the present practices of use and concentration in cosmetics (Elder 1985), reaffirmed safe for use in the present practices of use and concentration (Andersen 2005b);

Methyl Alcohol—safe as used to denature alcohol used in cosmetic products (Andersen 2001); and

Salicylic Acid, Sodium Salicylate, and Methyl Salicylate—safe as used when formulated to avoid irritation and when formulated to avoid increasing sun sensitivity, or, when increased sun sensitivity would be expected, directions for use include the daily use of sun protection (Andersen 2003).

The denaturants noted above will not be discussed further in this safety assessment. The safety of Alcohol Denat. and the SD Alcohols containing these ingredients may be inferred.

The remainder of this section will focus on three denaturants listed in the *International Cosmetic Ingredient Dictionary and Handbook* (Gottschalck and McEwen 2004), Quassin, Brucine Sulfate, and Denatonium Benzoate, which have not previously

TABLE 1
Specifications in the Code of Federal Regulations (27CFR§21) for specially Denatured Alcohol

Ingredient	100 Gallons of Ethanol Denatured With
SD Alcohol 3-A	5 gallons of methyl alcohol[a]
SD Alcohol 30	10 gallons of methyl alcohol[a]
SD Alcohol 39	9 pounds of sodium salicylate[a], U.S.P., or salicylic acid[a], U.S.P., and 1.25 gallons of fluid extract of quassia, N.F., and 1/8th gallon of t-butyl alcohol[a]
SD Alcohol 39-B	1/8th gallon of t-butyl alcohol[a] and 2.5 gallons of diethyl phthalate[a]
SD Alcohol 39-C	1 gallon of diethyl phthalate[a]
SD Alcohol 40	1/8th gallon of t-butyl alcohol[a] and 1.5 avoirdupois ounces of any combination of one or more of the following: brucine (alkaloid), brucine sulfate, N.F., or quassin
SD Alcohol 40-B	1/16th avoirdupois ounce of denatonium benzoate, N.F., and 1/8th gallon of t-butyl alcohol[a]
SD Alcohol 40-C	3 gallons of t-butyl alcohol[a]

[a]Denaturants for which CIR has completed a safety assessment - see text.

been addressed. Brucine is also included because it is specifically listed as an approved denaturant in Table 1.

CHEMISTRY

Definition and Structure

Quassin

According to the *International Cosmetic Ingredient Dictionary and Handbook* (Gottschalk and McEwen 2004), Quassin is a bitter alkaloid obtained from the wood of *Quassia amara*, CAS number 76-78-8 (the CAS number of *d*, *l*-quassin is 75991-65-0). Other names for Quassin include

2,12-dimethoxypicrasa-2,12-diene-1,11,16-trione; and
Picrasa-2,12-diene-1,11,16-trione,2,12-dimethoxy-.

Stojanac and Valenta (1991) reported that *d*, *l*-quassin can be synthesized via a 13-step process starting with ester hemiacetal.

The molecular formula of Quassin is $C_{22}H_{28}O_6$ (molecular weight is 388.45) and the structure is shown in Figure 1 (Budavari 1989). The melting point of Quassin is 222°C and it absorbs light at 255 nm (Budavari 2001). The calculated octonol/water partition coefficient for Quassin is 0.94 (Syracuse Research Corp. 2005).

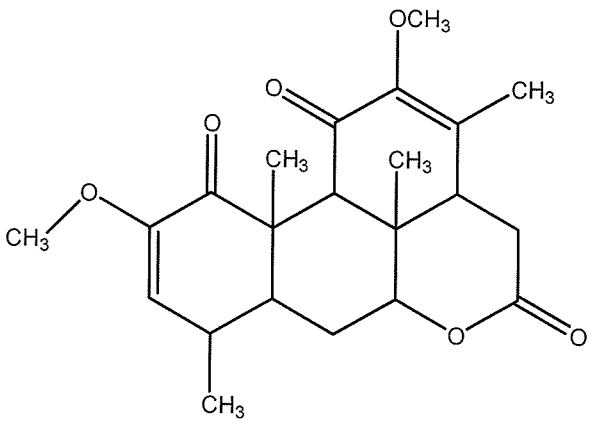

FIGURE 1
Structure of Quassin (Budavari 1989).

According to Hunt (1967), Quassin has been used for many years as a denaturant for alcohol used in cosmetic preparations. It is odorless and extremely bitter. Quassin is soluble in chloroform, ethyl acetate, and benzene, and sparingly soluble in ether or light petroleum. The concentration of Quassin may vary according to the different regulations governing different types of products as well as the alcohol content.

Brucine and Brucine Sulfate

Brucine Sulfate is an alkaloid that conforms to the formula: $(C_{23}H_{26}N_2O_4)_2 \cdot H_2SO_4 \cdot 7H_2O$ (CAS number 4845-99-2) with a molecular weight of 394.46 (Budavari 1989). Brucine Sulfate currently is listed as a denaturant in the *International Cosmetic Ingredient Dictionary and Handbook* (Gottschalck and McEwen 2004), with the following technical/other names:

- Brucine Sulfate Heptahydrate;
- 2,3-dimethoxystrychnidin-10-one Sulfate (2:1);
- Strychnidin-10-one,2,3-dimethoxy-, Sulfate (2:1); and
- Strychnidin-10-one,2,3-dimethoxy-, Sulfate (1:1), Heptahydrate.

The CAS number of Brucine is 357-57-3. Brucine currently is not listed in the *International Cosmetic Ingredient Dictionary and Handbook* as a cosmetic ingredient. This alkaloid resembles strychnine, with the formula $C_{23}H_{26}N_2O_4$, a molecular weight of 304.46, absorption maxima at 263 and 301 nm, a melting point of 178°C, a pH (saturated water solution) of 9.5, and the structure shown in Figure 2 (Budavari 2001).

The calculated octonol/water partition coefficient for Brucine is 1.49 (Syracuse Research Corp 2005).

Arena and Drew (1986) noted that Brucine Sulfate is intensely bitter, but they suggest that quantities present in denatured alcohol do not create any great hazard, unless an unusually large quantity is ingested.

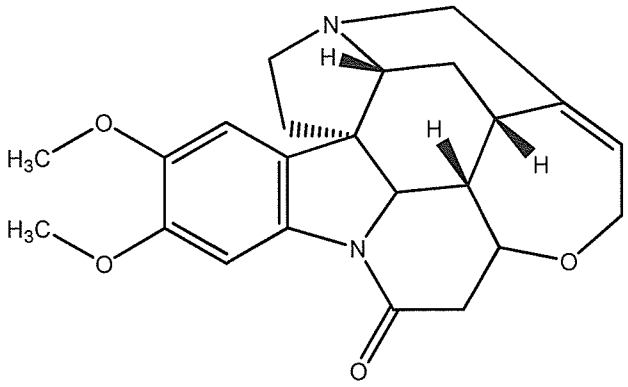

FIGURE 2
Structure of Brucine (Budavari 1989).

Denatonium Benzoate

As given in the *International Cosmetic Ingredient Dictionary and Handbook* (Gottschalck and McEwen 2004), Denatonium Benzoate (CAS number 3734-33-6) is an organic quaternary ammonium compound that functions as a denaturant and conforms to the following empirical formula: $C_{21}H_{29}N_2O \cdot C_7H_5O_2$. The structure of Denatonium Benzoate is shown in Figure 3. Technical/other names include:

- Benzenemethanaminium, N-[2-[(2,6-dimethylphenyl) amino]-2-oxoethyl]-N,N-diethyl-, benzoate;
- N-[2-[(2,6-dimethylphenyl)amino]-2-oxoethyl]-N,N-diethylbenzenementhanaminium benzoate (RIFM);
- N-[2-[(2,6-dimethylphenyl)amino]-2-oxoethyl]-N,N-diethylbenzene-methanaminium benzoate (RIFM); and
- N-[2-[(2,6-dimethylphenyl)amino]-2-oxoethyl]-N,N-diethylbenzene-methanaminium benzoate.

Denatonium Benzoate has a molecular weight of 446.58 (Budavari 1989). It is available as a white, odorless granule. The melting point ranges from 163°C to 170°C. Denatonium Benzoate is soluble in water and alcohol, somewhat in acetone, and nearly insoluble in ether (Hansen et al. 1993). Denatonium Benzoate absorbs radiation at 263 nm (at a concentration of 100 μg/ml in water) and the pH of a 3% solution in water is between 6.5 and 7.5 (Committee of Revision of the U.S. Pharmacopeial Convention 2004). The calculated octonol/water partition coefficient for Denatonium Benzoate is 1.78 (Syracuse Research Corp. 2005).

According to Jackson and Payne (1995), Denatonium Benzoate is one of the most bitter substances. It is added to a wide variety of products at required low levels, but does not interfere with the mode of action of the product. It is detectable by taste at concentrations as low as 10 ppb, discernibly bitter at 50 ppb, and at 10 ppm it is unpleasantly bitter.

For the purposes of denaturing alcohol, Denatonium Benzoate is added at a rate of 6 ppm in the USA and 10 ppm in the UK. In most other applications' 20 to 50 ppm is the recommended concentration to render a formulation aversively bitter; however, up to 100 ppm has been used (Hendersen et al. 1998; Klein-Schwartz 1991; Payne 1989).

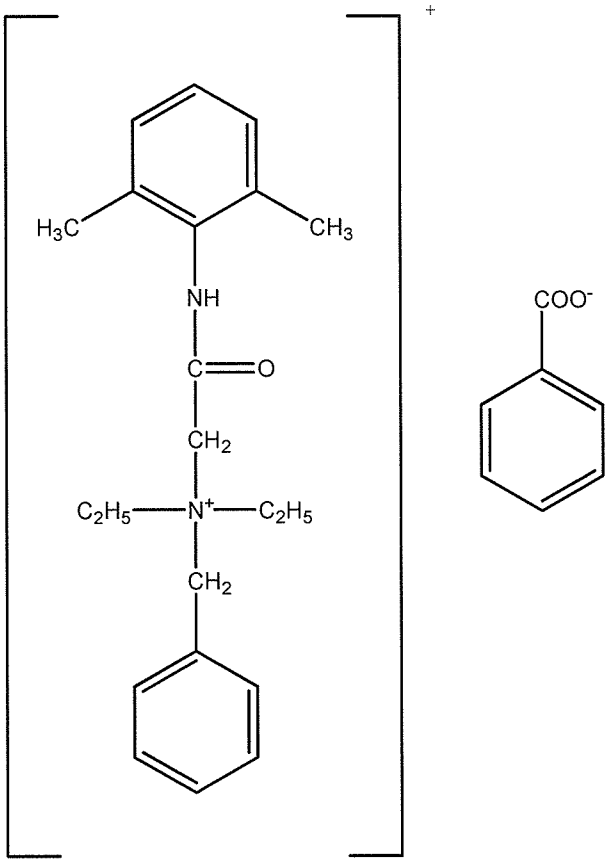

FIGURE 3
Structure of Denatonium Benzoate (Gottschalck and McEwen 2004).

As shown in Table 1, SD Alcohol 40-B is denatured with 1/16 avoirdupois ounce of Denatonium Benzoate, N.F., and 1/8 gallon of tert-butyl alcohol. As described by Equistar Chemicals, LP (2004), this results in SD Alcohol 40-B with approximately 0.0006% Denatonium Benzoate and 0.12% t-butyl alcohol.

The CIR Expert Panel also considered several pieces of data on lidocaine, a topical anesthetic structurally related to Denatonium Benzoate. A United States Patent (Hay 1963) provided the chemical structure of a quaternary carboxylate derived from lignocaine, a synonym for lidocaine.

The structure of lignocaine (aka lidocaine) is represented in Figure 4 and may be compared to the structure of Denatonium Benzoate in Figure 3. Lignocaine benzyl benzoate and lidocaine benzyl benzoate are synonyms for Denatonium Benzoate (Hay 1963).

Method of Manufacture

Information on specific CD or SD Alcohols was not found. The CIR Expert Panel presumed that Table 1 describes a simple mixing process.

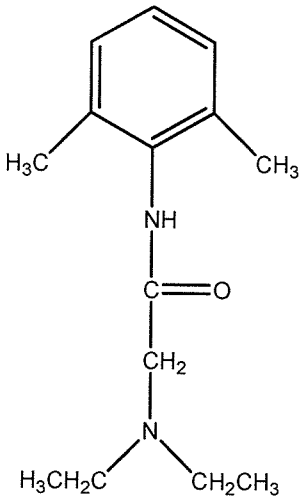

FIGURE 4
Structure of lignocaine, aka lidocaine (Hay 1963).

Quassin

Clark (1937) stated that quassin is prepared by extracting *Quassia* chips (20 kg) and allowing them to stand in hot water for 3 h. The extract is poured off and the process is repeated three times. The extract is treated with solutions of lead acetate and carbon (Carbex) to absorb the Quassin. The amount of lead acetate and carbon that is added is in a ratio of 4 to 1. The carbon is removed, air-dried, and mixed with chloroform. The mixtures are concentrated to dryness under reduced pressure, dissolved in 150 cc of methanol, and mixed with 950 cc of hot water. A crystallization of crude Quassin begins once the mixture is filtered. The crystallization is complete within 2 days.

Brucine

Tedeschi et al. (1968) reported that Brucine (2,3-dihydroxystrychnine) is prepared by reacting 2-hydroxystrychnine in acetyl alcohol with a solution of Fremy salt in water. This reaction is allowed to complete, and 2 N $HClO_4$ and $Na_2S_2O_4$ are added. The pH is adjusted with NH_4OH to 7 to 7.5. This mixture is then dissolved in $CHCl_3$/Methyl Alcohol and treated with ethereal diazomethane. The two products that emerge are β-colubrin and Brucine.

According to the *Merck Index*, Brucine also can be isolated from Strychnos seeds (Budavari 2001).

Denatonium Benzoate

Lignocaine benzyl benzoate (a synonym for Denatonium Benzoate) is described by Hay (1963) in a United States Patent describing the use of this quaternary carboxylate as a denaturant for Ethyl Alcohol. The patent presents multiple synthetic approaches to the preparation of Denatonium Benzoate. The patent states that the method of denaturing Ethyl Alcohol comprises adding 0.01% to 0.001% Denatonium Benzoate (note: as described earlier, 1/16 avoirdupois ounce per 100 gallons of Ethyl Alcohol is the currently approved level of Denatonium Benzoate as given in the Code of Federal Regulations).

Analytical Methods

Quassin

Hunt (1967) described a method by which Quassin may be determined when used as a denaturant for the alcohol contained in cosmetic products. The two bitter principles, neoquassin and Quassin, are determined together as Quassin, after oxidation with a solution of sodium dichromate in glacial acetic acid. Perfume oils are first removed by solvent extraction and the aqueous phase is then evaporated to dryness and the residue oxidized. Further solvent-extraction steps yield a solution of Quassin in chloroform, which is applied to a thin-layer chromatographic plate. The spots are compared visually with standards prepared from an oxidized "quassin" solution to permit determinations within the range of 5 to 36 ppm in samples.

Brucine

Sondack and Koch (1973) noted that there are many methods in which Brucine and strychnine are estimated, including gravimetric and titrimetric analyses, paper chromatography, thin-layer chromatography (TLC), ion-exchange chromatography, spectrophotometry, and nuclear magnetic resonance (NMR) spectroscopy. Brucine and strychnine have been separated by gas–liquid chromatrography (GLC).

Zhang et al. (2002) reported a new capillary electrophoresis procedure with field-enhanced stacking concentration for the analysis of strychnine and Brucine. These two alkaloids can be separated after the optimization of the separation and concentration within 5 min and quantified for high sensitivity. The detection limits for strychnine and Brucine were 1.0 ng ml^{-1} and 1.4 ng ml^{-1}, respectively. The method was useful for qualitative and quantitative analysis of strychnine and Brucine in *Strychnos nux-vomica* L with recovery of 105.1% for strychnine and 98.4% for Brucine.

Denatonium Benzoate

The concentration of Denatonium Benzoate was measured by high-pressure liquid chromatography (HPLC). The analysis of standards showed a linear curve in the range of 1.25 to 50 ppm (Henderson et al. 1998).

Damon and Pettitt (1980) reported a method for the determination of Denatonium Benzoate in rapeseed oil. The method utilizes a simple extraction and concentration technique followed by reverse-phase high performance liquid chromatography. The column eluent was monitored at 210 nm and peak area data were generated by computing integrator. Detection was possible below 5 ng and calibration curves were linear to 100 ng or more.

Impurities

Brucine

Brucine alkaloid (27CFR§21.99) must pass two tests in order for its use to be authorized: strychnine test and sulfate test. The strychnine test states that Brucine alkaloid shall be free of strychnine when tested by the method listed under Brucine Sulfate,

N.F. IX. If the Brucine contains as much as 0.05% strychnine, a clear distinctive violet color, characteristic of strychnine, will be obtained. The sulfate test states that no white precipitate is formed that is not dissolved by hydrochloric acid when several drops of a 1 N barium chloride solution are added to 10 ml of a solution of the alkaloid.

Quassin

Quassin (27CFR§21.124) must pass two tests in order for its use to be authorized: optical assay and solubility. The optical assay states that when 1 g of Quassin (in solution in a small amount of 95% alcohol) is dissolved in 10,000 ml of water, the absorbance of the solution in a 1-cm cell at a wavelength of 258 mμ shall not be less than 0.400. The solubility test states that 0.5 g of Quassin dissolves completely in 25 ml of 190 proof alcohol.

Denatonium Benzoate

The Committee of Revision of the United States Pharmacopeial Convention (2004) states that Denatonium Benzoate, dried at 105°C for 2 h, contains one molecule of water of hydration or is anhydrous. When dried at 105°C for 2 h, it contains no less than 99.5% and no more than 101.0% of $C_{28}H_{34}N_2O_3$. When Denatonium Benzoate is dried at 105°C for 2 h, the monohydrate loses between 3.5% and 4.5% of its weight, and the anhydrous form loses no more than 1.0% of its weight. The residue on ignition cannot be more than 0.1%. When 900 mg of Denatonium Benzoate is dissolved (dried and accurately weighed) in 50 ml of glacial acetic acid, 1 drop of crystal violet TS is added, and titrated with 0.1 N perchloric acid VS to a green end point. A blank determination is performed, and any corrections are made. Each milliliter of 0.1 N perchloric acid is equivalent to 44.66 mg of $C_{28}H_{34}N_2O_3$.

USE

Cosmetic

According to the *International Cosmetic Ingredient Dictionary and Handbook* (Gottschalk and McEwen 2004), Alcohol Denat. functions as an antifoaming agent, cosmetic astringent, solvent, and viscosity-decreasing agent in cosmetics, and all of the SD alcohols function as cosmetic astringents, solvents, and viscosity decreasing agents. Table 2 lists the authorized uses for the denatured alcohols in the Code of Federal Regulations (27CFR§20 and 21).

As reported by industry to the Food and Drug Administration (FDA), Table 3 gives the reported frequency of use as a function of product category, along with the total number of products in that category. Table 3 also includes current concentration of use information provided to CIR directly by the industry. If a particular denaturant was reportedly used in that product category, it is listed (in bold type, if the denaturant has been considered previously in a CIR safety assessment).

For example, Alcohol Denat. was reported to be used in 9 out of a total of 215 bubble baths (4.2%) at a concentration of 0.1%, but the denaturants used were not reported; whereas Alcohol Denat. was reported to be used in 264 of 684 colognes and toilet waters (38.6%) at concentrations from 79% to 96%, with diethyl phthalate, t-butyl alcohol, and Denatonium Benzoate given as examples of denaturants used in this category (although not together in one product or necessarily in all products).

For each SD alcohol in Table 3 for which there is a formula for denaturants in Table 1, the denaturants used are given next to the name. For example, SD Alcohol 3-A (denatured with Methyl Alcohol only) is used in a total of 16 cosmetic products at a concentration range from 0.1% to 5%.

Table 3 also includes ingredient use in categories for which no reports were provided to FDA, but for which a current concentration of use was provided by the Cosmetic, Toiletry and Fragrance Association (CTFA). For example, Alcohol Denat. was not reported to FDA to be used in eye shadow products, but industry reported that it is currently being used in this product category in the concentration range of 0.008% to 6%.

Denaturing agents are regulated in the United States, as given in Table 1, and Europe. Alcohol Denat. is not included among the substances listed as prohibited, restricted, or provisionally allowed in the use of cosmetic products marketed in Europe (European Commission 1999) or in Japan (Ministry of Health, Labor, and Welfare [MHLW] 2001a, 2001b). Brucine is listed in Annex II (substances which must not form part of the composition of cosmetic products) of the European Union regulations (European Commission 2005).

Noncosmetic

Alcohol Denat. is used in medical, industrial, and domestic products. For example, methylated spirit contains 5% methyl alcohol in ethanol; Solox (Shellac solvent) contains 5% methyl alcohol, 1% gasoline, 1% ethyl acetate, and 1% methyl isobutyl ketone in ethanol; Sterno-canned heat contains 3.7% methanol and 4% acetone in ethanol; and rectified spirit contains 90% ethanol (Bastani et al. 1976). Although FDA/CFSAN has listed Alcohol Denat. as one of the food additives in the "Everything Added to Food in the United States" (EAFUS) list, there is no reported use of it as a food additive (FDA, 2003).

GENERAL BIOLOGY

Absorption, Metabolism, and Distribution

Quassin

Garcia et al. (1997) reported that an intestinal transit test was performed using Quassin extract. Thirty male mice were distributed into three equal groups. After 6 h of fasting, each group was administered one of the following treatments: 0.5 ml of distilled water and Quassin extract in doses of 500 and 1000 mg/kg. The data were recorded and expressed as the mean value ± the standard error. With the 500 mg/kg dose, the average percentage of intestinal transit was 63.86% ± 7.15, which did not constitute a statistically different value upon comparison with

TABLE 2
Authorized uses as a solvent for SD Alcohols in personal care products in the Code of Federal Regulations (27CFR§20 and 21)

Ingredient	Authorized Uses as a Solvent in Personal Care Products
SD Alcohol 3-A	Shampoos, soap and bath preparations
SD Alcohol 30	Soap and bath preparations
SD Alcohol 39	Hair and scalp preparations, bay rum, lotions and creams (hand, face, and body), perfume and perfume tinctures, toilet waters and colognes, shampoos, soap and bath preparations
SD Alcohol 39-B	Hair and scalp preparations, bay rum, lotions and creams (hand, face, and body), deodorants (body), perfumes and perfume tinctures
SD Alcohol 39-C	Hair and scalp preparations, lotions and creams (hand, face, and body), deodorants (body), perfumes and perfume tinctures, toilet waters and colognes, soaps and bath preparations
SD Alcohol 40	Hair and scalp preparations, bay rum, lotions and creams (hand, face, and body), deodorants (body), perfumes and perfume tinctures, toilet waters and colognes, shampoos, soaps and bath preparations
SD Alcohol 40-B	Hair and scalp preparations, bay rum, lotions and creams (hand, face, and body), deodorants (body), perfumes and perfume tinctures, toilet waters and colognes, shampoos, soaps and bath preparations
SD Alcohol 40-C	Hair and scalp preparations, bay rum, lotions and creams (hand, face, and body), deodorants (body), perfumes and perfume tinctures, toilet waters and colognes, shampoos, soaps and bath preparations

the control group (58.19% ± 8.82). The group that received 1000 mg/kg had an average percent intestinal transit of 71.96% ± 4.96.

Lidocaine

As seen in Figures 3 and 4, lidocaine and Denatonium Benzoate are chemically related.

Kearns et al. (2003) used an iontophoretic drug delivery system to give lidocaine to 12 children (ages 5 to 15). The children were exposed to lidocaine (100 mg of a 10% solution) for 0, 0.3, and 3.5 h. Plasma levels of lidocaine, measured for up to 10 h after this treatment, were below 10 ng/ml at all time points. This method, which enhances entry into the skin, did not result in a measurable systemic dose.

CTFA (2005) offered the commentary that lidocaine is a smaller molecule than is Denatonium Benzoate. Even with an iontophoretic drug delivery system, little of the lidocaine was found in plasma, suggesting that the larger Denatonium Benzoate, in the absence of a delivery system other than its presence as an alcohol denaturant, would be unlikely to result in any systemic exposure.

Insect Antifeedant and Insecticidal Activities

Quassin

Daido et al. (1995) noted that many cytotoxic quassinoids have been shown to have insect antifeedant activity. The quassinoids were isolated from *P.ailanthoides* and the antifeedant and insecticidal activities were measured against the diamondback moth, *Plutella xylostella*. These activities were compared to a known antifeedant, chlordimeform.

The quassinoid tested was applied to the cabbage leaf discs fed to the moth at 63.7, 31.9, and 16.0 $\mu g/cm^2$ and observations were made on days 1, 2, and 5. The results of the antifeedant activity test showed Quassin produced 90% to 100% damage with all applications and on all days. Chlordimeform also produced this result. For the insecticidal activity test, protocol was the exact same, in which Quassin produced potent insecticidal activity as well. It was concluded that the death of the larvae was not due to any antifeedant activity of the compounds tested, but that these compounds had lethality.

Park et al. (1987) reported that Quassin, among other quassinoids, was tested upon acute insecticidal activity against the American cockroach. The quassinoids were subcutaneously injected into the cockroach and observations were made at 3, 24, and 48 h with recordings of the minimum effective dose ($\mu g/g$ of insect body weight) to cause ataxia. All of the quassinoids, including Quassin, had strong acute paralytic activity at dosages as low as 25 $\mu g/g$ of insect body weight, but after 24 h most of the insects recovered and the specific activity fell to 100 $\mu g/g$ of insect body weight or more. When a higher dosage was applied, acute insecticidal activity resulted and there was no recovery. The authors noted that Quassin and neoquassin were not effective as an insecticide against the cockroach under a different evaluation method that was focused on delayed toxicity.

Leskinen et al. (1984) reported in a study where 13 different quassinoids were tested for antifeedant activity against the Mexican bean beetle (*Epilachna varivestis Mulsant*) and the southern armyworm (*Spodoptera eridania*). Quassin was tested at 500, 250, 100, 50, and 10 ppm with the Mexican bean beetle and 500, 250, and 100 ppm with the southern armyworm. Five trials at each concentration were performed by coating one leaf with the compound and one with just a solvent. The subjects were placed on the leaves, and after 24 h the percentage eaten for each leaf was estimated. In every test conducted with the Mexican bean beetle, more of the control leaf was eaten compared to the leaf with Quassin applied to it. When leaves eaten

TABLE 3
Alcohol Denat. and SD Alcohol use in cosmetics

Product Category (Total formulations in product category - FDA 2002) (Denaturant, if known [a] - CTFA 2003)	Number of Formulations Containing Ingredient (FDA 2002)	Use Concentration (CTFA 2003)
Alcohol Denat.		
Bath Preparations		
Bath Oils, Tablets, and Salts (143)	6	0.005%
Bubble Baths (215)	9	0.1%
Other Bath Preparations (196)	3	0.07%
Eye Makeup Preparations		
Eyeliner (548) (t-Butyl Alcohol; Denatonium Benzoate)	3	2–10%
Eye Shadow (576) (Denatonium Benzoate)	—	0.008–6%
Eye Makeup Remover (100) (Denatonium Benzoate)	—	3%
Mascara (195) (Denatonium Benzoate)	—	3–7%
Other Eye Makeup Preparations (152) (Diethyl Phthalate; Denatonium Benzoate)	—	5–10%
Fragrance Preparatons		
Colognes and Toilet Waters (684) (Diethyl Phthalate; t-Butyl Alcohol; Denatonium Benzoate)	264	79–96%
Perfumes (235) (Diethyl Phthalate;t-Butyl Alcohol; Denatonium Benzoate)	79	70–97%
Dusting and Talcom Powders (273)	—	0.008%
Sachets (28) (t-Butyl Alcohol)	4	86%
Other Fragrance Preparations (173) (t-Butyl Alcohol; Denatonium Benzoate)	20	30–98%
Non-coloring Hair Preparations		
Hair Conditioners (651) (Denatonium Benzoate)	9	0.1–40%
Hair Sprays (aerosol fixatives) (275) (Denatonium Benzoate)	60	76–90%
Rinses (non-coloring) (42)	2	—
Shampoos (non-coloring) (884) (Denatonium Benzoate)	11	0.1–25%
Tonics, Dressings, and Other Hair Grooming Aids (598) (Denatonium Benzoate)	53	35–91%
Wave Sets (53) (Denatonium Benzoate)	6	89%
Other Hair Preparations (277) (Denatonium Benzoate)	27	29–83%
Hair Coloring Preparations		
Hair Bleaches (120)	3	—
Other Hair Coloring Preparations (55) (Denatonium Benzoate)	—	0.2–16%
Makeup Preparations		
Blushers (245)	—	0.008%
Face Powders (305)	—	0.008%
Leg and Body Paints (4)	—	0.03%
Foundations (324) (t-Butyl Alcohol; Denatonium Benzoate)	5	4–10%
Other Makeup Preparations (201) (t-Butyl Alcohol; Denatonium Benzoate)	1	7–12%
Nail Care Products		
Basecoats and Undercoats (44)	1	10%
Cuticle Softerners (19)	—	3%
Nail Polish and Enamel (123)	—	10–52%
Nail Polish and Enamel Removers (36)	2	—
Other Manicuring Preparations (55) (Diethyl Phthalate; Denatonium Benzoate)	4	3–48%
Oral Hygiene Products		
Mouthwashes and Breath Fresheners (46)	20	—

TABLE 3
Alcohol Denat. and SD Alcohol use in cosmetics

Product Category (Total formulations in product category - FDA 2002) (Denaturant, if known [a] - CTFA 2003)	Number of Formulations Containing Ingredient (FDA 2002)	Use Concentration (CTFA 2003)
Personal Cleanliness Products		
Bath soaps and detergents (421)	—	0.2%
Deodorants (underarm) (247) (t-Butyl Alcohol; Denatonium Benzoate)	47	72–99%
Douches (5)	2	—
Feminine Deodorants (4)	1	—
Other Personal Cleanliness Products (308)	17	9–82%
Shaving Preparations		
Aftershave Lotion (231) (Diethyl Phthalate; t-Butyl Alcohol)	77	55–97%
Preshave Lotions (all types) (14)	10	87%
Shaving Cream (134)	—	0.07%
Other Shaving Preparation Products (63) (t-Butyl Alcohol; Denatonium Benzoate)	11	5–70%
Skin Care Preparations		
Skin Cleansing Products (cold creams, lotions, liquids, and pads) (775) (Diethyl Phthalate and Denatonium Benzoate; Denatonium Benzoate)	15	0.7–51%
Depilatories (34) (Diethyl Phthalate)	-	1%
Face and Neck skin care (excl. shaving) (310) (Diethyl Phthalate; Denatonium Benzoate)	3	2–80%
Body and Hand skin care (excl. shaving) (840) (Diethyl Phthalate and t-Butyl Alcohol; Diethyl Phthalate; Denatonium Benzoate)	28	2–40%
Foot Powders and Sprays (35)	4	0.01%
Moisturizers (905) (Diethyl Phthalate; Denatonium Benzoate)	15	0.1–26%
Night skin care (200) (Denatonium Benzoate)	1	0.005–25%
Paste Masks (mud packs) (271) (Diethyl Phthalate; Denatonium Benzoate)	11	1–5%
Skin Fresheners (184)	38	10%
Other Skin Care Preparations (725) (Diethyl Phthalate; t-Butyl Alcohol; Denatonium Benzoate)	32	0.6–90%
Suntan Preparations		
Suntan Gels, Creams, and Liquids (131) (Diethyl Phthalate; t-Butyl Alcohol)	3	2–74%
Indoor Tanning Preparations (71)	—	0.0008%
Other Suntan Preparations (38) (Denatonium Benzoate)	1	50%
2002 total/range for Alcohol, Denat.	821	0.0008–99%
SD Alcohol 3-A(denatured with Methyl Alcohol only)		
Bath Preparations		
Bubble Baths (215)	5	4%
Other Bath Preparations (196)	3	—
Non-coloring Hair Preparations		
Shampoos (non-coloring) (884)	1	3%
Personal Cleanliness Products		
Bath Soaps and Detergents (421)	5	—
Skin Care Preparations		
Skin Cleansing Products (cold creams, lotions, liquids, and pads) (775)	2	5%
Skin Fresheners (184)	—	0.01–2%
2002 total/range for SD Alcohol 3-A	16	0.01–5%
SD Alcohol 39-B (denatured with t-Butyl Alcohol and Diethyl Phthalateonly)		
Fragrance Preparations		
Colognes and Toilet Waters (684)	1	—

(Continued on next page)

TABLE 3
Alcohol Denat. and SD Alcohol use in cosmetics *(Continnued)*

Product Category (Total formulations in product category - FDA 2002) (Denaturant, if known [a] - CTFA 2003)	Number of Formulations Containing Ingredient (FDA 2002)	Use Concentration (CTFA 2003)
Shaving Preparations		
Other Shaving Preparation Products (63)	1	—
1998 totals/ranges for SD Alcohol 39-B	2	—
SD Alcohol 39-C(denatured with Diethyl Phthalateonly)		
Bath Preparations		
Bath Oils, Tablets, and Salts (143)	1	2%
Other Bath Preparations (196)	—	52%
Eye Makeup Preparations		
Eye Makeup Remover (100)	1	—
Mascara (195)	4	5–10%
Other Eye Makeup Preparations (152)	—	5%
Fragrance Preparatons		
Colognes and Toilet Waters (684)	104	86%
Perfumes (235)	32	78–88%
Other Fragrance Preparations (173)	10	76–81%
Non-coloring Hair Preparations		
Hair Conditioners (651)	1	10%
Hair Sprays (aerosol fixatives) (275)	5	—
Rinses (non-coloring) (42)	1	—
Shampoos (non-coloring) (884)	1	0.3%
Tonics, Dressings, and Other Hair Grooming Aids (598)	12	0.3%
Other Hair Preparations (277)	6	74%
Hair Coloring Preparations		
Hair Dyes and Colors (1690)	58	—
Makeup Preparations		
Foundations (324)	1	0.003%
Other Makeup Preparations (201)	—	28%
Personal Cleanliness Products		
Bath Soaps and Detergents (421)	—	0.0002%
Deodorants (underarm) (247)	18	0.001–87%
Other Personal Cleanliness Products (308)	1	0.0005–65%
Shaving Preparations		
Aftershave Lotion (231)	35	88%
Preshave Lotions (all types) (14)	1	—
Other Shaving Preparation Products (63)	3	72%
Skin Care Preparations		
Skin Cleansing (cold creams, lotions, liquids, and pads) (775)	4	8%
Face and Neck skin care (excl. shaving) (310)	12	0.05–12%
Body and Hand skin care (excl. shaving) (840)	4	5%
Foot Powders and Sprays (35)	—	73%
Moisturizers (905)	3	—
Night skin care (200)	1	—
Paste Masks (mud packs) (271)	2	—
Skin Fresheners (184)	4	0.006%
Other Skin Care Preparations (725)	8	58%

TABLE 3
Alcohol Denat. and SD Alcohol use in cosmetics

Product Category (Total formulations in product category - FDA 2002) (Denaturant, if known [a] - CTFA 2003)	Number of Formulations Containing Ingredient (FDA 2002)	Use Concentration (CTFA 2003)
Suntan Preparations		
Suntan Gels, Creams, and Liquids (131)	2	—
Indoor Tanning Preparations (71)	1	—
Other Suntan Preparations (38)	1	—
2002 totals/ranges for SD Alcohol 39-C	337	0.0002–88%
SD Alcohol 40(denatured with t-butyl alcohol and Brucine, Brucine Sulfate, or quassin)		
Baby Products		
Other Baby Products (34)	1	—
Bath Preparations		
Bath Oils, Tablets, and Salts (143)	1	—
Bubble Baths (215)	1	0.07%
Other Bath Preparations (196)	3	—
Eye Makeup Preparations		
Eyeliner (548)	1	—
Mascara (195)	6	2–10%
Fragrance Preparations		
Colognes and Toilet Waters (684)	66	79–98%
Perfumes (235)	5	—
Powders (273)	1	—
Other Fragrance Preparations (173)	1	59%
Non-coloring Hair Preparations		
Hair Conditioners (651)	15	10%
Hair Sprays (aerosol fixatives) (275) (t-Butyl Alcohol and Brucine Sulfate)	147	3–64%
Hair Straighteners (63) (t-Butyl Alcohol and Brucine Sulfate)	-	0.00005%
Permanent Waves (207) (t-Butyl Alcohol and Brucine Sulfate)	3	5%
Rinses (non-coloring) (42)	1	—
Shampoos (non-coloring) (884)	4	0.004–0.4%
Tonics, Dressings, and Other Hair Grooming Aids (598) (t-Butyl Alcohol and Brucine Sulfate)	114	3–47%
Wave Sets (53) (t-Butyl Alcohol and Brucine Sulfate)	4	18%
Other Hair Preparations (277) (t-Butyl Alcohol and Brucine Sulfate)	67	45–55%
Hair Coloring Preparations		
Hair Dyes and Colors (1690)	164	—
Hair Color Sprays (aerosol) (5)	2	—
Hair Bleaches (120)	6	—
Other Hair Coloring Preparations (277)	5	6%
Makeup Preparations		
Foundations (324)	1	—
Other Makeup Preparations (201)	6	24%
Nail Care Products		
Basecoats and Undercoats (44)	1	—
Nail Polish and Enamel (123)	3	0.2–11%
Nail Polish and Enamel Removers (36)	2	—
Other Manicuring Preparations (55)	—	52%

(Continued on next page)

TABLE 3
Alcohol Denat. and SD Alcohol use in cosmetics *(Continued)*

Product Category (Total formulations in product category - FDA 2002) (Denaturant, if known [a] - CTFA 2003)	Number of Formulations Containing Ingredient (FDA 2002)	Use Concentration (CTFA 2003)
Personal Cleanliness Products		
Bath Soaps and Detergents (421)	13	—
Deodorants (underarm) (247) (t-Butyl Alcohol and Brucine Sulfate)	15	0.4–73%
Douches (5) (t-Butyl Alcohol and Denatonium Benzoate; Brucine Sulfate)	—	0.9–8%
Other Personal Cleanliness Products (308) (t-Butyl Alcohol and Brucine Sulfate)	8	0.0001–10%
Shaving Preparations		
Aftershave Lotion (231) (t-Butyl Alcohol and Brucine Sulfate)	27	0.0009–5%
Preshave Lotions (all types) (14)	2	—
Other Shaving Preparation Products (63)	4	—
Skin Care Preparations		
Skin Cleansing Products (cold creams, lotions, liquids and pads) (775) (t-Butyl Alcohol and Brucine Sulfate; t-Butyl Alcohol and Brucine Sulfate)	16	15–66%
Face and Neck skin care (excl. shaving) (310)	1	—
Body and Hand skin care (excl. shaving) (840) (t-Butyl Alcohol and Brucine Sulfate)	12	0.0002–15%
Foot Powders and Sprays (35) (t-Butyl Alcohol and Brucine Sulfate)	—	18–67%
Moisturizers (905) (t-Butyl Alcohol and Brucine Sulfate)	4	0.3–30%
Night Creams, Lotions, and Powders (200)	—	70%
Paste Masks (mud packs) (271) (t-Butyl Alcohol and Brucine Sulfate)	8	0.6%
Skin Fresheners (184) (t-Butyl Alcohol and Brucine Sulfate)	15	20–30%
Other Skin Care Preparations (725) (t-Butyl Alcohol and Brucine Sulfate)	15	10–70%
Suntan Preparations		
Suntan Gels, Creams, and Liquids (131) (t-Butyl Alcohol and Brucine Sulfate)	4	5–76%
2002 totals/ranges for SD Alcohol 40	775	0.00005–98%
SD Alcohol 40-B(denatured with Denatonium Benzoate and t-Butyl Alcoholonly)		
Bath Preparations		
Bath Oils, Tablets, and Salts (143)	6	—
Bubble Baths (215)	14	8%
Other Bath Preparations (196)	25	3–33%
Eye Makeup Preparations		
Eyebrow pencil (102)	—	5%
Eyeliner (548)	1	0.003%
Eye Lotion (25)	1	—
Eye Makeup Remover (100)	1	0.006%
Mascara (195)	6	0.003–5%
Other Eye Makeup Preparations (152)	1	5%
Fragrance Preparations		
Colognes and Toilet Waters (684)	141	71–96%
Perfumes (235)	37	69–86%
Powders (273)	5	—
Sachets (28)	1	—
Other Fragrance Preparations (173)	25	50–70%
Non-coloring Hair Preparations		
Hair Conditioners (651)	12	10–11%
Hair Sprays (aerosol fixatives) (275)	41	48–80%

TABLE 3
Alcohol Denat. and SD Alcohol use in cosmetics

Product Category (Total formulations in product category - FDA 2002) (Denaturant, if known [a] - CTFA 2003)	Number of Formulations Containing Ingredient (FDA 2002)	Use Concentration (CTFA 2003)
Shampoos (non-coloring) (884)	7	0.05%
Tonics, Dressings, and Other Hair Grooming Aids (598)	23	5–95%
Wave Sets (53)	5	—
Other Hair Preparations (277)	36	32–94%
Hair Coloring Preparations		
Hair Color Sprays (aerosol) (5)	—	6%
Hair Lighteners with Color (5)	—	0.03%
Other Hair Coloring Preparations (55)	4	—
Makeup Preparations		
Blushers (245)	—	5%
Foundations (324)	2	3–19%
Lipstick (962)	1	—
Makeup Bases (141)	3	26%
Makeup fixatives (20)	—	15%
Other Makeup Preparations (201)	4	30%
Nail Care Products		
Basecoats and Undercoats (44)	2	23%
Nail Polish and Enamel (123)	3	14–51%
Other Manicuring Preparations (55)	3	22–32%
Personal Cleanliness Products		
Bath Soaps and Detergents (421)	14	—
Deodorants (underarm) (247)	11	21–99%
Other Personal Cleanliness Products (308)	4	—
Shaving Preparations		
Aftershave Lotion (231)	27	60–86%
Preshave Lotions (14)	—	60–82%
Shaving Cream (134)	1	63%
Other Shaving Preparations (630)	2	77–87%
Skin Care Preparations		
Skin Cleansing Products (cold creams, lotions, liquids and pads) (775)	11	5–47%
Face and Neck skin care (excl. shaving) (310)	2	5–42%
Body and Hand skin care (excl. shaving) (840)	17	6–70%
Foot Powders and Sprays (35)	5	60–91%
Moisturizers (905)	30	0.003–8%
Night skin care (200)	1	3–5%
Paste Masks (mud packs) (271)	18	10%
Skin Fresheners (184)	14	32–46%
Other Skin Care Preparations (725)	15	35–63%
Suntan Preparations		
Suntan Gels, Creams, and Liquids (131)	1	25–49%
Indoor Tanning Preparations (71)	2	—
Other suntan preparations (38)	—	5–37%
2002 totals/ranges for SD Alcohol 40-B	585	0.003–99%

[a]Denaturants reported to be used in some products in this category, but not necessarily all products. If two or more denaturants are used in combination, their names are separated by "and." CIR has completed safety assessments on the denaturing agents highlighted in bold type.

by the southern armyworm were compared at 500 and 250 ppm, the percentage eaten for the control leaf was greater than the leaf treated with Quassin; however, when the concentration was lowered to 100 ppm, more of the treated leaf was eaten compared to the control.

Cytotoxicity

Brucine and Quassin

Brine shrimp were used in a microplate assay for cytotoxicity. Many compounds were tested, including a group of quassinoids (0.6 mg of samples were tested). Among the quassinoids were Bruceine A, Bruceine C, Bruceine D, Brusatol, and Quassin, all which were made up to 1 mg/ml in artificial sea water except for water insoluble compounds which were dissolved in 50 μl DMSO prior to adding sea water. Once the brine shrimp were exposed to the testing material, they were incubated for 24 h, examined under a binocular microscope, and the numbers of dead nauplii in each well were counted. It was concluded that all of the quassinoids (Bruceine A, Bruceine C, Bruceine D, and Brusatol) were toxic to the brine shrimp except for Quassin. The authors concluded that Quassin did not possess cytotoxic or antiplasmodial activity (Solis et al. 1993).

ANIMAL TOXICOLOGY

Acute Oral Toxicity

Quassin

Garcia et al. (1997) reported an acute toxicity test on Quassin extract by the oral route using 25 albino male mice. Mice were distributed into five groups evenly and each group received a single treatment of 250, 500, 750, or 1000 mg/kg of extract. The control group received 0.5 ml of distilled water. There was no mortality, nor was there any sign of toxicity at the end of the 48 h of observation.

Brucine

Malone et al. (1992) conducted a study to determine the LD_{50} indices in male Swiss-Webster mice. Brucine base, 30 ml/kg, was administered orally to 7 to 12 randomly distributed mice. The LD_5, LD_{50}, and the LD_{95} values were 78.0, 150, and 290 mg/kg, respectively. It was discovered that in all instances, the mice died in tonic seizures preceded by clonic convulsions. It was noted that not all clonic convulsions lead to tonic seizures, however, if a tonic seizure did occur, death was attributed to respiratory arrest. When the mice were exposed to the Brucine base orally, they experienced central nervous system depression prior to the onset of convulsions.

Denatonium Benzoate

The International Research and Development Corporation (1976) conducted three individual acute oral toxicity studies using Denatonium Benzoate.

The first study was conducted using male and female Charles River CD albino rats weighing 150 to 196 g. Denatonium Benzoate (10 ml/kg) was administered orally to five rats of each sex at each dosage level. The dosages were 127.1, 201.7, 320.2, 508.4, 807, and 1281 mg/kg. The rats were observed and LD_{50} values were determined for each sex. The acute oral toxicity (LD_{50}) of Denatonium Benzoate in male albino rats was calculated to be 640 mg/kg (confidence limits of 554 to 740 mg/kg). The LD_{50} value for female albino rats was 584 mg/kg (confidence limits of 485 to 702 mg/kg). The combined male and female albino rat acute oral toxicity was 612 mg/kg, with confidence limits of 558 to 671 mg/kg.

The second study was conducted with neonatal albino rats that were derived from pregnant Charles River CD female albino rats. They weighed 5.7 to 9.6 g and were dosed orally with Denatonium Benzoate by means of a polyethylene catheter attached to a syringe. Denatonium Benzoate (10 ml/kg) was administered to 10 neonatal rats at dosages of 7.9, 12.5, 19.8, 31.5, 50.0, 79.4, 125, and 315 mg/kg. The acute oral toxicity (LD_{50}) for Denatonium Benzoate was calculated to be 23 mg/kg with confidence limits of 19 to 27 mg/kg.

The third acute oral toxicity study with Denatonium Benzoate was performed using male and female New Zealand white rabbits, weighing 2518 to 2886 g. Two rabbits of each sex were orally treated with 10 ml/kg of Denatonium Benzoate at dosages of 201.7, 320.2, 508.4, 807.1, or 1281 mg/kg. The calculated LD_{50} value for male albino rabbits was 508 mg/kg with confidence limits of 202 to 1281 mg/kg. The LD_{50} value for female albino rabbits was 640 mg/kg with confidence limits of 508 to 807 mg/kg. The combined acute oral toxicity value for Denatonium Benzoate in male and female rabbits was 593 mg/kg with confidence limits of 483 to 728 mg/kg (International Research and Development Corporation 1976).

Acute Inhalation Toxicity

Denatonium Benzoate

In a review article, Klein-Schwartz (1991) stated that no acute inhalation toxicity was found in a study using 10 rats exposed to 0.1% Denatonium Benzoate solution (no further details provided).

Acute Ocular Toxicity

Denatonium Benzoate

Denatonium Benzoate concentrations of 0.005% to 0.05% (solvent not mentioned) were not irritating to ocular mucosa in 6 mature albino rabbits (Klein-Schwartz 1991).

Acute Parenteral Toxicity

Quassin

Garcia et al. (1997) reported an acute toxicity test of Quassin extract, by the intraperitoneal (i.p.) route, in which 30 albino male mice were distributed into 3 groups of 10 animals each. The first treatment group received 500 mg/kg of extract, the second received 1000 mg/kg of extract, whereas the control group received 0.5 ml of distilled water. After a 4-h period, the group

that was administered 500 mg/kg had the following signs of toxicity: piloerection, decrease in motor activity, and partial loss of righting reflex. All animals recovered after 24 h. The group that was administered 1000 mg/kg had the following signs of toxicity after 4 h: piloerection, loss of posterior prehensile reflex, and decrease in motor activity. All of the animal died within 24 h of receiving the extract.

Brucine

Malone et al. (1992) determined the LD_{50} indices of mice. Male Swiss-Webster mice (7 to 12 animals) received 15 ml/kg of Brucine base i.p. The LD_5, LD_{50}, and LD_{95} values were calculated to be 45.0, 62.0, and 86 mg/kg, respectively. When Brucine base was administered via this route especially, it was found that the mice developed clonic convulsions without subsequent tonic extension seizures. The authors also noted that the mice experienced central nervous system depression prior to the onset of convulsions, just as when the Brucine was administered orally.

Brucine base also was administered to the mice intravenously (i.v.) mg/kg. The LD_5, LD_{50}, and LD_{95} values were 5.1, 12.0, and 28.0, respectively. Additionally, Brucine Sulfate heptahydrate (7.5 ml/kg) was administered i.v. into the tail vein. The LD_5, LD_{50}, and LD_{95} values were 6.5, 15.4, and 36.0 mg/kg, respectively. The authors noted that when the Brucine was administered i.v., the onset of convulsions was instantaneous (Malone et al. 1992).

Short-Term Toxicity

Quassin

Garcia et al. (1997) reported on a study that included four separate tests on the extract of *Quassia amara*, Quassin. The first test was a hippocratic assay in which 12 Wistar female rats were distributed evenly into three groups. Two of the groups were given the extract orally, prepared based on a stock solution in doses of 500 and 1000 mg/kg, whereas the control group received 0.5 ml of distilled water. This particular experiment focused on the effects that the extract had on the central nervous system's activity. After 9 days of treatment, the animals were not observed to have any central nervous system abnormalities.

Chronic Toxicity

Denatonium Benzoate

The International Research and Development Corporation (IRDC) performed chronic toxicity studies of Denatonium Benzoate using rats and monkeys (IRDC 1977a, 1977b, 1978); the full 2-year study report using rats is presented under Carcinogenesis. In the study using cynomolgus monkeys (IRDC 1977a), Denatonium Benzoate was administered to eight male and eight female monkeys (same as control group) by gavage at 1.6, 8, and 16 mg/kg/day. They were observed daily, and at the end of the study, it was concluded that there were no changes in general behavior and appearance, ophthalmoscopy, electrocardiograms, body weights, hematological and biochemical studies, or urinalysis. There was one death in the control group, one death at the 1.6 mg/kg/day dose, three deaths at the 8 mg/kg/day dose, and four deaths at the 16 mg/kg/day dose. The authors stated that deaths at 8 and 16 mg/kg/day doses were the result of compound effects.

Dermal Irritation

Denatonium Benzoate

CTFA (1996) reported a modified Draize rabbit primary dermal irritation test using a cream formula that contained 55.65% SD Alcohol 40-B. New Zealand white rabbits were used in the study. The study group consisted of six animals, weighing 2 to 3 kg and at 2 to 3 months old. The animals were observed 24 and 72 h after application of the cream (amount of cream used was not specified). There was an intact site and an abraded treatment site present on each animal, of which each was observed for erythema and edema. Each of the six animals was given a score each time they were observed, and the mean was reported for the entire study group. The erythema scores for the group after 24 h were 1.3 (both intact and abraded sites). The erythema scores after 72 h were 0.2 (intact site) and 0.7 (abraded site). The edema scores for the group after 24 h were 0.3 (both intact and abraded sites). The edema scores after 72 h were 0.0 (for both sites as well). The primary dermal irritation score was 1.0 for the cream formula containing 55.65% SD Alcohol 40-B.

Vaginal Irritation

Denatonium Benzoate

NAMSA (2004) evaluated the potential of a spray formula containing 12% SD Alcohol 40-B to cause vaginal mucosal irritation. Female New Zealand white rabbits were used as the subjects of study. Twelve rabbits total were used in the study; six rabbits received a daily 0.4-ml intravaginal treatment of the test material, and the other six rabbits were dosed daily with 0.4 ml of sesame oil, NF (SO) to serve as the control. Both groups received treatment for 10 days. All of the animals were euthanized the day following the last treatment. Each vagina was removed and evaluated grossly and histologically. Both the test and control animals had a slight reddening of the vaginal orifice prior to doses 8 and 9. At dose 10, results showed both reddening and swelling of the vaginal orifice. The microscopic examination resulted in an irritation index of 0. It was concluded that the spray formula was nonirritating to the vaginal mucosal tissue of the rabbit.

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

Quassin

Njar et al. (1995) reported on the effect of crude methanol extracts of *Quassia amara* extracts, Quassin, and 2-methoxycanthin-6-one on testosterone production of rat Leydig cells in vitro. Extracts were tested at concentrations that

ranged from 50 to 250 μg/ml, but only one had an effect, so that crude methanol extracts of *Quassia amara* were used throughout. The crude methanol extract of *Quassia amara* was fractionated by chromatography. The result was the isolation of Quassin and 2-methoxycanthin-6-one, which were then tested.

The crude methanol extracts of *Quassia amara* (50 to 250 μg/ml) and Quassin (5 to 25 ng/ml) inhibited basal and leutinizing hormone (LH)-induced testosterone secretion in a dose-related fashion, while 2-methoxycanthin-6-one (5–25 ng/ml) had no effect. Leydig cell viability before and after treatment was not different. The authors concluded that the inhibition of testosterone production cannot be due to cytotoxic effects of the crude methanol extracts of *Quassia amara* or Quassin (Njar et al. 1995).

Raji and Bolarinwa (1997) conducted an experiment using Wistar albino rats to evaluate the effect of the crude methanol extract of *Quassia amara*, Quassin, and 2-methoxycanthin-6-one on male reproductive functions. Quassin and 2-methoxycanthin-6-one were prepared by fractionation of the crude extract.

Crude methanol extract was provided in drinking water for 8 weeks to yield doses of 100, 1000, and 2000 mg/kg (5 animals in each group), with a phosphate buffered saline control group. A second set of rats were treated in the same manner, except that they were allowed a recovery period of 8 weeks with no further treatment. In a third treatment, rats were administered 0.1, 1.0, and 2.0 mg/kg of Quassin daily for 8 weeks, and in a fourth treatment, 2-methoxycanthin-6-one was administered at 0.1, 1.0, and 2.0 mg/kg daily for 8 weeks; as above, control groups received phosphate buffered saline. Final treatment groups (5 animals each) received ovine LH (0.5 μg/ml via i.p. injecton) alone, crude methanol extract of *Quassia amara* (2000 mg/kg oral) plus ovine LH (0.5 μg/ml i.p. injecton), or Quassin (2.0 mg/kg oral) plus the ovine LH (0.5 μg/ml i.p. injection) daily for 8 weeks.

All of the rats were killed, and the seminal vesicles, epididymides, testes, and anterior pituitary glands were removed and weighed. Epididymal sperm counts, motility, and morphology were evaluated. There was no significant effect of the crude methanol extract of *Quassia amara*, Quassin, or 2-methoxycanthin-6-one on the body weight of the rats. However, the mean weights in the testes, seminal vesicles, and epididymides were significantly reduced in the crude methanol extract of *Quassia amara*– and Quassin-treated groups. The weights of the anterior pituitary glands were significantly increased in these groups. It was found that regardless of the dosages of the treated groups, the sperm counts did not differ, but groups that were treated with the crude methanol extract of *Quassia amara* (mean value = $3.38 \pm 0.20 \times 10^6$/ ml) and Quassin (mean value = $3.15 \pm 0.15 \times 10^6$/ ml) were significantly reduced when compared to 2-methoxycanthin-6-one (mean value = $4.88 \pm 0.15 \times 10^6$/ ml) and control rats (mean value = $5.1 \pm 0.28 \times 10^6$/ ml).

In the recovery groups and the groups that were treated with crude methanol extract of *Quassia amara* plus ovine LH and Quassin plus ovine LH, the sperm counts as well as the serum testosterone concentrations were not different from the control. Groups treated with the crude methanol extract of *Quassia amara* and Quassin did have significantly decreased concentrations of LH, follicle-stimuating hormone (FSH), and testosterone (Raji and Bolarinwa 1997).

Lidocaine

Although lidocaine has not been studied in pregnant women, it has not been shown to cause birth defects or other problems in animal studies (National Library of Medicine 2005).

GENOTOXICITY

Brucine

The National Toxicology Program (NTP) reported an Ames assay for Brucine mutagenesis completed in 1982 (NTP 2004). The strains of Salmonella used were: TA100, TA1535, TA1537, and TA98. All of the strains were dosed up to 6666 μg/plate, with and without metabolic reaction. Brucine was negative in all tests.

Denatonium Benzoate

CTFA (2005) provided the results of two studies in which plant materials extracted with SD Alcohol 40-B were evaluated in a chromosome aberration assay using Chinese hamster ovary (CHO) cells in culture. In both studies, CHO cells were incubated in growth medium, with or without Aroclor-induced S9 fraction metabolic activation. Positive controls were mitomycin C for the "without activation" comparison and cyclophosphamide for the "with activation" comparison. The second study used a vehicle control, whereas the first study relied on historical control values.

Macerated roots (plant species not identified) were extracted in SD Alcohol for 2 weeks. The final level of SD Alcohol 40-B in the plant preparation was 45% (0.006% Denatonium Benzoate and 0.12% t-butyl Alcohol).

Replicate CHO cultures were incubated with 33.9, 48.4, 69.1, 98.7, 141, 202, 288, 412, 589, 841, 1200, 1720, 2450, 3500, and 5000 μg/ml of each plant extract for 3 h, with and without metabolic activation. In each case, the final SD Alcohol 40-B concentration at the highest dose was 0.225% (0.013 ppm Denatonium Benzoate). Cells from the four highest dose groups in each study were analyzed for chromosome aberrations.

In the first study, reductions in the mitotic indices were noted in the five highest dose groups without metabolic activation, but the effect was not dose-related. A smaller reduction in mitotic indices was seen for only three dose levels with metabolic activation, but again the effect was not dose-related. No increase in cells with chromosome aberrations, polyploidy, or

endoreduplication was observed in the four highest dose groups, compared to historical controls.

In the second study, there were no reductions in mitotic indices in cultures at the five highest dose levels without metabolic activation, and only slight reductions (<10%) for the three highest dose levels with metabolic activation. The authors reported a statistical anomaly in this study because the vehicle control group with metabolic activation had no chromosome aberrations. Treatment groups, however, were within the range of historical controls, so the authors concluded that there were no increases in cells with chromosome aberrations, polyploidy, or endoreduplication in the four highest dose groups compared to historical controls (CTFA 2005).

CARCINOGENECITY

Denatonium Benzoate

IRDC (1977b, 1978) conducted a 2-year chronic toxicity study using Charles River CD rats. The doses of Denatonium Benzoate administered by gavage were 1.6, 8, and 16 mg/kg/day. Sixty-five males and 65 females (same in the control group) were observed weekly for any changes. General behavior and appearance were observed, ophthalmoscopy performed, body weights measured, food consumption and efficiency determined, and hematological, biological, and urinalysis studies conducted.

The coagulation times for female rats (1.6 mg/kg/day dose levels) and male and female rats (8 and 16 mg/kg/day dosage levels) were slightly higher than the control groups. Twenty control rats and 20, 26, and 19 rats at the 1.6, 8, and 16 mg/kg/day dose levels, respectively, died during the first 78 weeks of the study. There were no compound-related gross pathologic lesions or variations in organ weights of the rats.

The authors concluded that there were no changes that were related to Denatonium Benzoate in general behavior and appearance, ophthalmoscopy, body weights, food consumption and efficiency, biochemical studies, or urinalyses. A histopathologic evaluation concluded that the types and incidences of neoplasms did not indicate any tumorigenic effect related to administration of Denatonium Benzoate (IRDC 1977b, 1978).

CLINICAL ASSESSMENT OF SAFETY

Dermal Sensitization and Irritation

Brucine

AMA Laboratories Inc. (1998a) tested a product with SD Alcohol 40 at 95% in a product at 47% (~ 44.7% SD Alcohol 40) in a repeated insult patch test (RIPT) with 110 subjects. Only 104 subjects, aged 18 to 65, completed the study. A dose measuring 0.2 ml or 0.2 g of SD Alcohol 40 was dispensed onto a semiocclusive, hypoallergenic patch. After 24 h, the patch was removed by the subject. This procedure was repeated for nine consecutive 24-h exposures. The area of erythema and edema was measured and reactions were scored approximately 24 h after removal of the patch. Subjects were then subjected to a period of 10 to 14 days without treatment and then a challenge was applied once to a previously unexposed test site. The dose was equivalent to that used in the previous 9 exposures, and the reactions were scored 24 and 48 h after application.

None of the subjects that completed the study had any adverse reactions to SD Alcohol 40 (95%). The product with SD Alcohol 40 (95%) was described as a non-primary irritant and a non-primary sensitizer to the skin (AMA Laboratories Inc. 1998a).

AMA Laboratories Inc. (1998b) tested a product with SD Alcohol 40 (95%) in product at 47% (~ 44.6% SD Alcohol 40) using a repeat-insult patch test. This study included 109 subjects (aged 18 to 64) with 105 of them completing it. The exact same procedure was performed as above (AMA Laboratories Inc. 1998a). During observations, no adverse reactions were noted during the course of the study and since there were no reactions to the product, SD Alcohol 40 (95%) was again classified by the testing laboratory as a nonprimary irritant and a nonprimary sensitizer to the skin.

Two RIPTs were performed using products containing 74% and 72% SD Alcohol 40-2 with Brucine (CTFA 1998).

The first RIPT was conducted on 204 subjects, 200 of them completing the test. Nine consecutive, 48-h occlusive patches of a product with 74% SD Alcohol 40-2 were used in the induction phase, followed by a challenge phase after 10 to 14 days of nontreatment consisting of a 48-h occlusive patch on the induction site, with skin evaluations at 48 and 96 h post application and a 48-h occlusive patch on naive site with skin evaluation at 48-h post application.

Two subjects reacted during the challenge phase. One subject had a +1 reaction (patchy erythema less than 50% of test site) at the 96-h reading to the first challenge patch application. There was no reaction to a second challenge patch test. The other subject had a +2 reaction (erythema of the entire site) at the 48-h reading of the first challenge patch application and a +3 (erythema and induration) at the 96-h reading of the same patch site, but did not have a reaction to the second challenge patch application. This particular subject also had Induction scores of +1 and +2, which led to a rechallenge with the intact product 5 weeks after the completion of the RIPT. The results of this test were negative.

It was concluded that the particular subject's reactions were due to skin fatigue rather than sensitization. It was also concluded that the sensitization potential of the product with 74% SD Alcohol 40-2 is very low if at all existent.

The second RIPT was conducted on 202 subjects. A product containing 72% SD Alcohol 40-2 was used, and the exact same protocol as above was used. One panelist had a +1 reaction at the 48-h reading of both challenge patch applications, but did not have a reaction at the 96-h reading of the first challenge patch application. Again, it was concluded that the product with 72% SD Alcohol 40-2 has a low potential for irritation and sensitization (CTFA 1998).

AMA Laboratories Inc. (1999a) performed an RIPT on SD Alcohol 40 (95%) in a product at concentration of 40%. There were 110 subjects in the study, but only 106 of them completed the study (ages ranged from 18 to 64). AMA Laboratories Inc. performed the test, in the same manner as the previous two studies, by dispensing 0.2 ml or 0.2 g onto an occlusive, hypoallergenic patch. The patch was removed by the subject 24 h later, and the procedure was repeated for nine consecutive 24-h periods. Erythema and edema were measured and reactions were scored 24 h after each patch removal. A 10- to 14-day period of nontreatment was then followed by a challenge period where a previously unexposed test site was tested with the same dose as before. Reactions were then scored 24 and 48 h after application. None of the subjects had any adverse reactions to the product with SD Alcohol 40 (95%), and it was classified as a nonprimary irritant and a nonprimary sensitizer to the skin by the testing laboratory.

AMA Laboratories Inc. (1999b) used an RIPT to evaluate the skin irritation/sensitization potential of a product (tonic, dressing, other hair-grooming aid product category) containing 47% SD Alcohol 40 (95%) denatured with Brucine Sulfate. One hundred and seven subjects enrolled, but only 104 completed the test (71 females and 36 males). A semiocclusive, hypoallergenic patch containing 0.2 ml or 0.2 g of the test material was applied to the subjects on their upper left back. The patches were removed 24 h later, and the procedure was repeated for a series of nine consecutive 24-h exposures over 3 consecutive weeks. The test sites were scored just before applications 2 to 9 and the next test date following application 9. There was a 10- to 14-day rest period, which was then followed by a challenge dose. There were no adverse reactions noted during the entire course of the study, so it was concluded that the product containing 47% SD Alcohol 40 (95%) denatured with Brucine Sulfate may be classified as a non-primary irritant and a non-primary sensitizer to the skin.

AMA Laboratories Inc. (2001) tested SD Alcohol 40 (95%) present in a product at 47% in an RIPT. The tests originated with 110 subjects, but 108 subjects (aged 19 to 65) completed the patch tests. The testing laboratory used the exact same protocol as in the studies described above. No adverse reactions were observed, and the product with SD Alcohol 40 (95%) was classified as a non-primary irritant and a non-primary sensitizer to the skin.

Denatonium Benzoate

CTFA (1984a) reported results of an RIPT conducted on 91 individuals (84 female and 7 male) to determine if a deodorant body spray containing 98.2% SD Alcohol 40-B induces skin irritation and/or allergic sensitization. Ten patches were placed on the upper backs of the subjects (five on each side). About 0.1 ml of the test material was added to the patches before applications. The patches were applied every Monday, Wednesday, and Friday for 3 consecutive weeks, for 24 h. A challenge was conducted in week 6 of the study, and the reactions were scored 24 and 48 h after removal.

Only four reactions occurred throughout the entire study, including the challenge phase, in which those subjects received a mark of "barely perceptible; minimal faint (light pink) uniform or spotty erythema." It was concluded that the deodorant body spray containing 98.2% Alcohol 40-B does not exhibit any potential for inducing allergic sensitization (CTFA 1984a).

CTFA (1984b) reported that another RIPT was conducted with a night cream containing 61.3% SD Alcohol 40-B. One hundred and six individuals participated in the study, of which 101 were females and only 5 were males. Ten patches were applied to the left and right side of the upper back every Monday, Wednesday, and Friday for 3 consecutive weeks. About 0.1 ml of the test material was added to each of the patches. A challenge was conducted in week 6 of the study.

The highest score that any individual received throughout the entire study was "mild; pink uniform erythema covering most of the contact site"; however, this score was not very common. It was concluded from the study that the night cream with 61.3% Alcohol 40-B does not exhibit any potential for inducing allergic sensitization (CTFA 1984b).

AMA Laboratories Inc. (1996) conducted an RIPT using 33% SD Alcohol 40-B (denatured with Denatonium Benzoate and t-butyl Alcohol), which was used in a foot massage gel (percentage not stated). Of the112 subjects enrolled, only 103 completed the study (ages ranged from 16 to 76). SD Alcohol 40-B (0.2 ml or 0.2 g) was dispensed onto the semiocclusive, hypoallergenic patch, and placed on the infrascapular regions of the back of the subject. The subject removed the patch 24 h later, and the procedure was repeated for nine consecutive 24-h periods. Any signs of a reaction (erythema or edema) were measured and recorded 24 h after patch removal. The subjects were given a 10- to 14-day period of nontreatment, which was followed by a challenge dose. This dose was applied once to a previously unexposed area and was equivalent to the previous nine exposures. Any sign of reaction was scored 24 and 48 h after application.

No adverse reactions of any kind to 33% SD Alcohol 40-B were noticed during the study, which led the conclusion that SD Alcohol 40-B at 33% is a nonprimary irritant and a nonprimary sensitizer (AMA Laboratories Inc. 1996).

Harrison Research Laboratories, Inc. (2002a) conducted a Draize sensitization RIPT using a gel formula that contained 29% SD Alcohol 40-B. There were 235 subjects inducted into the study, but only 216 completed the test. A webril/adhesive patch was used semiocclusively. The patch contained 0.2 g of test material and was placed on the left side of the subject's back on Monday, Wednesday, and Friday. The patches were left in place for 24 h and then removed and reactions were scored. This cycle was continued for a total of nine induction patchings over a period of 3 weeks. A rest period of 2 weeks was followed by a challenge phase, during which a patch was placed on a virgin site and removed 24 h later. Subjects were observed at 48, 72, and 96 h. During the induction and challenge phases, low-level, transient (±/1) reactions were noted. The gel formulation containing 29% SD Alcohol 40-B did not induce dermal sensitization.

Harrison Research Laboratories, Inc. (2003a, 2003b, and 2003c) conducted three separate RIPTs for SD Alcohol 40-B (82%) in a preshave lotion. During each test a webril/adhesive patch was used semiocclusively with 0.2 g of SD Alcohol 40-B applied to each patch. The left side of the back was the test area on each subject for the induction phase. The patches remained in place for 24 h and then removed by the subject. The cycle of patch removal was every 24 h during the weekday and 48 h over the weekend. Nine induction patches were completed over a period of 3 consecutive weeks. The nontreatment rest period lasted for 2 weeks, and was followed by a challenge phase. The right side of the back was used as the testing area, and patches were removed 24 h later, with recording made at 48, 72, and 96 h.

The first test (Harrison Research Laboratories, Inc. 2003a) started with 115 subjects, of which 103 completed the test. No reactions occurred during the induction or challenge phase.

The second test (Harrison Research Laboratories, Inc. 2003b) started with 115 subjects, with 103 of them completing the test. During the induction phase, one subject had a low-level, transient (±) reaction (faint, minimal erythema). At the challenge, an additional subject had a low-level, transient (±/1) reaction (faint, minimal erythema/erythema).

The third test (Harrison Research Laboratories, Inc. 2003c) started with 121 subjects, of which 102 completed the test. During the induction phase, one subject had a low-level (±) reaction (faint, minimal erythema). At the challenge, no reactions were noted.

All three RIPTs concluded that the product with 82% SD Alcohol 40-B did not induce dermal sensitization in human subjects (Harrison Research Laboratories, Inc. 2003a, 2003b, 2003c).

Harrison Research Laboratories, Inc. (2003d) conducted an RIPT on 115 subjects using an "other shaving preparation" containing 82% SD Alcohol 40-B (95%). Approximately 0.2 g of the test material was applied to a semiocclusive patch and adhered to the left side of the subject's upper back. The patches were removed after 24 h and repatched again after 24 h on a weekday and 48 h after a weekend patch removal. This cycle continued for 3 consecutive weeks until 9 induction patchings were completed. A challenge phase was conducted approximately 2 weeks after the final induction patch. A total of 103 subjects completed the test: 24 males and 79 females aged from 19 to 69. There were no reactions observed on the 103 subjects by the "other shaving preparation" containing 82% SD Alcohol 40-B (95%).

Harrison Research Laboratories, Inc. (2004a) conducted an RIPT on a spray formula that contained 12.0% SD Alcohol 40-B. The exact same protocol was used in this study as mentioned above (Harrison Research Laboratories, Inc. 2002a). There were 240 subjects inducted into the study, but only 209 completed the test (withdrawals were not due to reactions test material). During the induction phase, 4 subjects had low-level, transient reactions. At the challenge, no reactions were noted. Harrison Research Laboratories, Inc. concluded that there was no dermal sensitization in human subjects.

Taste Aversion

Denatonium Benzoate

Berning et al. (1982) stated in a taste test among 108 children aged 18 to 47 months that addition of 11.4 ppm Denatonium Benzoate to detergents containing alcohol resulted in immediate and intense aversion, indicating that addition of denaturant to alcohol-containing products would reduce the probability of an accidental ingestion.

Sibert and Frude (1991) conducted a study in 33 children (ages 17–36 months old) in which a 10 μl sample of orange juice, with & without 10 ppm of Denatonium Benzoate, was placed on the back of the tongue. Volunteers then rated how tolerable the test substances were. The children expressed their reactions to the Denatonium Benzoate solution with facial grimaces, expressions of shock, pronounced mouth movements, crying, and vomiting.

Jackson and Payne (1995) tested 20 normal males aged 18 to 45 years old with ethylene glycol and methanol samples with and without Denatonium Benzoate. A 10-μl sample was put on the back of their tongue and the volunteers rated how tolerable it was. The authors concluded that the addition of Denatonium Benzoate to products would reduce the tolerance level and make the product less likely to be ingested.

Photoallergy

Brucine Sulfate

A sunscreen product containing 35% SD Alcohol 40 (denatured with Brucine Sulfate) was evaluated for photoallergy among 103 fair-skinned subjects. Occlusive patches were applied for 24 h, the patches were removed, and 15 min later the sites were irradiated with 3 minimal erythema dose (MED) of ultraviolet A/B (UVA/UVB). Six patches were irradiated over a period of 3 weeks. A challenge phase followed after a 14-day rest period. The sites were irradiated after the removal of the patch with 10 J/cm^2 UVA + 0.5 MED UVA/UVB. The challenge sites were scored at 48 and 72 h following irradiation. All 103 subjects received a score of 0 "normal skin." The test product does not induce photoallergy (CTFA 2000c).

A sunscreen product containing 72.4% SD Alcohol 40 (denatured with Brucine Sulfate) was evaluated for photoallergy among 103 fair-skinned subjects. This study used the same protocol as the previous study. It was concluded that this test product does not induce photoallergy because all reactions were scored as 0 "normal skin" (CTFA 2000d).

A sunscreen product containing 74.5% SD Alcohol 40 (denatured with Brucine Sulfate) was evaluated for photoallergy among 103 fair-skinned subjects. The protocol for this study was the same as the previous two studies. The results of this study coincided with the previous two studies; all reactions to the test material were scored as normal skin, and it was concluded that the test product did not induce photoallergy (CTFA 1999b).

Denatonium Benzoate

Harrison Research Laboratories, Inc. (2002b) evaluated the potential of a gel formula that contained 29% SD Alcohol 40-B to induce dermal photoallergy in human subjects. There were 29 fair-skinned subjects used in the study. Approximately 0.2 g of the gel formula was applied to each patch and placed on the subject's volar forearm or back. There were additional sites patched; one was to be irradiated, but no test material was applied to the patch, and one to be patched with the test material but not irradiated. Twice a week, for the first 3 weeks, patches were applied to the identical sites for a total of six induction patchings. After 24 h the subjects returned to the lab to be irradiated with both UVB and UVA, and immediately thereafter the test site was scored and recorded. Subjects were exposed to the UVA radiation source for 17 min (95% of the output for the UVA light was in a wavelength range between 320 and 400 nm). The UVB radiation source had an output in the range of 280 to 320 nm. The duration of exposure was based on each subject's skin type and MED as determined by the first irradiation. A challenge phase followed a rest period of 2 weeks in which a patch was placed on a virgin site for 24 h. The sites were irradiated with UVA light only and reactions were scored immediately. Subjects were also observed at 48 and 72 h.

During the induction phase, low-level, transient reactions occurred at the irradiated test material contact site. One subject had a low-level, transient reaction on the nonirradiated test material site. Low-level, transient reactions occurred on the irradiated (no test material) control site. During the induction phase, the irradiated sites (with and without test material) were observed to have slight tanning responses. At the challenge, no reactions occurred on either the irradiated or the nonirradiated test material sites. No reactions occurred on the irradiated (no test material) control site and the gel formulation containing 29% SD Alcohol 40-B did not induce dermal photoallergy (Harrison Research Laboratories, Inc. 2002b).

Harrison Research Laboratories, Inc. (2004c) used a spray liquid formula containing 12% SD Alcohol 40-B to induce dermal photoallergy in human subjects. There were 33 subjects initially at the start of the study, but only 30 subjects completed the test (dropout not due to test material reaction). The protocol described above was used, including amount of test material applied to patch (0.2 g), patch timing (24 h), duration of study (six induction patchings followed by rest period and challenge), and the amount and time of UV light exposure (UVA: peak output 369 nm/17 min; UVB: peak output 313 nm/time determined by subject's skin type and MED).

During the induction phase, low-level, transient reactions occurred at the irradiated test material site, but no reactions were present on the nonirradiated test material site. There were also low-level, transient reactions at the irradiated (no test material) control site. As in the study above as well, there was a slight tanning response at the irradiated sites (with and without test material).

At challenge, one subject had a low-level, transient reaction on the irradiated test material contact site. No reactions occurred at the non-irradiated test material site and irradiated (no test material) control site. It was concluded that this spray formula containing 12% SD Alcohol 40-B did not induce dermal photoallergy or dermal sensitization in the subjects (Harrison Research Laboratories, Inc. 2004c).

Phototoxicity

Brucine Sulfate

CFTA (1999a) reported results of phototoxicity evaluation of a sunscreen product containing 74.5% SD Alcohol 40 (denatured with Brucine Sulfate) in 22 fair-skinned subjects. An occlusive patch was applied for 24 h and followed by UVA irradiation (20 J/cm^2) and $^1/_2$ MED of UVA/UVB light. The reactions were scored on a scale of 0 to 5 and recorded immediately 24 and 48 h after irradiation.

At the site where the product was irradiated, two subjects at 24 h had a score of 1. There were two subjects that had a score of 1 at 24 h for the site that had product applied, but not irradiated. At the irradiated site where no product was applied, 16 subjects (at 24 h) and 6 subjects (at 48 h) had a score of 1. All other reactions in the subjects were 0. Based on this data, the test product was not phototoxic (CFTA 1999a).

CTFA (2000a) reported results of an evaluation of a sunscreen product containing 35% SD Alcohol 40 (denatured with Brucine Sulfate) for phototoxicity in 22 fair-skinned subjects using the protocol described above.

At 24 h, two subjects had a score of 1, which was "minimal visible erythema" at the irradiated product test site. At 24 h, two subjects had a score of 1 "minimal visible erythema" at the unirradiated site where the product was applied. At 24 h, 16 subjects received a score of 1 "minimal visible erythema" at the irradiated site where no product was applied. There were six subjects at 48 h with a score of 1 "minimal visible erythema" at the irradiated site to which no product was applied. All the irradiated and non-irradiated product test sites had a score of 0 "normal skin." Based on this data the sunscreen product was not phototoxic (CTFA 2000a).

CTFA (2000b) reported results of a sunscreen product containing 72.4% SD Alcohol 40 (denatured with Brucine Sulfate) evaluated for phototoxicity among 22 fair-skinned subjects. The protocol of this study was the same as above. At 24 h, there were five subjects that had a score of 1, and at 48 h, there were two subjects that had a score of 1 at the irradiated product site. At the site where the product site was nonirradiated, at 24 h, there were six subjects that had a score of 1. At 48 h, two subjects had a score of 1. At the irradiated site where no product was applied, 16 subjects (at 24 h) and 6 subjects (at 48 h) had a score of 1. All other reactions were given a score of 0. Based on this data, it was concluded that the test product was not phototoxic in humans (CTFA 2000b).

Denatonium Benzoate

Harrison Research Laboratories, Inc. (2001a) evaluated the potential of a gel formula, containing 29% SD Alcohol 40-B, to induce a phototoxic response in human subjects. Ten subjects participated in the study in which a webril/adhesive patch was used semiocclusively. A patch containing 0.2 g of the gel was adhered to the subject's volar forearm or back. The site that was not irradiated was on the subject's back. The patches were worn for 24 h, and then were exposed to UVA (95% output in a wavelength range between 320 and 400 nm) for 17 min. All subjects were observed and reactions were scored. At 48 and 72 h, the subjects were observed again and reactions were scored. There were no visible reactions to the UVA light, so Harrison Research Laboratories, Inc. stated that there was no dermal phototoxicity response in human subjects to the gel formula.

Harrison Research Laboratories, Inc. (2004b) tested the potential of a spray liquid formula that contained 12% SD Alcohol 40-B to induce a phototoxic response in humans. The protocol for this study was as above (Harrison Research Laboratories, Inc. 2001a). All 10 subjects completed the test. No reactions occurred on the irradiated or nonirradiated (both test material and no test material sites). It was concluded that this spray formula does not induce a dermal phototoxic response in humans.

Case Studies

Denatonium Benzoate

A 30-year-old male with no history of atopy, atopic symptoms, or any skin diseases developed pruritus on the face, arms, and hands along with asthma-like symptoms after working with several products. About 2 to 3 h after coming in contact with these products, small red papules were visible at the above mentioned sites. He was patch tested for 48 h with an insecticidal spray (Pyrex), an antifrost liquid (T-rod), an ethanol containing window cleaner, a carburetor spirit, an aftershave lotion, and a skin disinfectant (M-sprit). All of these products contained ethanol and Denatonium Benzoate as a denaturant. He tested positive for Denatonium Benzoate during an open patch test with petechia, wheals, and flare with concentrations of 2×10^{-2}, 2×10^{-3}, and 2×10^{-4} mg/L; petechia and erythema with 2×10^{-5} mg/L; and erythema with 2×10^{-6} mg/L, but a negative reaction was observed when diluted to 2×10^{-7}mg/L. Application of Denatonium Benzoate at the test site elicited a wheal and flare response after 20 min. All 10 control subjects were negative (Bjorkner 1980).

PART II: ETHANOL CHEMISTRY

Definition and Structure

The formula of ethanol is CH_3CH_2OH. The CAS number for ethanol is 64-17-5. By convention, ethanol used in cosmetics is called Alcohol (Gottschalck and McEwen 2004).

Chemical and Physical Properties

Ethanol is a clear, colorless, very mobile, flammable liquid with a pleasant odor and burning taste. Ethanol absorbs water rapidly from air. It has a molecular weight of 46.07, a closed-lid flash point of 13°C, density at 20°C of 0.789g/L, boiling point of 78.5°C, and melting point of −114.1°C. Ethanol is miscible with water and many other organic liquids (Budavari 1989).

Method of Manufacture

Ethanol can be manufactured by a variety of different methods and sources, including fermentation of starch, sugar, and other carbohydrates; isolated from ethylene, acetylene, sulfite waste liquors, synthesis gas (CO + H); made by hydrolysis of ethyl sulfate; and produced by oxidation of methane (Budavari 1989).

Analytical Methods

The purity of ethanol may be analyzed using gas chromatography with flame ionization detection (Fisher Scientific 2003).

Impurities

Some of the common impurities found in aqueous 70% to 95% ethanol are methyl isobutyl ketone, n-heptane, and acetone (Medical Chemical Corporation 1992; Americhem Sales Corporation 2000).

USE

Cosmetic

According to the *International Cosmetic Ingredient Dictionary and Handbook* (Gottschalk and McEwen 2004), Alcohol functions as an antifoaming agent, antimicrobial agent, cosmetic astringent, fragrance ingredient, and viscosity-decreasing agent. Table 4 includes cosmetic uses and concentrations for Alcohol that have been reported to FDA and CTFA.

GENERAL BIOLOGY

Absorption

Litovitz (1986) noted that ethanol is rapidly absorbed from the gastrointestinal tract, reaching peak levels within 30 to 60 min. Ethanol concentrations below 10% or above 40% are more slowly absorbed.

Dermal

In a study conducted in nonhuman primates and on human skin samples, Scott et al. (1991) found that there was no apparent relationship between skin structure and permeability to the most rapid penetrants, water and ethanol.

Schaefer and Redelmeier (1996) suggested that exposure of 1000 cm^3 of skin to 70% ethanol for less than 1 h will produce approximately 100 mg of absorption of ethanol, which can

TABLE 4
Current cosmetic product uses and concentrations for Alcohol

Product Category (Total number of products in each category (FDA, 2002))	Ingredient uses in each product category (FDA, 2002)	Use concentrations (CTFA, 2003) (%)
Bath products		
Oils, tablets, and salts (143)	6	0.03
Soaps and detergents (421)	1	0.06
Bubble baths (215)	—	0.2
Other (196)	14	0.01–0.3
Eye makeup		
Eyeliners (548)	3	0.2
Eye shadow (576)	—	0.0002
Eye lotions (25)	1	6.0
Mascara (195)	—	0.4–4
Fragrance products		
Colognes and toilet waters (684)	97	52–93
Perfumes (235)	40	82
Powders (273)	2	—
Other (173)	11	93–98
Noncoloring hair care products		
Conditioners (651)	5	0.08–91
Sprays/aerosol fixatives (275)	1	76
Shampoos (884)	4	0.03–0.09
Tonics, dressings, etc. (598)	19	6–82
Other (277)	10	—
Hair coloring products		
Color sprays (5)	1	—
Makeup		
Face powders (305)	—	0.003
Foundations (324)	—	0.1
Lipstick (962)	—	0.007–1
Makeup bases (141)	3	—
Other (201)	3	0.8
Nail care products		
Basecoats and undercoats (44)	1	—
Nail polishes and enamels (123)	4	4
Nail polish and enamel removers (36)	5	—
Other (55)	3	0.08
Oral hygiene products		
Dentifrices (40)	3	—
Mouthwashes and breath freshener sprays (46)	4	8–30
Other (6)	1	—
Personal hygiene products		
Underarm deodorants (247)	28	20–90
Douches (5)	—	0.9–2
Other (308)	8	—
Shaving products		
Aftershave lotions (231)	28	0.2–53
Other (63)	4	0.001

TABLE 4
Current cosmetic product uses and concentrations for Alcohol *(Continued)*

Product Category (Total number of products in each category (FDA, 2002))	Ingredient uses in each product category (FDA, 2002)	Use concentrations (CTFA, 2003) (%)
Skin care products		
Skin cleansing creams, lotions, liquids, and pads (775)	14	0.04–88
Depilatories (34)	—	17
Face and neck creams, lotions, powders, and sprays (310)	9	20–35
Body and hand creams, lotions, powders, and sprays (840)	13	0.004–65
Foot powders and sprays (35)	2	0.12
Moisturizers (905)	27	0.0008–3
Night creams, lotions, powders, and sprays (200)	6	—
Paste masks/mud packs (271)	17	0.2–0.5
Skin fresheners (184)	12	0.04–30
Other (725)	19	2–30[a]
Suntan products		
Suntan gels, creams, liquids, and sprays (131)	2	0.008
Indoor tanning preparations (71)	1	2
Total uses/ranges for Alcohol	435	0.0002–90

[a]one product containing 14% is a facial toner

be equated to 1.5 ml of wine containing 10% (*v*/*v*) ethanol. Therefore, the authors concluded that skin exposure to ethanol in cosmetics is not a safety concern.

Pendlington et al. (2001) found the half-life of evaporation of ethanol from whole pig skin to be 11.7 seconds. In the same study, skin penetration experiments revealed that the amount of ethanol that penetrated through the pig skin was greater in the occluded cells (with parafilm) than in the nonoccluded cells. The maximum flux through the skin in the nonoccluded cells was reached by the first hour. The flux rate in the occluded cells peaked at 2 h. The total recovery for the nonoccluded cells was less than 3%, indicating that most of the ethanol had evaporated before being able to penetrate the skin.

Human use experiments were conducted in 16 adult volunteers by Pendlington et al. (2001). An aerosol-based ethanol-preparation was sprayed over the body for 10 seconds followed by a 15-min waiting period. Blood alcohol concentration was determined using two different columns in gas chromatography. Rig 1 was a 30-m megabore capillary column of DB wax (a Polyethylene 400 equivalent) and Rig 2 was a 1/4-inch glass column of 0.2% Carbowax 1500 on Carbopak C60–80 mesh. Only 22 samples out of 96 showed presence of ethanol. The maximum concentration recorded was 1.3 mg/100 ml. However, no blood sample was positive for the presence of alcohol using both the columns. The authors reached a conclusion that use of alcohol-based sprays will not lead to a toxicologically significant blood alcohol level.

According to a material safety data sheet prepared by the Union Carbide Corporation (2000), there was no evidence of harmful effects from skin contact of ethanol (200 proof).

Inhalation

Mangione (1985) observed that inhalation of vapors of ethanol during use of rubbing alcohol can result in sufficient absorption to result in alcohol intoxication in children.

Kruhoffer (1983) studied the rate of uptake of ethanol from prolonged periods of inspiration of air containing ethanol at a constant concentration of 10 to 12 mg/L (about 6000 ppm) in adult, male human subjects. The study indicated that, when ethanol is absent from the systemic blood, prolonged inspiration of ethanol-containing air results in absorption of 55% of the inspired ethanol and return of remainder with the expired air. There is a breath-by-breath increase in the expiratory concentration of ethanol until a constant level is approached after some 15 to 20 respirations. This phenomenon represents a gradual washout by ethanol-free alveolar air of an airway depot built up during the preceding inspirations of ethanol containing air. The author also concluded that during the ethanol inhalation period, ethanol will be eliminated from the body at a slightly lower rate than in the preinhalation period because the inhalation of ethanol will abolish elimination by way of the respiratory organs.

Distribution

Parenteral

Most of the studies of acute toxicity have been conducted using ethanol. As noted earlier, ingestion of Alcohol Denat. is unlikely, so these data are considered of limited relevance to the safety of Alcohol Denat., and only representative reports are cited.

Wiberg et al. (1970) reported a comparison of LD_{50} between young (3 to 4 months of age) and old (10 to 12 months of age)

male albino rats of the Wistar strain (groups of 10 animals each, i.p. injection of 15% *w/v* or oral 40% *w/v*) and showed that the older rats had a lower threshold of LD_{50} than younger rats (5.1g/kg versus 6.7 g/kg for i.p., and 7.06 g/kg versus 10.6 g/kg for oral). In vivo metabolism in the same study showed that blood alcohol levels after 30 min of dosing 3 g/kg of 15% ethanol were higher in young rats, but declined more rapidly in young rats. Also, the brain concentrations of alcohol were consistently higher in old rats.

Inhalation

Freeman (1984) observed that rubbing alcohol, consisting of 70% to 90% ethanol or propanol, is known to cause toxic effects following inhalation of vapors from topical rubs and stated that absorption through skin may pose a significant route especially if the skin is damaged.

Pastino et al. (1997) exposed 10-week-old male and female F-344 rats and $B6C3F_1$ mice to 600 ppm of ethanol for 6 h and found that steady-state blood ethanol concentrations were reached within 30 min in rats and within 5 min in mice.

Maximum blood alcohol levels ranged from 71 μM in rats to 105 μM in mice. Exposure to 200 ppm ethanol for 30 min resulted in peak blood ethanol levels of approximately 25 μM for mice and 15 μM for rats.

Peak blood alcohol levels of 10 μM were measured following exposure to 50 ppm in female rats and male and female mice, whereas ethanol was undetectable in the blood of male rats. Other than this, no sex-dependent differences in blood alcohol concentrations were observed in rats and mice exposed to ethanol at any of the concentrations studied.

A physiologically based pharmacokinetic model predicted that peak blood alcohol levels lower than 25 μM would be achieved in rats following exposure to 50 ppm for 6 h and peak blood alcohol levels of 300 μM would be achieved in human males following 6-h exposure to 600 ppm ethanol. In humans, behavioral effects of ethanol become apparent at much higher blood alcohol concentrations. For example, a decrease in reaction time, diminished fine motor coordination, and impaired judgement are observed when blood alcohol concentrations reach approximately 4 to 7 mM (Pastino et al. 1997).

Oral

Cook et al. (1975) reported severe alcohol intoxication in a newborn whose mother had consumed bourbon whiskey 30 min prior to delivery for relief of labor pain, indicating acute cross-placental ethanol transport.

Penetration Enhancement

Scheuplein and Blank (1973) found that ethanol increases irreversibly the permeability of the skin more than twofold, and has a permeability coefficient of approximately 8×10^{-4} cm/h. The authors stated that since the permeation of stratum corneum is a passive diffusion process, there can be no fundamental differences between in vitro or in vivo permeation as the result of any vital process, e.g., active transport. Pure liquid ethanol altered the stratum corneum to much greater degree than higher alcohols such as butanol and propanol. The mobility of the alcohol molecules in the dermis was approximately 4 orders of magnitude greater than through stratum corneum.

Roberts and Anderson (1975) stated that the extent of hydration of the stratum corneum is significant in the penetration of most compounds through skin. The increase in the penetration ratio observed for water as a vehicle compared to that of ethanol may be attributed to this effect. They stated that ethanol also suppresses the barrier resistance of the stratum corneum, presumably as a result of structure alterations.

Addition of ethanol increases permeability of chemicals through the skin (Kitagawa and Li 1997; Kitagawa et al. 1997), at least partly by perturbation of the stratum corneum lipid lamella. Disruption of lipid packaging permits easier diffusion through the skin, which will promote the penetration of polar molecules.

While studying skin applications of levosimendan, Valjakka-Koskela et al. (2000) showed that dissolving the chemical in 10% to 90% ethanol enhances solubility of this moderately lipophilic compound, resulting in better penetration. They also proposed that better penetration through human cadaver skin is due to increased concentration gradient across the skin and that permeability changes due to ethanol were minimal.

Riviere et al. (2001) argued that current dermal risk assessment methodologies, which emphasize absorption of the chemical alone or at best from a single solvent, ignore interactions that can occur when exposure is to a mixture of chemicals. Using the mechanistically defined chemical mixture approach, mixtures can be developed to include components that alter the percutaneous absorption process through well-defined mechanisms. Surfactants like sodium lauryl sulfate are irritants and may alter the barrier properties of the skin and so affect absorption. Pentachlorophenol, a known skin penetrant, was minimally absorbed from an ethanol vehicle, the addition of water drastically increased the extent of absorption. Dermal risk assessments are commonly conducted using binary mixtures with ethanol or acetone, but the above results indicate that such studies may significantly underestimate the risks posed by real-world exposures in aqueous solutions, which would in turn directly impact on toxicity evaluations. The effects of mixture components on the percutaneous absorption process can be significant for some compounds that may possess certain structural and thermodynamic characteristics that limit the extent of their absorption.

Using human cadaver skin samples, Nielsen (2002) showed that 50% ethanol slightly enhances percutaneous transport properties without compromising the general integrity of the dermal barrier.

ANIMAL TOXICOLOGY

Acute Toxicity

Inhalation

Karanian and Salem (1988) studied the effect of ethanol inhalation on the cardiovascular state of male rats by exposing the

animals to alcohol vapors for one day. Plasma catecholamine levels and blood pressure were measured. The blood alcohol level was maintained at >120 mg% throughout the exposure period. The results indicated that the acute exposure led to elevated blood pressure with marked increase in plasma catecholamine levels.

Subcutaneous

Barlow (1935) reported that subcutaneous injections of 0.1 ml to 0.4 ml of 50% absolute alcohol in the rabbit ear led to whitened, and later, necrotic areas at the site of injection, and to inflammation in the surrounding areas. Two days later the healing was complete. At higher concentrations, sloughing and perforation along with inflammation and necrosis were observed at the site of injection.

Ocular

Carpenter and Smyth (1982) reported that the instillation of 0.1 ml of undiluted ethanol in eyes of five normal albino rabbits caused severe injury. Severe injury was defined by authors as necrosis covering about three-fourths of the surface of the cornea as observed after staining with fluorescein dye or severe necrosis in smaller area that may be visible without staining. The severity of eye irritation increased when the amount of alcohol applied was increased to 0.5 ml.

In a study by Guillot et al. (1982), the ocular irritancy of ethanol (50% *v/v* solution, pH 8.3) on six male albino rabbits was tested with and without rinsing of the eye following the exposure. The results indicated that ethanol was a severe irritant, characterized by less readily reversible lesions. Rinsing of the eye after 30 s of exposure reduced the severity and accelerated reversibility of the eye lesions.

Short-Term Toxicity

Inhalation

Karanian and Salem (1988) studied the effect of ethanol inhalation on the cardiovascular state of male rats by exposing the animals to alcohol vapors for 7- to 14-day periods followed by in vivo measurements of plasma catecholamine levels and blood pressure. The blood alcohol level was maintained at >120 mg% throughout the exposure period. The results indicated that the short-term alcohol exposure did not significantly alter blood pressure, but there was a mild increase in plasma catecholamine levels.

Groups of six male Sprague-Dawley rats were exposed to air containing ethanol vapors 24 h/day for 35 days (Rikans and Gonzalez 1990). Ethanol flow rates were adjusted to maintain behavioral levels of intoxication to cause ataxia. This required a gradual increase in ethanol flow rates over a period of 12 days, during which the blood ethanol levels reached 200 mg/dl. For the remainder of the exposure period, the blood alcohol levels were between 200 and 300 mg/dl. Ethanol-exposed rats gained less weight during the 5-week exposure period. Lung and liver weights were unaffected by the treatment, but a moderate decrease (24%) in hepatic total protein and a small decrease (9%) in pulmonary soluble protein were observed. Chronic exposure to ethanol vapors did not enhance lipid peroxidation in lung or liver, but increased the activities of catalase and Cu/Zn superoxide dismutase in lung. Also, the blood ethanol concentrations in rats inhaling ethanol vapors at levels that produce ataxia were three to four times higher than those reported for rats ingesting an ethanol-containing liquid diet. The authors concluded that chronic ethanol inhalation did not induce a significant degree of oxidative stress in rat lung.

Subchronic Toxicity

Oral

Many studies evaluated the short-term oral toxicity of alcohol. For example, Watanabe and Yanagita (1983) reported, after a 10-week study of oral alcohol administration in one male and two female Rhesus monkeys, severe elevation in the serum triglyceride level along with accumulation of fat droplets in the hepatocytes at 6 g/kg/day of 15% *v/v* ethanol. However, the same study showed no hypertriglyceridaemia in male SD rats exposed to 8 g ethanol/kg/day intragastically. Because the intent of denaturing alcohol is to make it unsuitable for consumption, further short-term oral toxicity studies are not presented.

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

Reproductive and developmental toxicity studies have been reported using *Drosophila*, mice, rats, rabbits, dogs, swine, ewes, and lambs. Table 5 summarizes the available animal reproductive and developmental toxicity data.

Drosophila

Chronic exposure of *Drosophila melanogaster* females to 10% or 15% (*v/v*) of ethanol as well as acute exposure of 75% (*v/v*) ethanol for 60 min resulted in a significant increase ($p < .05$) in the frequency of X-chromosome disjunction and number of malformed flies. Ovary analysis showed cessation of egg production after chronic treatment (Rey et al. 1992).

Mice

A single i.p. injection of 0.03 ml of 25% ethanol in pregnant mice on gestation days between 7 and 12 resulted in increased fetal death and incidence of congenital anomalies (Kronick 1976).

Short-term oral exposure of pregnant C57BL/6J mice from gestation days 5 to 11 to ethanol (15 to 22 g/kg) in the diet resulted in decreased numbers of pups, and a higher incidence of hydronephrosis in the offspring. The authors concluded that the teratogenic effects of alcohol can lead to renal anomalies in the offspring (Boggan and Randall 1979).

When pregnant mice were exposed to a single i.p. dose of ethanol (25% ethanol as 5.8, 4.3, and 2.9 g ethanol/kg),

TABLE 5
Reproductive and developmental studies using Ethanol

Test system	Concentration of ethanol	End point	Results	References
Mice				
Mice	0.03 ml of 25% ethanol	Teratogenic and fetotoxic effects	Single intraperitoneal injection between gestation days 7–12 resulted in fetal death and increased congenital anomalies	Kronick, 1976
C57BL/6J mice	15–22 g/kg	Teratogenicity	Decreased number of pups, higher incidences of hydronephrosis in neonates indicating renal toxicity	Boggan and Randall, 1979
Mice	25% ethanol at 5.8, 4.3, and 2.9 g/kg	Developmental toxicity	Single, intraperitoneal maternal exposure produced dose-dependent defects such as exencephaly (exposure on gestational day 7), maxillary hypoplasia(exposure on gestational day 8) and limb defects in fetuses (exposure on gestational day 9 through 11)	Webster et al., 1980
Mice	10, 12.5, or 15% ethanol - 1 ml	Embryotoxicity	Induction of non-dysjunction in recently fertilized mouse eggs	Kaufman, 1983
CD-1 mice	0.94%, 1.88%, 3.75%, 7.5%, and 15% in water	Multi generation/ reproductive toxicity	Fo generation showed no adverse effect on mating or fertility; F1 mice had reduced weights, increased kidney and liver weights and reduced sperm motility in 15% group	NTP, 1985
Swiss Webster mice	9.9 mg/l of air	Neonatal brain development	Neonatal mice without maternal separation, after 12 days of inhalational exposure (blood levels 160–290 mg/dl) did not result in mortality; physical dependence was produced without any effect of weight gain; whole brain and cerebellar weights were significantly reduced on postnatal day 45	Pal and Alkana, 1997

(Continued on next page)

TABLE 5
Reproductive and developmental studies using Ethanol

Test system	Concentration of ethanol	End point	Results	References
Rats				
Sprague-Dawley derived barrier-sustained rats	1000 mg ethanol/kg/day	Fetotoxicity and neonatal toxicity	During lactation exposure of mothers resulted in reduced weight gain and survival rate of litters; exposure before, during and after pregnancy resulted in death of all litter by postnatal 5 days; increased length of gestation for mothers and growth retardation of offsprings to postnatal day 72	Martin et al., 1976
Long-Evans rats	2, 3, and 4 g/kg	Developmental toxicity	Maternal exposure during fetal organogenesis period resulted in reduction in body weights of fetuses	Samson et al., 1979
Albino rats	80–90% ethanol	Abortion	Intrauterine instillation during mid-pregnancy results in abortion, lower concentrations not effective	Malaviya et al., 1975
Wistar white rats	40% ethanol, 2 ml	Developmental toxicity	After maternal intragastric injection (exposure on gestational day 13), cytotoxic effects in fetus appeared within an hour, reaching its peak at 24 hours.	Barilyak and Kozachuk, 1981
CF rats	75,200,300 mg/kg	Teratogenicity	Maternal exposure during organogenesis resulted in fetuses showing microcephaly, micrognathia, micromelia, hemorrhage, digital abnormalities, reduced body weights and tail length and impaired skeletogenesis	Sreenathan et al., 1983
Sprague-Dawley rats	10000, 16000 or 20000 ppm	Reproductive toxicity	After inhalational exposure of pregnant rats (gestation days 1 through 19) to 16000 and 20000 ppm, male fetuses showed reduced weight. No behavioral effects were seen in fetuses when both the parents were exposed to these levels of ethanol by inhalation	Nelson et al., 1990

(Continued on next page)

TABLE 5
Reproductive and developmental studies using Ethanol *(Continued)*

Test system	Concentration of ethanol	End point	Results	References
Rabbits				
Rabbits	7.5% ethanol at 5 ml/100g	Contraception	Oral administration resulted in inhibition of spermatozoa through fallopian tubes and blocking of ovulation	Sharma and Chaudhury, 1970
Guinea Pigs				
Nulliparous Dunkin-Hartley guinea pigs	4 g/kg	Developmental toxicity	Chronic prenatal ethanol exposure in young adult offspring increased spontaneous locomotor activity in the open-field and impaired task acquisition in the Morris water maze; increased expression of the β2/3-subunit of the $GABA_A$ receptor in the hippocampus; produced cognitive and behavioral deficits; altered the expression pattern and pharmacological properties of hippocampal $GABA_A$ receptors	Iqbal et al., 2004
Swine				
Sinclair miniature swine	Voluntary alcohol consumption	Fetotoxicity	Reduction in consecutive litter size and birth weights and increased number of abortions in alcohol consuming mothers	Dexter et al., 1983
Sheep				
Sheep	25% ethanol as 2 mg/kg	Fetotoxicity	After maternal intravenous injection (gestational day not specified), maternal and fetal plasma levels of ethanol correlated; fetal weights and fetal pituitary-thyroid function reduced	Rose et al., 1981
Sheep	1 gm/kg	Fetotoxicity	Maternal exposure decreases fetal breathing activity 30 minutes later, lasting till 9 hours; fetal brain blood flow reduced	Patrick et al., 1984

TABLE 5
Reproductive and developmental studies using Ethanol

Test system	Concentration of ethanol	End point	Results	References
Fruit Flies				
Drosophila melanogaster	10% or 15% (chronic) and 75% (acute)	Reproductive toxicity	After acute exposure, increase in the frequency of X-chromosome dysjunction and number of abnormal flies; caseation of egg production after chronic exposure	Rey et al., 1992
Multiple Species				
Sprague-Dawley rats and dogs	25–50 μL (rats) or 100–200 μL (dogs)	Sterilization	After injection of 95% ethanol directly in vas deferens, complete sterility was observed	Freeman and Coffey, 1973

malformations in the fetus were observed based on the day of the exposure. The main malformations were exencephaly (exposure on day 7), maxillary hypoplasia (exposure on day 8), and limb defects (exposure on one of the days 9 through 11). The frequency of defects increased with an increase in the dose (Webster et al. 1980).

Recently mated mice were given 1 ml of either 10%, 12.5%, or 15% solution of ethanol by mouth to study the effect of ethanol on the first few hours after fertilization. The results indicated that in vivo exposure of parthenogenetically activated and recently fertilized mouse eggs to ethanol while they are completing the second meiotic division may induce non-dysjunction (Kaufman 1983).

In an NTP study, administration of ethanol in drinking water at various concentrations (0%, 0.94%, 1.88%, 3.75%, 7.5%, and 15%) to CD-1 mice showed that for F0 generation, these concentrations did not affect mating, fertility, or the number of litters. However, live litter size was significantly reduced in the 15% ethanol group. Body weights of F1 mice in the 15% ethanol group were lower than the control. F1 mice also had increased liver and kidney weight and reduced sperm motility in males suggesting the reproductive toxicity of ethanol. The litter weights of F1 mice were significantly depressed (NTP 1985).

Pal and Alkana (1997) studied the effect of ethanol inhalation on neonatal brain development in Swiss Webster mice without maternal separation. Two-day-old mice, with their mothers, were put in an inhalation chamber and continuously exposed to 9.9 ± 0.7 mg/L ethanol vapors for 12 days. The pups maintained a substantial blood alcohol concentration of 160 to 290 mg/dl and the mothers had a minimal blood alcohol concentration of <10 mg/dl. No mortality was observed during the ethanol exposure, but a physical dependence to ethanol was produced in the neonates. There was no effect on the weight gain of the pups or mothers. The authors also noted that 12 days of neonatal exposure to ethanol vapors significantly reduced whole brain and cerebellar weights on postnatal day 45 as compared to controls and altered normal behavior on postnatal day 40 to 41.

Rats

Complete sterility was observed in Sprague-Dawley rats after injection of 25 or 50 μl of 95% ethanol into the vas deferens (Freeman and Coffey 1973).

While studying induction of abortion in albino rats, Malaviya et al. (1975) showed that intrauterine instillation of 80% and 90% ethanol during mid-pregnancy resulted in abortion, whereas lower concentrations were not suitable for this purpose.

Forty-five Sprague-Dawley-derived, barrier-sustained rats were exposed to ethanol either before, during, after pregnancy (23-day nursing period), or for all three stages (Martin et al. 1977). Ethanol was administered orally as sole fluid source in sweetened water. In addition, the after pregnancy (23-day nursing period) group received subcutaneous injections of 1000 mg/kg/day of ethanol during the last 21 days of the nursing period; and the group exposed during all three stages received twice-daily, subcutaneous injections of 500 mg/kg of ethanol only during the pregnancy period. The study showed that the litters of dams exposed to alcohol during lactation had lower weight gain and survival rate. All litters from dams exposed to alcohol during all three stages did not survive past postnatal day 5. Other differences noted between the control and alcohol treated group included reduced maternal and fetal weight gain, increased length of gestation, and growth retardation of offspring to postnatal day 72.

Adult female rats of the Long-Evans strain were treated with three different doses of ethanol, 2, 3, and 4 g/kg ($n=4$ for each group), on days 9 to 12 of gestation. The results showed a significant reduction in body weight of fetuses at higher doses of ethanol, which could not be accounted for by decreased maternal

weight. These data suggest that acute doses of alcohol during pregnancy can result in some developmental retardation (Samson et al. 1979).

In another study, 60 white rats of the Wistar line were injected intragastrically with 2 ml of 40% ethanol on the 13th day of pregnancy. The results suggested that unlike the somatic cells in adult animals, cytotoxic effects of ethanol manifested in embryonic cells were apparent within an hour after administration and peaked at 24 h. These cytotoxic effects included induction of single and paired fragments, chromosomal translocation and deletions, and increased levels of polyploid cells (Barilyak and Kozachuk 1981).

Sreenathan et al. (1983) exposed pregnant CF rats to 75, 200, or 300 mg/kg of ethanol on days 8 to 15 of gestation. The fetuses had microcephaly, micrognathia, micromelia, hemorrhage, digital abnormalities, reduced body weight and tail length, and impaired skeletogenesis, which suggest that ethanol may be teratogenic at such low doses.

Nelson et al. (1990) administered ethanol by inhalation for 7 h per day on gestation days 1 to 19 to groups of 15 pregnant female Sprague-Dawley rats at 10,000, 16,000, or 20,000 ppm. No harmful maternal effects were observed at lower concentrations whereas at 20,000 ppm narcosis and reduced food consumption were observed in pregnant rats. Fetuses had no harmful effects at maternal exposure to 10,000 ppm. At higher concentrations, male fetuses had significantly reduced weight compared with controls. The authors concluded that ethanol was not teratogenic, even at concentrations that produced, in maternal animals, narcosis that persisted throughout the 7-h exposure period. Behavioral teratology was investigated in groups of 15 pregnant rats and 18 male rats, which were exposed to the same concentrations of ethanol for 6 weeks and then mated to untreated females. The results for this study were negative at all exposure levels.

Guinea Pigs

Iqbal et al. (2004) reported on a study that tested the hypothesis that chronic prenatal ethanol exposure (CPEE) increases γ-aminobutyric acid ($GABA_A$) receptor expression in the hippocampus of guinea pig offspring that exhibit cognitive deficits in a hippocampal-dependent spatial learning task. Ethanol (4 g/kg of maternal body weight) was administered to pregnant guinea pigs, which were compared to guinea pigs that were treated with isocalorie/sucrose pair feeding, or water throughout gestation. $GABA_A$ receptor subunit protein expression in the hippocampus was measured at two development ages: near-term fetus and young adult.

In young adult animals, CPEE increased spontaneous locomotor activity in the open field and impaired task acquisition in the Morris water maze. CPEE did not change $GABA_A$ receptor subunit protein expression in the near-term fetal hippocampus, but increased expression of the β2/3-subunit of the $GABA_A$ receptor in the hippocampus of young adult offspring. CPEE produced cognitive and behavioral deficits in adult guinea pig offspring, and altered the expression pattern and pharmacological properties of hippocampal $GABA_A$ receptors. The authors suggested the data demonstrated decreased responsiveness to the endogenous neurosteroid, allopregnanolone, which may play a role in the expression of both cognitive and behavioral dysfunction in offspring that received CPEE (Iqbal et al. 2004).

Rabbits

A study performed in rabbits showed that oral administration of 7.5% ethanol at 5 ml/100 g body weight in females resulted in slight inhibition in the transport of spermatozoa through the fallopian tubes and blocked ovulation in successfully mated rabbits (Sharma and Chaudhury 1970).

Dogs

Complete sterility was obtained after injection of 100 or 200 μl of 95% ethanol into the vas deferens of dogs indicating that rapid male sterility can be achieved using 95% ethanol (Freeman and Coffey 1973).

Swine

A comparison between three consecutive litter sizes for voluntary alcohol-consuming Sinclair (S-1) miniature swine showed a progressive decrease in mean litter size and birth weights, and an increase in perinatal deaths and number of abortions from controls (6.6, 719 $\pm$ 186 g, 10%, and 0, respectively) to alcohol consuming mothers ($L_1 = 4.85$, $L_2 = 2.0$, and $L_3 = 1.83$; $L_1 = 602 \pm 153$ g, $L_2 = 478 \pm 168$ g, and $L_3 = 167 \pm 122$ g; $L_1 = 17.5\%$, $L_2 = 41.4\%$, and $L_3 = 36.4\%$; and $L_1 = 0$, $L_2 = 0$, and $L_3 = 3$, respectively) (Dexter et al. 1983).

Sheep

Intravenous ethanol administration to pregnant ovine ewes (2 g/kg as 25% solution) resulted in a close correlation between the plasma levels of ethanol in the fetus and mother. Chronic alcohol exposure decreased the weights of the fetuses as well as reduced fetal pituitary-thyroid function (Rose et al. 1981).

A study performed on 12 fetal lambs after receiving 1 g/kg ethanol of maternal weight, had an abrupt decrease in fetal breathing activity 30 min later that lasted 9 h. The arterial glucose levels were slightly decreased and fetal brain blood flow was significantly reduced (Patrick et al. 1984).

GENOTOXICITY

The available data on genotoxicity are summarized in Table 6.

Human Cells

Human fibroblasts, exposed or treated with 1.2% ethanol, had a 9% increase in chromatid breakage over control values (Meisner and Inhorn 1972). This effect was completely eliminated 9 days after the exposure, indicating a non-specific cytotoxicity of ethanol rather than mutagenicity.

TABLE 6
Ethanol genotoxicity/mutgenicity

Test system	Dose/Concentration	Results	References
In vitro assays			
Human fibroblast	1.2% ethanol	Human cadaver skin samples showed that after 2 days exposure there were 11.5% of cells with chromatid breaks and 0.6% cells with chromosome aberrations. After 9 days, there were no cells with chromatid breaks or chromosome aberrations	Meisner and Inhorn 1972
Human fibroblast	1.2% + 1.2% ethanol	Human cadaver skin samples showed that after 1 day exposure there were 9.6% cells with chromatid breaks and 0% cells with chromosome aberrations. After 8 days there were 7.5% cells with chromatid breaks and 0.9% cells with chromosome aberrations	Meisner and Inhorn 1972
Human lymphocyte culture	1.16, 2.32, and 3.48 mg ethanol/ml	After 50 hours of incubation, exposed cultures showed increased chromatid-type and chromosome-type breaks and gaps and structural rearrangements	Badr et al. 1977
Human blood lymphocytes	0.05, 0.15, 0.5% (v/v)	A dose-dependent increase in the mean number of sister chromatid exchanges	Alverez et al. 1980
Chinese hamster embryonic diploid cell line	1% ethanol	Increase in achromatic lesions	Dulout and Furnus 1988
Bacterial and fungal assays			
Aspergillus nidulans	0.25, 5, 10, and 20% (v/v)	A dose-dependent decrease in conidial viability with complete inhibition at	Harsanyi et al., 1977

(Continued on next page)

TABLE 6
Ethanol genotoxicity/mutgenicity *(Continued)*

Test system	Dose/Concentration	Results	References
		20%, increased frequency of mitotic cross over and mid-distribution due to interference with mitotic spindle apparatus	
E. coli CHY832	140 and 180 mg ethanol/ml	RK test showed mutagenic effects of ethanol without S9 but mutatest showed negative results even with S9	Hayes et al., 1984
S.cereveciae	4% (0.85M) ethanol	4% ethanol has no effect but higher concentrations result in increase in single-stranded DNA breaks in repair deficient cells	Ristow et al., 1995
Animal assays			
CBA male mice	1.24 or 1.86 g ethanol/kg/d	Mice exposed to ethanol for 3 days showed Induction of mutations on late spermatid and on epididymal system	Badr et al., 1977
Parkes albino mice	0.62, 1.24, or 1.86 g ethanol/kg	Increased frequency of micronuclei in the exposed group, although no clear dose-response effect were seen	Badr et al., 1977
Male albino rats	6 to 10 %	After 5 weeks of oral exposure, presence of multi-nucleated giant cells, fewer mitotic figures, retarded cellular differentiation beyond the primary spermatocyte stage and reduced sperm count	Klassen and Persaud 1978
Male CBS Mice	0.5 ml of 10 and 20% v/v ethanol	After 3, 5, 12, or 16 weeks of exposure, significant increase in sister chromatid exchanges in bone marrow chromosome	Obe, at al. 1979
CBA/CA male mice	0.8 ml of 12.5 and 15% ethanol	2–6 hours after oral administration, frequency of aneuplidy increased with increase in concentration.	Hunt, 1987
Sprague-Dawley rats	4 g ethanol/kg of 20% v/v	Significant single-stranded DNA breaks	Singh, et al., 1995

Blood from five human volunteers was used to perform human lymphocyte culture testing using 1.16, 2.32, and 3.48 mg ethanol/ml of final culture medium. After 50 h of incubation, alcohol-treated cultures had higher incidences of chromatid-type as well as chromosome-type breaks and gaps, and structural rearrangements indicating mutagenic potential of ethanol (Badr et al. 1977).

Blood lymphocytes, collected from four human donors, were grown in the presence of ethanol at concentrations of 0.05%, 0.15%, or 0.5% (v/v) ethanol concentration to study effect on frequency of sister-chromatid exchanges (SCEs). At 0.05%, which is half the blood-level concentration used to define legal intoxication by the American Medical Association, the mean number of SCEs per cell increased by approximately 30% in three out of four samples. The mean number increased significantly ($p < .01$) over the control for all four samples at higher concentrations of alcohol, thereby indicating that ethanol may be a mild mutagen in human cells *in vitro* (Alverez et al. 1980).

Animal Cells

Exposure of Chinese hamster embryonic diploid cell line to 1% ethanol resulted in an increase in achromatic lesions/gaps (Dulout and Furnus 1988).

Bacterial/Fungal Cells

During germination, heterozygous diploid conidia of *Aspergillus nidulans* were exposed to ethanol concentrations of 0.25%, 5%, 10%, and 20% (v/v) for 72 h to detect events of crossing over, non-dysjunction, and mutation. With increased concentrations of ethanol, a decrease in conidial viability was observed with complete inhibition at 20% ethanol concentration. Genetic analysis suggested that the frequencies of mitotic cross over and misdistribution of chromosomes were increased by ethanol. On cytological examination of ethanol treated cells there was an interference with the mitotic spindle apparatus (Harsanyi et al. 1977).

Escherichia coli strain CHY832 was used to assess mutagenicity of ethanol using the RK test. In the absence of S9, 140 and 180 mg/ml of ethanol were found to be highly toxic and mutagenic. Mutatest results show ethanol to be non-mutagenic in presence of S9 (Hayes et al. 1984).

In another study, up to 4% (0.85 M) ethanol in the growth medium was not harmful to *Saccharomyces cerevisiae*, and at higher concentrations ethanol induced single-strand DNA breaks only in repair-deficient cells. This chromosomal damage, which was observed in normal cells, was thought to be induced by acetaldehyde, the primary metabolite of ethanol (Ristow et al. 1995).

Mice

To study dominant lethal mutation, Badr et al. (1977) treated male CBA mice with 1.24 g/kg/day or 1.86 g ethanol/kg/day for 3 days and then mated them with untreated females. Ethanol induced mutations at both doses by acting on late spermatids and on the epididymal system. In the same study, the micronucleus test was performed following injection of four male and four female albino mice of Parkes strain with 0.62, 1.24, or 1.86 g/kg ethanol. Significantly ($p < .01$) increased frequencies of micronuclei were observed in exposed groups compared to controls although no clear dose-response relationship was observed.

After oral exposure of male CBS mice to ethanol (10% and 20% v/v) as the sole source of liquid for 3, 5, 12, or 16 weeks, Obe et al. (1979) demonstrated that ethanol significantly ($p < .05$) increased SCEs in the bone-marrow chromosome. Most compounds that induce SCEs also induce chromosomal aberration and mutations.

Hunt (1987) showed that 2 to 6 hours after oral administration of approximately 0.8 ml of 12.5% and 15% ethanol to CBA/CA male mice (7 and 10 in number, respectively), the frequency of aneuploidy increased with the increase in concentration of ethanol and was significantly different ($p < .001$) from controls.

Rats

Klassen and Persaud (1978) noted that 6% to 10% alcohol orally administered to 12 albino rats resulted in signs of intoxication. The exposed group had reduced weight gain overall and reduced weight and size of the testes. Further tests revealed the presence of multinucleated giant cells, fewer mitotic figures, and retarded cellular differentiation beyond the primary spermatocyte stage along with reduced sperm counts. These results indicate that alcohol may disturb meiosis and has a mutagenic potential.

Singh et al. (1995) analyzed brain cells of male Sprague-Dawley rats for single-strand DNA breaks after intubation with 4 g/kg of 20% v/v solution of ethanol. The results indicated significant DNA damage ($p < .025$).

EPIGENETICS

Epigenetics refers to DNA and chromatin modifications that influence chromatin structure and change the state of gene expression without altering the nucleotide sequence.

Kim and Shukla (2006) reported the acute in vivo effect of ethanol (binge drinking) on histone H3 modifications in rat tissues. Ethanol was injected into the stomach of Sprague-Dawley rats (8 weeks old) using blunt tipped needle; water was used as a control. The rats were sacrificed after 1, 3, or 12 h, nuclei were isolated from tissue obtained from 11 organs, and analyzed for histones using an acid extraction method. In liver, the authors reported an increase in Ac-H3-Lys9, with maximal increase of sixfold after 12 h of exposure. The lung also showed a threefold increase. In the spleen, ethanol-induced Ac-H3-Lys9 in all three ethanol-treated groups with a similar increase (1.5- to 1.6-fold). The testes showed a significant increase (threefold increase) of Ac-H3-Lys9 only at 1 h of ethanol exposure. Ethanol had no affect on Ac-H3-Lys9 in other tissues: kidney, brain, heart, stomach, colorectum, pancreas, and vessels. Ethanol had little effect

on Me-H3-Lys9 in all rat tissues examined. The authors concluded that ethanol can impact the acetylations of H3-Lys9 in rat tissues, but that these epigenetic alterations in rat tissues are tissue-specific events.

CARCINOGENICITY

The International Agency for Research on Cancer (IARC 1988) has determined that alcoholic beverages are carcinogenic to humans and has designated them as a Group 1 carcinogen. Three studies which suggest mechanisms by which ethanol acts are described briefly below.

Fan et al. (2000) studied the mechanism(s) of alcohol-induced mammary cancer using human breast cancer cell lines, MCF-7 and T47D. Ethanol (at 0, 10, 20, 40, 60, 80, and 100 mM) caused a dose-dependent increase of up to 10- to 15-fold (100 mM) in the transcriptional activity of the ligand-bound estrogen receptor (ER-α), but did not activate the non–ligand-bound receptor. The authors speculated that inactivation of *BRCA1* and increased estrogen responsiveness might contribute to alcohol-induced breast cancer but could not rule out a role for theincrease in the ER-α levels.

Izevbigie et al. (2002) postulated that ethanol may alter the growth rate of human breast tumor epithelial cells. Using the MCF-7 cell line exposed to a physiologically relevant concentration of ethanol (0.3% or 65 mM), these authors measured [^{3}H]thymidine incorporation, cell number, p44/42 mitogen-activated protein kinases (MAPKs), and activities in the presence or absence of an inhibitor of MAPK/ERK-1 (extracellular signal-regulated kinase 1) and MEK1 (MAPK kinase 1), PD098059. Treatment of MCF-7 cells with ethanol increased MAPK activities by an average of 400% ($p < .02$), and subsequent cell growth by 200% ($p < .05$) in a MEK1 inhibitor (PD098059)-sensitive fashion. The authors suggested that the Ras/MEK/MAPK signaling pathways are crucial for ethanol-induced MCF-7 cell growth.

Dhar and Plummer (2006) studied protein expression of G-protein inwardly rectifying potassium channels (GIRK) in breast cancer cells. Cells grown in culture were washed and centrifuged into pellets. Membrane protein was isolated to determine GIRK protein expression. GIRK1 protein expression at the expected molecular weight of 62 kDa was confirmed in MDA-MB-453 and ZR-75-1 cells, and at a lower molecular weight (40 to 42 kDa) in MDA-MB-361, MDA-MB-468, MDA-MB-453, MCF-7, and ZR-75-1 cells. MDA-MB-453 cells treated with 0.12% ethanol had a reduced protein expression of GIRK1. The authors concluded that GIRK channels involved in cellular signaling exist in breast cancer cells and that ethanol affect, that signaling.

CLINICAL ASSESSMENT OF SAFETY

Case Studies

Two patients, treated with tetraethylthiuramdisulfide (also known as disulfiram; it inhibits aldehyde dehydrogenase, which leads to the buildup of acetaldehyde) for alcoholism, had an adverse reaction to aftershave containing 50% alcohol. The patients experienced warmth, flushing, and blotching of the face and nausea. A similar response was shown after inhalation of 70% ethanol (Murcurio 1952).

A 3-year-old boy and 4-year-old girl, who accidentally ingested mouthwash containing 16% alcohol, developed hypoglycemia. This effect was independent of the blood alcohol concentration. If this had not been treated promptly, death could have resulted (Varma and Cincotta 1978).

A 56-year-old alcoholic man, subjected to disulfiram therapy, developed generalized pruritic macular rash after using a shampoo. A closed patch test later confirmed the reaction to alcohol, one of the ingredients in the shampoo (Stoll and King 1980). These results suggest an adverse interaction of alcohol-containing personal care products to individuals taking certain medications.

Sperry and Pfalzgraf (1989) reported three different case studies of fatal alcohol intoxication in which intentional ingestion of mouthwash and hair spray by alcoholics occurred. The blood concentration ranged from 0.340 to 565 g/dl, indicating alcohol as the primary cause of death.

Data obtained from the toxic exposure surveillance system for 2004 (Watson et al. 2005) are shown in Table 7.

Effects of Chronic Ingestion of Alcohol

Although the intent of denaturing alcohol is to make it unfit for consumption, the case studies presented above document the ingestion of Alcohol Denat.

It was stated in the material safety data sheet by Union Carbide Corporation (2000) that chronic ingestion of ethanol, 200 Proof Spirits Synthetic Alcohol could lead to the development of progressive liver injury with fibrosis. It was also stated that repeated ingestion by pregnant women could be shown to adversely affect the central nervous system of the fetus, causing mental and physical retardation, disturbance of learning, motor and language deficiencies, behavioral disorders, and small sized head.

According to a review of available data by Brooks (1997), chronic alcohol abuse is associated with liver and brain damage, and with an increase in certain types of cancers.

According to Obe and Ristow (1979), the genotoxic effects of ethanol were mediated via its metabolite, acetaldehyde. Because this metabolite is formed in very small amounts in specific tissues in vivo, Phillips and Jenkinson (2001) suggested that ethanol should not be classified as a mutagen.

While acknowledging that acetaldehyde is one mechanism of action, Brooks (1997) and Portari et al. (2003) provided a more current explanation of the effects of ethanol. The microsomal ethanol oxidizing system is induced by ethanol, beginning with the specific ethanol-inducible cytochrome P450CYP2E1 is linked to liver damage and inhibitors of this cytochrome can

TABLE 7
Demographic Profile of Exposure Cases of Alcohol (Watson et al., 2005).

Category	No. of Exposures	Age (year)			Reason				Treated in Health Care Facility	Outcome				
		<6	6–19	>19	Unint[a]	Int[b]	Other[c]	Adv Rxn[d]		None	Minor	Moderate	Major	Death
Ethanol cleansers	289	151	83	55	275	9	4	1	37	57	65	3	0	0
Non-beverage Ethanol	8264	6340	721	1170	7927	274	36	19	522	2266	866	76	7	5
Rubbing alcohol: Ethanol with methyl salicylate	12	11	1	0	12	0	0	0	1	3	1	0	0	0
Rubbing alcohol: Ethanol without methyl salicylate	280	194	18	68	257	18	1	4	40	82	25	7	1	0
Ethanol containing mouthwash	15727	4442	2586	8614	14222	1386	47	37	1422	2802	1172	268	35	2
Perfume/cologne/ aftershave	17627	14917	1468	1184	16919	540	111	39	1183	4107	3701	110	11	1

[a]Unintentional (includes general, therapeutic error, misuse, environmental, food poisoning, occupational and unknown)
[b]Intentional (includes suicidal, abuse, misuse and unknown)
[c]Others (includes malicious, contamination/tampering, withdrawal)
[d]Adv Rxn = Adverse reaction (includes drug, food, others)

reduce liver damage. Other data link the induction of this cytochrome to damage in the brain as well. The effects of high levels of P450CYP2E1 may be further traced to a generation of reactive oxygen species (oxygen radical, superoxide anion, and hydroxyl radical). These reactive oxygen species, in turn, are linked to DNA damage. In an experimental animal study in rats, Portari et al. (2003) reported that β-carotene does not have a beneficial effect as an antioxidant in chronic ethanol consumption.

Effects on the Central Nervous System

Union Carbide Corporation (2000) also stated that mild alcohol intoxication occurs at blood levels between 0.05% and 0.15%, with 25% of individuals showing signs of intoxication at these levels. Above 0.15% the person is definitely under the influence of ethanol, and 50% to 95% of individuals at this level are clinically intoxicated. Severe poisoning occurs when the blood ethanol level is 0.3% to 0.5%. Above 0.5% the individual becomes comatose and death can occur.

McBay (1973) reported judgement impairment in humans after oral ingestion of alcohol occurs at a blood alcohol concentration of about 1 g/L (0.20%). Intoxication occurs at 2 g/L, and coma and death can occur at blood alcohol concentrations of 4 to 5 g/L or higher.

Representative of other reports, Baker et al. (1986) evaluated human behavioral tests using ethanol as a model for central nervous system (CNS) depressant effect. Twenty-one adult males received between 0 and 1.4 ml vodka/kg body weight, and the blood alcohol concentrations 15 and 65 min after the administration were 0.054% and 0.052%, respectively. The results indicated that tachistoscopic perception, which measures the ability to reproduce patterns of dots embedded within a three-by-three grid, showed high reliability and sensitivity to ethanol indicated by increase in number of errors after alcohol consumption, indicating an effect on the CNS. However, the Archimedes spiral after-effect, used to measure the duration of apparent motion of spiral after it has stopped rotating, was not significantly different before and after consumption of alcohol and the authors concluded that this test is not a reliable model to assess the effects of alcohol on the CNS.

The substantial lethal dose of ethanol in adults without supportive therapy is 5 to 8 g/kg, and in children, 3 g/kg (Scherger et al. 1988).

Fetal Alcohol Syndrome (FAS)

Obe and Ristow (1979) described fetal alcohol syndrome (FAS) as characterized by a variety of developmental and mental defects that is found in infants born to women who are chronic alcoholics. Brooks (1997) suggested that reactive oxygen species production of DNA damage, as described above, could be associated with FAS.

Chiriboga (2003) described how alcohol abuse by pregnant women is harmful to the developing embryo and fetus; however, it wasn't until 1973 the term FAS was coined to describe a constellation of abnormalities seen among offspring of chronic alcoholic women. FAS was also reported with binge drinking as defined as 5 drinks or more on one occasion. Statistically, FAS is found in 1.9 per 1000 live births (worldwide). In the United States it varies from 2.9/1000 to 4.8/1000 live births. FAS is characterized by three major categories: intrauterine and postnatal growth retardation, cranial facial dysmorphology, and central nervous system effects.

This author also reported that the teratogenic effects of FAS are well documented. Fetal susceptibility to the effects of alcohol is greatest during the first trimester of pregnancy, being the time of organogenesis. Common FAS malformations include hemangiomas, cardiac defects, minor joint and limb abnormalities, genital abnormalities, and single palmar creases. The author noted that a fetus exposed to 1 to 1.5 drinks of alcohol per day can suffer from fetal alcohol effects (FAEs), which is less severe than FAS; however, children affected suffer from slower cognitive processing of information and developmental delays. It was also stated that alcohol transferred through breast milk impairs motor development but not mental development at age 1. Ingestion of alcohol by children may lead to hypoglycemic seizures (Chiriboga 2003).

O'Leary (2004) stated in a review article that diagnosing FAS is very difficult because there is no laboratory testing for it. Diagnosis is based upon the pattern of abnormalities seen in the subject and self reports given voluntarily by the mothers. It was noted that not all children exposed to alcohol are affected or even affected to the same degree as others. Children affected by FAS often display the following features: growth retardation (prenatal growth deficiency, postnatal growth deficiency, and low weight-to-height ratio), characteristic facial features (short palpebral fissures, maxillary hypoplasia, epicanthal folds, thin upper lip, and flattened philtrum), and CNS anomalies or dysfunction (microcephaly, developmental delay, intellectual disability, and neonatal problems including irritability and feeding difficulties).

Factors such as pattern and quantity of alcohol consumption, timing of intake, and stage of development of fetus at the time of exposure play a role in the severity of FAS. Major risk to the fetus is reported to require chronic, daily alcohol consumption of 6 or more drinks per day or at least 5 to 6 drinks per occasion, with a monthly intake of 45 drinks. The authors did not identify any increase in fetal malformations with moderate alcohol consumption (2 drinks per week to 2 drinks per day) during the first 3 months of pregnancy. The authors did mention the long-term effects of FAS as being behavior and developmental abnormalities, poor motor development skills, problems with communication, and hearing disorders (O'Leary 2004).

SUMMARY

Alcohol Denat. and various specially denatured (SD) alcohols are used as a cosmetic ingredients in a wide variety of products.

A denaturant is a chemical added to alcohol to make it impotable. This safety assessment included data for Alcohol Denat. and the following SD Alcohols: SD Alcohol 3-A, SD Alcohol 30, SD Alcohol 39, SD Alcohol 39-B, SD Alcohol 39-C, SD Alcohol 40, SD Alcohol 40-B, and SD Alcohol 40-C.

This report is divided into two parts in order to separately address the denaturants of alcohol and ethyl alcohol itself. Each of the SD alcohols are prepared by mixing ethanol with quantities of a wide variety of denaturing agents. In the USA, the addition of denaturants is prescribed in federal regulations. In Europe, each member state describes the denaturants and their addition to ethanol to produce Alcohol Denat.

Previously considered denaturants. Many of the denaturants have been considered previously as individual cosmetic ingredients by the CIR Expert Panel.

Safety assessments were already issued for t-Butyl Alcohol, Diethyl Phthalate, Methyl Alcohol, Alcohol Salicylic Acid, Sodium Salicylate, and Methyl Salicylate. The conclusion for t-Butyl stated it was safe as used in cosmetic products. Diethyl Phthalate was regarded safe for topical application in the present practices of use and concentration in cosmetics in 1985 and reaffirmed safe for use in the present practice of use and concentration in 2002. Methyl Alcohol's conclusion stated it was safe as used to denature alcohol used in cosmetic products. Salicylic Acid, Sodium Salicylate, and Methyl Salicylate were all assessed safe as used when formulated to avoid irritation and when formulated to avoid increasing sun sensitivity, or, when increased sun sensitivity would be expected, directions for use include the daily use of sun protector.

Quassin is a bitter alkaloid obtained from the wood of *Quassia amara*. The concentration of Quassin required for denaturing may vary according to the different regulations governing different types of products, as well as the alcohol content.

The manufacturing of Quassin starts with an extraction from *Quassia* chips and treating the extract with a solution of lead acetate and carbon. The carbon is removed (having absorbed the extract) and is mixed with chloroform. The addition of methanol and hot water then induces a crystallization that after 2 days forms crude Quassin.

Quassin must pass the optical assay and solubility test specified in 27CFR§21.124 for its use to be authorized.

After 9 days of oral treatment, Wistar female rats did not display any effects from 500 and 1000 mg/kg of Quassin on CNS activity. Also, albino male mice did not show any signs of toxicity when treated orally with 250, 500, 750, and 1000 mg/kg of Quassin extract.

When 30 albino male mice were given 500 and 1000 mg/kg of Quassin intraperitoneally, there were some signs of toxicity. The group treated with 500 mg/kg showed piloerection, decrease in motor activity, and a partial loss of righting reflex, all which resumed back to normal after 24 h. However, the group that was exposed to 1000 mg/kg showed piloerection, loss of posterior prehensile reflex, and a decrease in motor activity, and all mice died within 24 h of receiving treatment.

In a cytotoxicity test with brine shrimp, 1 mg/ml of Quassin did not possess any cytotoxic or antiplasmodial activity.

Quassin has been used as an antifeedant and insecticide. When 63.7, 31.9, and 16.0 $\mu g/cm^2$ of Quassin was applied to the cabbage leaf discs of the diamondback moth, the results showed 90% to 100% damage on all days. For the insecticidal test, Quassin produced potent insecticidal activity. Quassin (25 $\mu g/g$ of insect body weight) was found to have strong paralytic activity against the American cockroach; however, when applied at higher concentrations, acute insecticidal activity resulted and there was no recovery. Quassin was tested at 500, 250, 100, 50, and 10 ppm as an antifeedant for the Mexican bean beetle, and at 500, 250, and 100 ppm with the southern armyworm. In all tests for the Mexican bean beetle, Quassin was an effective antifeedant, having more of the control leaf eaten compared to the leaf with Quassin applied to it. As for the southern armyworm tests, Quassin was an effective antifeedant at 500 and 250 ppm, but it was not at 100 ppm.

Quassin was administered to rat Leydig cells in vitro at concentrations of 5 to 25 ng/ml to test the testosterone production. Quassin inhibited both the basal and LH-stimulated testosterone secretion in a dose-related fashion. Leydig cell counts before and after treatment showed that there was no change in cell viability. The authors concluded that inhibition of testosterone production cannot be due to the cytotoxic effects of Quassin.

The previous study was taken a step further to examine the effect of Quassin on male reproduction when 0.1, 1.0, and 2.0 mg/kg of body weight of Quassin was administered to albino rats. There was no significant effect of Quassin on the body weights of the rats, but the mean weights of the testes, seminal vesicles, and epididymides were significantly reduced. The weights of the anterior pituitary glands were significantly increased. Regardless of the doses of Quassin, the sperm counts did not differ, but they were significantly lower when compared to the control. The levels of LH, FSH, and testosterone were significantly lower in groups treated with Quassin.

Brucine is prepared by the reaction of 2-hydroxystrychnine in acetyl alcohol and Fremy salt in water. $HClO_4$ at 2 N and $Na_2S_2O_4$ are added, pH is adjusted with NH_4OH to 7 to 7.5, and then dissolved in $CHCl_3$/methyl alcohol and treated with ethereal diazomethane.

Brucine and Strychnine can be estimated by gravimetric and titrimetric analyses, paper chromatography, TLC, ion-exchange chromatography, spectrophotometry, NMR spectroscopy, and a new capillary electrophoresis procedure.

According to Federal regulations, Brucine must pass the strychnine test and sulfate test in order for its use to be authorized.

In an acute oral toxicity test, male Swiss-Webster mice were administered 30 ml/mg Brucine. The LD_5, LD_{50}, and LD_{95} were 78.0, 150, and 290 mg/kg, respectively. All of the mice experienced clonic convulsions that led to tonic seizures in some cases. If a tonic seizure did occur, death by respiratory arrest

followed. It was also noted that the mice experienced central nervous system depression prior to the convulsions.

Male Swiss-Webster mice were given 15 ml/kg of Brucine intraperitoneally. The reported LD_5, LD_{50}, and LD_{95} were 45.0, 62.0, and 86.0 mg/kg, respectively. The mice experienced clonic convulsions and central nervous system depression. Additionally, another group of male Swiss-Webster mice were dosed intravenously (amount not specified). The LD_5, LD_{50}, and LD_{95} were 5.1, 12.0, and 28.0 mg/kg, respectively. Next, another group of mice were intravenously given 7.5 ml/kg of Brucine Sulfate heptahydrate. The reported LD_5, LD_{50}, and LD_{95} were 6.5, 15.4, and 36.0 mg/kg, respectively. The onset of the clonic convulsions was instantaneous.

Brucine was tested among four strains of *Salmonella* in an Ames assay (TA100, TA1535, TA1537, and TA98). All strains, dosed up to 6666 μg/plate, with and without metabolic activation, were negative.

In a RIPT for a "tonic, dressing, other hair-grooming aid" containing 47% SD Alcohol 40 (95%), it was reported that Brucine Sulfate may be considered a non-primary irritant and a nonprimary sensitizer.

Three different sunscreen products (35% SD Alcohol 40-B, 72.4% SD Alcohol 40, and 74.5% SD Alcohol 40) did not show any signs of photoallergy in human subjects. Also, these three formulas did not exhibit any evidence of phototoxicity in humans.

Denatonium Benzoate is a bitter substance that functions as a denaturant added to a variety of products at required low levels, but does not interfere with the mode of action of the product. It was found to be detectable at a concentration of 10 ppb, discernibly bitter at 50 ppb, and unpleasantly bitter at 10 ppm. In order to denature SD Alcohol 40-B (100 gallons of ethyl alcohol), 0.12% t-butyl alcohol and 0.0006% Denatonium Benzoate are sufficient according to current Federal regulations.

The addition of Denatonium Benzoate to a liquid product reduces the tolerance level of that product. A 10-μl sample of orange juice with 10 ppm Denatonium Benzoate was placed on the back of children's tongues. All the children made facial grimaces, had expressions of shock, showed pronounced mouth movements, cried, and/or vomited.

A United States patent for the denaturant lignocaine benzyl benzoate (also known as Denatonium Benzoate) stated that it is a quaternary carboxylate derived from lignocaine and demonstrated several approaches to its synthesis. Lidocaine, a topical anesthetic, is synonymous with lignocaine. A study of the distribution of topically applied lidocaine, with an iontophoretic drug delivery system, demonstrated that virtually no lidocaine appears in the plasma, suggesting that the larger Denatonium Benzoate molecule in ethanol also would have little or no systemic exposure.

The concentration of Denatonium Benzoate was measured by HPLC. The analysis of standards showed a linear curve in the range of 1.25 to 50 ppm.

Denatonium Benzoate (0.1%) did not show adverse effects in 10 rats in an acute inhalation toxicity test. Also, Denatonium Benzoate (0.005% to 0.05%) was nonirritating to ocular mucosa in six albino rabbits.

Male and female Charles River CD albino rats were orally dosed with 127.1, 201.7, 320.2, 508.4, 807, and 1281 mg/kg of Denatonium Benzoate. The LD_{50} for the males was 640 mg/kg and for the females it was 584 mg/kg.

Neonatal rats derived from Charles River CD albino rats had an LD_{50} of 23 mg/kg when orally dosed with 10 ml/kg of Denatonium Benzoate at 7.9, 12.5, 19.8, 31.5, 50, 79.4, 125, and 315 mg/kg.

Female New Zealand white rabbits were orally administered 10 ml/kg of Denatonium Benzoate at doses of 201.7, 320.2, 508.4, 807.1, and 1281 mg/kg. The LD_{50} for the male rabbits was 508 mg/kg and for the female rabbits it was 640 mg/kg.

In two chronic toxicity studies, Denatonium Benzoate was administered (by gavage) at 1.6, 8, and 16 mg/kg/day. The first test was performed on cynomologus monkeys, and the other was on Charles River CD rats. In both cases no changes among the groups were considered related to the compound.

SD Alcohols. A modified Draize rabbit primary dermal irritation test was conducted on New Zealand white rabbits using a cream formula that consisted of 55.65% SD Alcohol 40-B denatured with Denatonium Benzoate. The erythema scores after 24 h were 1.3 for the intact and abraded sites. At 72 h, the scores were 0.2 (intact) and 0.7 (abraded). The edema scores after 24 h were 0.3 for intact and abraded sites. At 72 h, the edema score was 0.0 for both sites. The primary dermal irritation index was 1.0.

A spray formula containing 12% SD Alcohol 40-B was found to be a nonirritant when evaluated for vaginal mucosal irritation in New Zealand white rabbits.

Many RIPTs were conducted using SD Alcohol 40-B (denatured with Denatonium Benzoate). The materials tested in these studies were a deodorant spray: 98% SD Alcohol 40-B; a night cream: 61.3% SD Alcohol 40-B; other shaving preparation: 82% SD Alcohol 40-B (95%); a gel formula: 29% SD Alcohol 40-B; and a spray formula: 12.0% SD Alcohol 40-B. In all of the tests performed, there was no evidence of dermal sensitization.

A gel formula containing 29% SD Alcohol 40-B and a spray liquid containing 12% SD Alcohol 40-B did not induce photoallergy or dermal sensitization in human subjects. Also, these two formulas did not induce a dermal phototoxic response in humans.

Ethanol is also known as Alcohol for purposes of cosmetic ingredient labeling. Although absorption occurs through skin, ethanol does not appear to affect the integrity of the skin barrier nor reach a very high systemic concentration following dermal exposure. Ethanol may be found in the bloodstream as a result of inhalation exposure and ingestion. Also, ethanol can act as a penetration enhancer.

In rat acute oral toxicity studies, ethanol was not very toxic, with an LD_{50} of 7.06 g/kg in older rats (10 to 12 months)

compared to 10.6 g/kg in younger rats (3 to 4 months). Acute inhalation studies in rats demonstrated an increase in plasma catecholamine levels. Acute ocular toxicity studies demonstrated that ethanol can cause severe injury.

Short-term oral and inhalation studies were not considered relevant to the question of the safety of denatured alcohol in cosmetics. Most of the systemic toxicity of ethanol appears to be associated with chronic abuse of alcohol.

Ethanol is a reproductive and developmental toxicant. Inhibition of sperm transport in rabbits, sterility in rats and dogs, fetal death in mice, cessation of egg production in fruit flies, and abortion in rats are among the reproductive effects reported. Non-dysjunction in fertilized eggs, congenital anomalies (exencephaly, microcephally, maxillary hypoplasia, and limb defects), and growth retardation are among the developmental effects reported.

An increase in chromatid breakage in human fibroblasts exposed in vitro to ethanol was reversed 9 days after the exposure terminated. Human lymphocyte cultures treated with ethanol had chromatid and chromosome breaks, gaps, and rearrangements. In Chinese hamster cells in vitro, ethanol increased the frequency of achromatic lesions. Ethanol was mutagenic in *A. nidulans* and *E. coli*, but not in repair competent *S. cerevisiae*. In vivo genotoxicity has been demonstrated in mice and rats. It has been proposed that the genotoxic effects of ethanol were mediated via its metabolite, acetaldehyde. Because this metabolite is formed in very small amounts in specific tissues in vivo, it has also been suggested that ethanol should not be classified as a mutagen. The microsomal ethanol oxidizing system is induced by ethanol, beginning with the specific ethanol-inducible cytochrome P450CYP2E1. The effects of high levels of cytochrome P450CYP2E1 may be further traced to generation of reactive oxygen species (oxygen radical, superoxide anion, and hydroxyl radical). These reactive oxygen species, in turn, are linked to DNA damage.

No data on the carcinogenicity of topically applied or inhaled denatured alcohol were available.

Occupational exposure to ethanol along with dyes and petrol has been linked to anencephaly, meningocele, and hydrocephally. Ingestion of cosmetics containing denatured alcohol in children younger than 6 years of age produces intoxication-like effects related to the quantity ingested, with a threshold of 50 mg/dl in blood.

Alcohol abuse by pregnant women highly affects a developing embryo and fetus. FAS is characterized by three major categories: intrauterine and postnatal growth retardation, cranial facial dysmorphology, and CNS effects. FAEs can occur if the fetus is exposed to just 1 to 1.5 drinks of alcohol per day by the mother. The fetus is at major risk to FAS if 6 or more drinks per day or occasion (45 drinks per month) are consumed by the pregnant woman.

Although ethanol is denatured to make it unfit for consumption, there have been reports of intentional and unintentional consumption of products containing denatured alcohol.

A brief summary of the effects of chronic ingestion of alcohol documents the intoxication effects, liver damage, brain damage, and possible carcinogenicity. Although acetaldehyde, an ethanol metabolite, is implicated in these effects, the induction of the microsomal ethanol oxidizing system and the ethanol-inducible CYP2E1, and the subsequent formation of reactive oxygen species, is thought to play a major role in DNA damage.

DISCUSSION

Alcohol Denat. and SD Alcohols can be prepared using a wide variety of denaturants.

Because dermal application or inhalation of cosmetic products containing these ingredients will not produce significant systemic exposure to ethanol (also known as Alcohol for purposes of cosmetic ingredient labeling), the CIR Expert Panel concluded that safety of the ingredients should be predicated on the safety of the denaturants used. The Panel considered that the adverse effects known to be associated with Alcohol ingestion included in this safety assessment do not suggest a concern for Alcohol Denat. or SD Alcohols because of the presence of the denaturants, which are added for the express purpose of making the Alcohol unpotable.

The CIR Expert Panel has previously conducted safety assessments of t-Butyl Alcohol, Diethyl Phthalate, Methyl Alcohol, Salicylic Acid, Sodium Salicylate, and Methyl Salicylate, in which each was affirmed safe or safe with qualifications. Given their use as denaturants are at low concentrations of use in Alcohol, the CIR Expert Panel determined that Alcohol Denat. denatured with t-Butyl Alcohol, Diethyl Phthalate, Methyl Alcohol, Salicylic Acid, Sodium Salicylate, and Methyl Salicylate is safe as used in cosmetic formulations with no qualifications. Likewise, because they are denatured with either t-Butyl Alcohol, Diethyl Phthalate, or Methyl Alcohol, SD Alcohols 3-A, 30, 39-B, 39-C, and 40-C all are considered safe as used.

Brucine, Brucine Sulfate, Denatonium Benzoate, and Quassin had not previously been considered as alcohol denaturants. The Panel considered that safety test data on these denaturants themselves is relevant to the assessment of their safety, along with safety test data on SD Alcohols that contain these denaturants.

Accordingly, the Panel considered the available data for Denatonium Benzoate and SD Alcohol 40-B to be sufficient to support the safety of these ingredients in cosmetics. Denatonium Benzoate is sufficiently bitter that it is an effective denaturant at only 0.0006%. The Panel recognizes that data on dermal penetration of Denatonium Benzoate were not available, but considered that the available data on lidocaine, a smaller structurally related chemical, indicates that dermal exposure does not result in measurable systemic exposure. Denatonium Benzoate is even less penetrating than lidocaine, due in part to the charged quaternary ammonium. Given the low concentration of use and the lack of dermal penetration, use of Denatonium Benzoate in denatured alcohol in cosmetics applied to the skin would not result

in significant systemic exposure. Animal toxicity tests using inhalation or gavage exposures, which bypass the skin barrier, showed low systemic toxicity, suggesting that dermal exposure would be nontoxic systemically. SD Alcohol 40-B was not a dermal irritant, sensitizer, or photosensitizer.

The CIR Expert Panel recognizes that certain ingredients in this group are reportedly used in a given product category, but the concentration of use is not available. For other ingredients in this group, information regarding use concentration for specific product categories is provided, but the number of such products is not known. In still other cases, an ingredient is not in current use, but may be used in the future.

Although there are gaps in knowledge about product use, the overall information available on the types of products in which these ingredients are used and at what concentration indicate a pattern of use. Within this overall pattern of use, the Expert Panel considers all ingredients in this group to be safe.

The available data, however, were not sufficient to support the safety of Quassin, Brucine, and Brucine Sulfate, Alcohol Denat. denatured with those denaturants, or SD Alcohol 39 and SD Alcohol 40 (because these SD Alcohols are denatured with Quassin, Brucine, and/or Brucine Sulfate).

In order for the Expert Panel to reach a conclusion for Quassin, Brucine, and Brucine Sulfate, or Alcohol Denat. denatured with these denaturants, or SD Alcohol 39 and SD Alcohol 40 denatured with Quassin, Brucine, or Brucine Sulfate, additional data are needed.

The additional data needs for Quassin, Alcohol Denat. denatured with Quassin, or SD Alcohol 39 denatured with Quassin are genotoxicity data; dermal absorption of the denaturant, and if the denaturant is absorbed at significant level, dermal reproductive and developmental toxicity data; and dermal sensitization and irritation.

The additional data needs for Brucine or Brucine Sulfate, Alcohol Denat. denatured with Brucine or Brucine Sulfate, or SD Alcohol 40 denatured with Brucine or Brucine Sulfate are mammalian genotoxicity data, and dermal absorption of the denaturant, and if the denaturant is absorbed at a significant level, dermal reproductive and developmental toxicity data.

CONCLUSION

The CIR Expert Panel concluded that Alcohol Denat., SD Alcohol 3-A, SD Alcohol 30, SD Alcohol 39-B, SD Alcohol 39-C, SD Alcohol 40-B, and SD Alcohol 40-C denatured with t-Butyl Alcohol, Denatonium Benzoate, Diethyl Phthalate, or Methyl Alcohol are safe in the practices of use and concentration as described in this safety assessment, and, that Denatonium Benzoate is safe as a denaturant.

The CIR Expert Panel concluded that the available data are insufficient to support the safety of Alcohol Denat., SD Alcohol 39, and SD Alcohol 40 denatured with Quassin, Brucine, and Brucine Sulfate in cosmetic products, and that the available data are insufficient to support the safety of Quassin, Brucine, and Brucine Sulfate as denaturants.

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