JOURNAL OF THE AMERICAN COLLEGE OF TOXICOLOGY
Volume 8, Number 4, 1989
Mary Ann Liebert, Inc., Publishers

5

Final Report on the Safety Assessment of Ethyl Acetate and Butyl Acetate

Ethyl and Butyl Acetate are used as solvents in nail polish, nail polish removers, basecoats, and other manicuring preparations.

Ethyl and Butyl Acetate were relatively nontoxic when administered orally, dermally, or by inhalation to rabbits, rats, mice, and guinea pigs. A nail polish containing 10% Ethyl Acetate and 25% Butyl Acetate was a moderate to severe irritant in unrinsed rabbit eyes and a mild irritant in rinsed rabbit eyes. Butyl Acetate was not a sensitizer in either mice or guinea pigs.

Ethyl and Butyl Acetate were nonmutagenic when tested by the Ames procedure, Rec-assay, and micronucleus assay. Neither compound induces mitotic aneuploidy in yeast or chromosomal aberrations in Chinese hamster fibroblasts. Butyl Acetate was not teratogenic when inhaled.

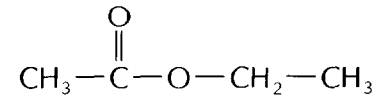
Ethyl Acetate and Butyl Acetate were mild skin irritants but not sensitizers to humans. Ethyl Acetate was neither phototoxic or photoallergenic in human tests.

It is concluded that Ethyl Acetate and Butyl Acetate are safe as cosmetic ingredients in the present practices of use and concentration.

CHEMISTRY

Definition and Structure

Ethyl Acetate (CAS No. 141-78-6) is the ester of ethyl alcohol and acetic acid. Its formula is $C_4H_8O_2$ and it has a molecular weight of 88.10. Ethyl Acetate conforms to the structure[1]:



Butyl Acetate (CAS No. 123-86-4) is the ester of butyl alcohol and acetic acid. It has a molecular weight of 116.16 and the formula $C_6H_{12}O_2$. Butyl Acetate conforms to the structure[1]:

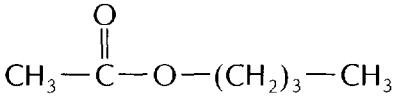


TABLE 1. Chemical and Physical Properties for Ethyl and Butyl Acetate

	Ethyl acetate	*Butyl acetate*
Description	Clear liquid[2]	Clear, colorless liquid[4]
Odor	Fruity[2]	Fruity[2]
Specific gravity (25°C/25°C)	0.898[2]	0.870–0.879[4]
n_D^{20}	1.3719[2]	1.3951[2]
Boiling point (°C)	77[2]	125–126[4]
Melting point (°C)	−83[2]	−77[2]
Flash point (°C)	7.2[2]	22[2]
Acidity	—	0.01% max[4]
Impurities	—	10.0% max[4] (as *n*-butyl and isobutyl alcohol, *n*-propyl, and isobutyl acetate)
Residue on evaporation	—	50 ppm max[4]

Synonyms for Ethyl Acetate include acetic acid, ethyl ester, acetic ether, acetidin, acetoxyethane, ethyl acetic ester, ethyl ethanoate, and vinegar naptha. Butyl Acetate is also known as butyl ethanoate and acetic acid, butyl ester.[2,3]

Chemical and Physical Properties

Ethyl Acetate is a clear volatile, flammable liquid with a characteristic fruity odor and a pleasant taste when diluted. It is slowly decomposed by moisture and then acquires an acid reaction due to the acetic acid formed. It absorbs water up to 3.3% w/w. Ethyl Acetate is miscible with water, alcohols, acetone, chloroform, and ether[2] (Table 1).

Butyl Acetate is a clear colorless, flammable liquid with a characteristic odor. It is slightly miscible with water and miscible with ethanol, ethyl ether, and hydrocarbons. Cosmetic grade Butyl Acetate consists mainly of *n*-Butyl Acetate with lesser amounts of *n*-butyl alcohol and isobutyl alcohol and traces of *n*-propyl acetate and isobutyl acetate. A maximum of 10% is given for the sum of all possible impurities[4] (Table 1).

Method of Manufacture

Ethyl Acetate is obtained by the slow distillation of ethanol and acetic acid in the presence of sulfuric acid. Butyl Acetate is prepared by the slow distillation of butanol and acetic acid in the presence of sulfuric acid. Both reactions are acid-catalyzed nucleophilic additions of the respective alcohol to the acetic acid.[2]

Analytical Methods

Analytical methods for the separation and/or determination of Ethyl and Butyl Acetate include infrared and ultraviolet spectroscopy,[5] gas chromatography,[6] gas chromatography/mass spectroscopy,[7] and head space gas chromatography.[8]

TABLE 2. Product Formulation Data for Ethyl Acetate[10]

Product category	Total no. of formulations in category	Total no. containing ingredient	No. of product formulations within each concentration range (percentage)				
			> 50	> 25–50	> 10–25	> 5–10	> 1–5
Basecoats and undercoats	38	12	—	8	4	—	—
Nail enamel and extenders	535	505	—	6	125	332	42
Nail polish remover	45	17	8	4	5	—	—
Other manicuring preparations	51	25	—	6	8	9	2
1987 TOTALS		559	8	24	142	341	44

USE

Cosmetic

Ethyl and Butyl Acetate are used as solvents in nail polish, nail polish removers, basecoats, and other manicuring preparations. Both are solvents for nitrocellulose, which is the basic film-forming material in nail polish. Ethyl Acetate is a low-boiling solvent that gives the necessary mobility to the polish to enable it to spread easily and dry quickly. In manicuring preparations, Butyl Acetate gives body to the polish and the slower evaporation gives the polish time to key onto the nail surface and flow in a more even film.[9]

Data submitted to the Food and Drug Administration (FDA) in 1987 by cosmetic firms participating in the voluntary cosmetic registration program indicated that Ethyl and Butyl Acetate were used in a total of 559 and 566 formulations, respectively. As mentioned above, the majority of the formulations containing Ethyl and Butyl Acetate were nail polishes and enamels, nail basecoats and undercoats, nail polish and enamel removers, and other manicuring preparations. Butyl Acetate was also used in one formulation of a hand cleanser. The majority of the formulations containing Ethyl Acetate used it in the > 5–10% range while the > 10–25% range was the majority for uses of Butyl Acetate[10] (Tables 2, 3).

The FDA cosmetic product formulation computer printout[10] is compiled through voluntary filing of such data in accordance with Title 21 part 720.4 of

TABLE 3. Product Formulation Data for Butyl Acetate[10]

Product category	Total no. of formulations in category	Total no. containing ingredient	No. of product formulations within each concentration range (percentage)					
			> 50	> 25–50	> 10–25	> 5–10	> 1–5	≤ 0.1
Basecoats and undercoats	38	31	—	28	3	—	—	—
Nail enamel and extenders	535	505	2	74	418	10	1	—
Nail polish remover	45	3	—	1	1	—	—	1
Other manicuring preparations	51	25	—	12	8	3	2	—
Hand cleansers	247	2	1	—	1	—	—	—
1987 TOTALS		566	3	115	431	13	3	1

the Code of Federal Regulations.[11] Ingredients are listed in preset concentration ranges under specific product type categories. Since certain cosmetic ingredients are supplied by the manufacturer at less than 100% concentration, the value reported by the cosmetic formulator may not necessarily reflect the actual concentration found in the finished product; the actual concentration would be a fraction of that reported to the FDA. Data submitted within the framework of preset concentration ranges provide the opportunity for overestimation of the actual concentration of an ingredient in a particular product. An entry at the lowest end of a concentration range is considered the same as one entered at the highest end of that range, thus introducing the possibility of a 2–10-fold error in the assumed ingredient concentration.

Cosmetic products containing Ethyl and Butyl Acetate are typically applied to the nails or skin. During application of these products, Ethyl and Butyl Acetate may come in contact with nasal mucosa and eyes as a result of evaporation from the formulation. Cosmetics formulated with this solvent have the potential for repeated application over the course of many years.

Noncosmetic

Both Ethyl and Butyl Acetate are cited as direct and indirect food additives as detailed in the Code of Federal Regulations. Ethyl Acetate is generally recognized as safe (GRAS) for use as a synthetic flavor and/or adjuvant; limitations on concentrations of use were not specified.[11] It was also approved for use as a diluent in color additive mixtures of inks for marking fruit and vegetables.[11] Both Ethyl and Butyl Acetate are used as adjuvants (release agents, waxes, or dispersants) for resinous and polymeric coatings used as the food-contact surface of articles intended for use in producing, preparing, or holding food.[11] They are both used in cellophane for packaging food; Butyl Acetate's use is limited to 0.1%.[11] Butyl Acetate is also an approved synthetic flavoring substance and/or adjuvant that may be used in food and is required to be used in the minimum quantity needed to produce its intended effect and in accordance with good manufacturing practice.[11] It is also a component of adhesives used in articles intended for use in packaging, transporting, or holding food; providing there is a functional barrier between the food and the article, or the adhesive is used within the limits of good manufacturing practice.[11]

Ethyl Acetate is also used as a solvent for varnishes, lacquers, and aeroplane dopes and in the manufacture of smokeless powders, artificial leather, photographic films and plates, artificial silk, and for cleaning textiles. Butyl Acetate, in addition to the uses described above, is used in the manufacture of lacquer, artificial leather, photographic films, plastic, and safety glass.[2]

GENERAL BIOLOGY

Biochemical Effects

Biochemical alterations in the blood and liver of the rat, following the administration of Ethyl Acetate, were examined. Ten male albino rats received

served as controls. Following a 20 h fast, the animals were killed and blood and liver were collected in 10% trichloroacetic acid solutions. Pyruvic and lactic acid levels were estimated in the blood and liver; liver glycogen levels were also assayed. The activities of acid phosphatase, 5'-nucleotidase, glucose-6-phosphatase, lactic dehydrogenase, succinic dehydrogenase, and adenosine triphosphatase were measured. The pyruvic acid content of the blood continued to increase steadily from day 1 to day 7 and then plateaued. Lactic acid content of the blood was higher for experimental animals than for controls. There was a significant decrease in hepatic glycogen content while the pyruvic and lactic acid concentrations increased in the liver. The increases in the liver were of the same order as the increases in the blood. The activity of glucose-6-phosphatase was decreased while that of lactic dehydrogenase was increased in experimental animals relative to the controls. The activities of adenosine triphosphatase, 5'-nucleotidase, succinic dehydrogenase, and acid phosphatase were not significantly changed by the administration of Ethyl Acetate. The authors suggested that the increase in pyruvic acid contents of the blood and liver might be due to an increased rate of glycolysis, especially when the aforementioned changes in enzymatic activity are taken into account.[12]

Cytotoxicity

Ethyl Acetate was tested for its cytotoxic potential in cultures of human lymphocytes. The effect of Ethyl Acetate on DNA synthesis was measured by the uptake of [^{3}H]thymidine. Short-term toxicity of a culture grown for 4 h was determined by the trypan-blue exclusion technique. The cultures were grown with and without the presence of rat liver metabolic activation. Ethyl Acetate, in concentrations of 10^{-2}, 10^{-3}, and 10^{-4} M, caused a significant inhibition of [^{3}H]thymidine uptake in cultures without rat liver metabolic activation. The viabilities of the cells at these concentrations were unaffected. This suggests that Ethyl Acetate exerted a cytotoxic action that was not immediately followed by cell death. It was stated that Ethyl Acetate "might inhibit some step of the DNA synthesis and/or interfere with thymidine uptake processes."[13]

In another study Ethyl Acetate was tested in five cell lines to determine the highest tolerated dosage in an effort to correlate in vitro tests with ocular irritancy. The cell lines included mouse 3T3 fibroblasts and RAW246.7 macrophages, hamster CHv79 fibroblasts, rabbit epithelial corneal cells, and human $HepG_2$ hepatoma cells. Of the 24 chemicals tested, Ethyl Acetate had the highest tolerated dosage in the human cells and second most tolerated dosage in the other four cell types. The values of the highest tolerated dosages, for Ethyl Acetate, ranged from 500 to 612 mM. The authors noted a good positive correlation between these values and a previously reported mild ocular irritancy value for Ethyl Acetate.[14]

Butyl Acetate was tested for its cytotoxicity in Ehrlich-Landschultz diploid ascites tumor cells propagated in outbred albino mice. Lissamine green B dye was added to samples taken at various times during the 5 h incubation period of the cell cultures grown with Butyl Acetate. The frequency of cells diffusely stained by the dye was taken as an index of cell injury. Butyl Acetate did not

induce an increase in the frequency of irreversibly injured cells as compared with controls. At the end of the 5 h incubation period, concentrations of 50 and 100 ppm of Butyl Acetate produced cell death values of 3.0 and 2.5%, respectively, compared to a control value of 4.2%.[15]

Physiological Effects

The cardiovascular effects of Ethyl Acetate were studied in dogs. Six dogs were anesthetized and the chest cavity was opened. Systemic and pulmonary arterial pressures, heart rate, and myocardial contractile force were measured continuously during the 1 h experimental period. Blood concentrations of Ethyl Acetate were measured by gas–liquid chromatography in arterial samples drawn every 4–5 min from a femoral artery. At a dosage of either 5 or 20 mg/kg/min, administered intravenously, Ethyl Acetate significantly decreased both the heart rate and mean systemic arterial pressure while the mean pulmonary arterial pressure increased gradually. The myocardial contractile force initially increased but then significantly decreased as the concentrations of Ethyl Acetate in the blood became more than 0.3 mg/ml.[16]

The myocardial depressant action of Ethyl Acetate was measured in ventricular strips from guinea pigs. Guinea pigs were killed and the heart removed and right ventricle excised. The ventricular strip was exposed to graded concentrations of Ethyl Acetate, 3×10^{-3}–3×10^{-2} M, and the force of ventricular contractions, stimulated at a rate of 90/min, was measured continuously. A negative inotropic action was observed with Ethyl Acetate, qualitatively similar to that seen with ethanol. Ethyl Acetate induced a magnitude of myocardial depressant action of approximately ten times that of ethanol. The authors suggested that this effect of Ethyl Acetate may have been due to its penetration into the lipid layer of the cell membrane, where it interfered with cellular functions.[17]

Jacobi[18] found that the application of nail polish or enamel considerably hinders the insensible perspiration of the nail, although the effect is easily reversed with the removal of the polish. However, the insensible perspiration of the nails was not notably changed when subjects dipped their nails in Ethyl and Butyl Acetate for 15 min.

ABSORPTION, METABOLISM, AND EXCRETION

Ethyl Acetate was hydrolyzed to ethyl alcohol and acetic acid by whole rat, rabbit, and human blood. The kinetics were studied in rat blood; 3.0 ml of the blood was mixed with 0.10 ml of a 7% v/v solution of Ethyl Acetate in distilled water. This dose provided an initial concentration of 0.20 g/100 ml per incubation tube. The tubes were shaken for 5 h and aliquots of 0.2 ml were withdrawn every half hour for 2 h and every hour thereafter. The aliquots were diluted with 1.0 ml of distilled water containing 0.01% butyl alcohol as an internal standard and analyzed for Ethyl Acetate and ethyl alcohol by gas chromatography. The whole blood hydrolyzed Ethyl Acetate to ethyl alcohol and acetic acid by simple stoichiometric hydrolysis. The data indicated first-

order kinetics and a half-time of 65–70 min was calculated. The half-life of Ethyl Acetate was not calculated for the reaction in rabbit or human blood.[19]

Four male Sprague-Dawley rats, weighing between 300 and 450 g, were used in a study to detect the hydrolysis of Ethyl Acetate. Each rat received an i.p. injection of Ethyl Acetate in a 25% solution in corn oil. The dosage administered, 1.6 ml/kg, was chosen to produce an approximate blood concentration of 0.20 g/100 ml Ethyl Acetate. Blood samples were drawn from the femoral artery 5, 10, 20, 30, 40, 50, 60, 90, 120, 150, and 180 min after the injection and were diluted and analyzed as above. High concentrations of ethyl alcohol in the blood were observed within 5 min following the injection. Ethyl Acetate levels in the blood were low for the first 20 min and undetectable thereafter. The half-life of Ethyl Acetate was estimated as approximately 5–10 min.[19]

The enzymes involved in the hydrolysis of Ethyl Acetate by whole blood are not known. Gallaher and Loomis[19] suggest that the involvement of the cholinesterases and pseudocholinesterases was likely because they were of a nonspecific nature. However, it was unlikely that the blood enzymes were solely responsible for this reaction because the half-life of Ethyl Acetate was much shorter in vivo (5–10 min) than in vitro (65–70 min).

The excretion of three volatile liquids, ether, Ethyl Acetate, and acetone, was examined by short duration washin and washout experiments in four subjects. Carbon dioxide was used as the reference gas. The subjects, 4 men 25–38 years of age, breathed air with low (0.01 vol %) tracer concentrations of the chemicals during washin and room air during washout. The gas concentrations in the air were measured by mass spectrometry. The subjects inhaled 2 breaths of room air followed by 12 breaths of air with Ethyl Acetate followed by 10 washout breaths of room air. To establish different ventilatory conditions, the subjects underwent the experiment both at rest and while riding an ergometer pedaling at about 70 cycles/min at work loads of 0, 50, 100, and 150 W. The excretion values for Ethyl Acetate were calculated from the mixed expired and inspired gas concentrations during washin. Ethyl Acetate had insignificant venous return due to its effective metabolism. No Ethyl Acetate was found in expired gas after a few breaths during washout. The excretion values for Ethyl Acetate and ether were consistently higher than those of acetone.[20]

Male and female subjects were exposed to seven different solvents, including Ethyl Acetate, to study their respiratory elimination. The concentration of the organic solvents in expired air was followed at intervals for up to 17 h after the cessation of exposure. After the cessation rapid decreases were observed in the concentrations of all the solvents in expired air. Ethyl Acetate and ethyl alcohol were eliminated faster than the other solvents. The amount of eliminated Ethyl Acetate was almost negligible and it was undetected 1 h after the cessation of exposure. The large rate constant for Ethyl Acetate might reflect its rapid decrease in blood concentration possibly due to its "rapid biotransformation."[21]

The respiratory retention, uptake, and excretion of Ethyl Acetate and other organic solvents were studied in a group of men and women, ages 18–25. A group of 10 subjects, 5 men and 5 women, was exposed to a concentration of

94–137 ppm Ethyl Acetate for 4 h. The respiratory retention of the solvents decreased with exposure time and was constant after 2 h of exposure. Retention was defined as the actual organic solvent retained in the body and was calculated as the difference between concentrations of the solvent in inhaled and exhaled air. Ethyl Acetate was easily retained and had an average retention value of 57%; during the first 2 h of exposure the male subjects retained more of the Ethyl Acetate (60%) than the female subjects (54%). A similar separation was seen with the uptake of Ethyl Acetate, male subjects had an average uptake value of 63% while female subjects had an average uptake value of 57%. The respiratory uptake was the sum of the retention and excretion of the solvent. An average of 3% of the Ethyl Acetate was excreted by the subjects in this study. The large retention of Ethyl Acetate was "presumably related to its high water solubility and rapid rate of metabolism."[22]

Ethyl Acetate had a strong stimulatory effect on sodium, glucose, and water transport in vitro in the small intestine of the golden hamster. The method of this stimulation was suggested as being a facilitated entry of glucose and sodium into the cell and a stimulation of glucose and sodium extrusion across the basolateral membrane of the cell, thus resulting in more sodium and glucose being transported. The authors concluded that Ethyl Acetate "is a substance which can easily penetrate into the cell and supply energy to it, so that the net transepithelial transport of Na^+ and glucose is greatly enhanced."[23]

The percutaneous absorption of [^{3}H]water in half-saturated solutions in organic solvents, including Butyl Acetate, was measured in human epidermis in vitro. Discs of human epidermal membrane tissue were clamped between the two chambers of a glass diffusion cell. The amount of [^{3}H]water that passed through the skin was measured by assaying, in a liquid scintillation counter, the radioactivity of samples drawn from the "receptor" chamber. An outside to inside water diffusion rate of 0.71 $\mu l/cm^2/h$ was determined for a half-saturated solution of Butyl Acetate and [^{3}H]water. The predicted rate of absorption of water was approximately 0.65 $\mu l/cm^2/h$. The results were consistent with predictions that absorption rates will be proportional to the thermodynamic activity of the penetrant in the vehicle if the properties of the stratum corneum are not changed by said vehicle.[24]

ANIMAL TOXICOLOGY

Acute Toxicity

Oral

Ethyl and Butyl Acetate were tested to calculate the LD_{50} and ND_{50} values in rabbits. The ND_{50} value was defined as the quantity that produced stupor and loss of voluntary movements in half of the rabbits. Each ingredient was administered by gavage to groups of 10–35 rabbits each weighing between 1.5 and 2.5 kg. LD_{50} values of 4.9 g/kg and 7.4 g/kg and ND_{50} values of 4.5 g/kg

TABLE 4. Acute Toxicity of Ethyl and Butyl Acetate

Ingredient	*Route of administration*	*Animal*	*No. of animals*	*Comments*	*References*
Ethyl Acetate	Oral	Rabbits	10–35	LD_{50} = 4.9 g/kg; ND_{50} = 4.5 g/kg	25
Ethyl Acetate	Oral	Rats	—	Median LD_{50} for all samples = 9.8 g/kg	27
Ethyl Acetate	Oral	Rats	—	LD_{50} = 5.6 g/kg	28
Ethyl Acetate	Oral	Rats	—	LD_{50} = 10.1 g/kg	3
Ethyl Acetate	Oral	Mice	—	LD_{50} = 0.44 g/kg	28
Ethyl Acetate	Inhalation	Mice	24	RD_{50} = 580 ppm	29
Ethyl Acetate	Inhalation	Mice	16	RD_{50} = 614 ppm	30
Ethyl Acetate	Inhalation	Rats	—	LC_{50} = 16,000 ppm after 6 h	28
Ethyl Acetate	Intraperitoneal	Mice	—	LD_{50} = 709 mg/kg	3
Ethyl Acetate	Subcutaneous	Cats	—	LD_{50} = 3 g/kg	3
Ethyl Acetate	Subcutaneous	Guinea pigs	—	LD_{50} = 3 g/kg	3
Ethyl Acetate, 97% in product	Oral	Rats	10	LD_{50} > 5.01 g/kg	31
Ethyl Acetate, 16.5% in nail polish remover	Oral	Rats	—	LD_{50} > 5.0 ml/kg	32
Ethyl Acetate, 10% in nail polish	Oral	Rats	10	One dose of 5 ml/kg, no deaths	33
Butyl Acetate, (25%) Ethyl Acetate, (10%) in nail polish	Oral	Rats	10	LD_{50} > 5 g/kg, 0/10 rats died at a dosage of 5 g/kg	34
Butyl Acetate	Oral	Rabbits	10–35	LD_{50} = 7.4 g/kg; ND_{50} = 2.2 g/kg	25
Butyl Acetate	Oral	Rats	5 per dose	LD_{50} = 14.13 g/kg	26
Butyl Acetate	Oral	Mice	—	LD_{50} = 7.1 g/kg	35
Butyl Acetate	Oral	Mice	—	LD_{50} = 7.1 g/kg	3
Butyl Acetate	Inhalation	Rats	—	LC_{50} = 2000 ppm after 4 h	3
Butyl Acetate	Intraperitoneal	Guinea pigs	8	Doses of 750 and 1500 mg/kg. Slightly elevated hepatic enzyme activity at lower dose. Two of four animals died and hepatic enzymatic activity elevated at higher dosage.	36
Butyl Acetate	Intraperitoneal	Mice	—	LD_{50} = 1.23 g/kg	3
Butyl Acetate	Dermal	Rabbits	10	LD_{50} > 5 g/kg; 1 of 10 animals died at a dosage of 5 g/kg.	37
Butyl Acetate	Dermal	Rabbits	4	LD_{50} > 20 ml/kg	26

and 2.2 g/kg were reported for Ethyl and Butyl Acetate, respectively[25] (Table 4).

Butyl Acetate was tested for its acute oral toxicity in rats. A logarithmic series of doses was given by intubation to groups of five Carworth-Wistar rats weighing between 90 and 120 g. The Butyl Acetate was diluted with either water, corn oil, or a 1% solution of sodium 3,9-diethyl-6-tridecanol sulfate if it was necessary to bring the volume given one rat to between 1 and 10 ml. The rats were observed for 14 days after the dosing. An oral LD_{50} value of 14.13 g/kg was obtained[26] (Table 4).

In a study conducted to explore the joint toxic action of 27 industrial solvents, a median oral LD_{50} value was reported for all the samples of Ethyl Acetate used. All animals used in the testing were female, Wistar strain, albino rats weighing between 90 and 120 g. The median LD_{50} value of 9.8 g/kg was reported for all the samples of Ethyl Acetate used[27] (Table 4).

The oral toxicity of a product containing 97.0% Ethyl Acetate was evaluated using 10 Sprague-Dawley rats (weights 200–255 g). After a 16 h fast, the product was administered (single dose) to each animal via oral intubation. The animals were observed for a total of 14 days after administration. Only one animal died during the study, and the LD_{50} was said to have been greater than 5.01 g/kg[31] (Table 4).

The oral toxicity of a nail polish remover containing 16.5% Ethyl Acetate was evaluated using male and female rats (number, weights, and strain not stated). Following the administration of a single oral dose (5.0 ml/kg), the LD_{50} was not achieved[32] (Table 4).

A nail polish formulation containing 10% Ethyl Acetate and 25% Butyl Acetate was also tested for acute oral toxicity in rats. Wistar albino rats, 5 male and 5 female, were fasted for 16 h prior to the test. The rats, weighing between 245 and 346 g, were given an oral dose of the nail polish of 5 ml/kg. None of the rats tested died during the 14 day observation period following the administration of the nail polish[34] (Table 4).

A study was conducted to determine the acute oral toxicity of a nail polish formulation containing 10% Ethyl Acetate. The nail polish was fed, at a dosage of 5 ml/kg, to a group of 5 male and 5 female Sprague-Dawley rats. The rats were fasted for 18 h before the administration of the nail polish. No toxic effects were observed and none of the animals died during the 14 day observation period following the administration of the nail polish.[33]

In other studies, Ethyl Acetate had oral LD_{50} values in rats of 5.6 g/kg,[28] and 10.1 g/kg,[3] and 0.44 g/kg in mice.[28] Butyl Acetate had an oral LD_{50} value of 7.1 g/kg in mice[3,35] (Table 4).

Inhalation

Male albino rats, five for each concentration, were exposed to air containing concentrations of 2.5, 5, and 10% Ethyl Acetate and to air containing mixtures of toluene and Ethyl Acetate. The concentrations of Ethyl Acetate, ethanol, and toluene were measured in the blood, liver, and brain by gas chromatography. All five animals died after 38–70 min of exposure to 2.5% Ethyl Acetate, after 28–36 min of exposure to 5% Ethyl Acetate, and after 19–32 min of exposure to 10% Ethyl Acetate. Ethyl Acetate was not detected in the liver of any animal. In all groups tested, the concentrations of Ethyl Acetate in the brain were higher than those in the blood. The maximum concentration in the brain, 0.4 mg/g, was detected within the group exposed to 10% Ethyl Acetate. The average concentrations of ethanol in the blood were 1.32, 1.40, and 1.24 mg/g for rats exposed to 2.5, 5, and 10% Ethyl Acetate, respectively.[38]

A short-term inhalation experiment to study sensory irritation was conducted using 22 industrial airborne solvents including Ethyl Acetate. The index for sensory irritation was the reflex decrease in respiratory rate. Male Swiss

OF_1 mice that weighed 25–27 g were used in the study. Stainless steel 200 L inhalation chambers were used and each received adjustable airflows of 10 to 30 m^3/h. At least four different concentrations and six mice per concentration were used to calculate the reflex decrease in respiratory rate for Ethyl Acetate. The mice were secured in body plethysmographs and exposed to a predetermined concentration for about 5 min. The RD_{50} value, the concentration associated with a 50% decrease in respiratory rate was calculated as 580 ppm for Ethyl Acetate[29] (Table 4).

Ethyl Acetate was evaluated along with ten other industrial solvents for its sensory irritation potential in another inhalation experiment. The method used was similar to that described above, with the exception that four male Swiss-Webster mice were used for each concentration tested and the animals were exposed to the experimental atmospheres for 10 min. The authors reported that the time–response relationship of Ethyl Acetate was different from that of the other industrial solvents tested. The decrease in respiratory rate was typical of a sensory irritation pattern but the reaction was slower and reached a plateau before recovery after the termination of exposure. The pattern observed was similar to the most common pattern previously observed with sensory irritants. Ethyl Acetate was the most potent solvent tested in the series of solvents, with an RD_{50} value of 614 ppm[30] (Table 4).

In both of the inhalation studies described above, the authors reported that the threshold limit value (TLV) for Ethyl Acetate, determined as 400 ppm, was too high.[39] This was based on the assumption of a model, which was applicable to most of the other solvents tested, that the TLV should be between 0.1 and 0.01 of the RD_{50} values. According to this model, the TLV should be between 5.8 to 58 ppm and 6.14 to 61.4 ppm, respectively, for the two RD_{50} values reported.[29,30] Alarie[40] proposed an alternative model in which the TLV should be calculated as 0.03 of the RD_{50}, the midpoint on a logarithmic scale between 0.1 and 0.01 of the RD_{50}, as suggested above. This would lead to a TLV of 17.4 or 18.4 ppm from the RD_{50} values of 580 ppm and 614 ppm, respectively.

An inhalation LC_{50} value in rats of 16,000 ppm after 6 h was also reported for Ethyl Acetate.[28] A TLV value of 150 ppm was recommended for Butyl Acetate with a Short-Term Exposure Limit (STEL) of 200 ppm also recommended.[39] Butyl Acetate also had a reported LC_{50} value of 2000 ppm after 4 h in rats[3] (Table 4).

Intraperitoneal

Butyl Acetate was injected undiluted into the peritoneal cavity of mature male guinea pigs. One day after the injection, 2 ml of blood was withdrawn from each animal by heart puncture to determine the activity of serum ornithine carbamyl transferase (OCT). The OCT assay is proposed as a method of determining incipient hepatic damage. Doses of 750 and 1500 mg/kg were each administered to groups of four guinea pigs. At the higher dosage, two of four animals died. Enzymatic activity was slightly increased at the lower dosage and elevated to 23.9 IU in the two surviving animals from the higher dosage group. Normal OCT activity is approximately 2 IU. A hepatic lesion was a slight accumulation of intracellular lipid. Butyl Acetate had a low order

of hepatotoxicity that correlated with increased OCT activity only at a dosage greater than 500 mg/kg[36] (Table 4).

An intraperitoneal LD_{50} value of 1.23 g/kg was also reported for Butyl Acetate in mice.[3] Ethyl Acetate, tested in mice, had an i.p. LD_{50} value of 709 mg/kg[3] (Table 4).

Dermal

Four male New Zealand albino rabbits were used to test the skin penetration and toxicity of Butyl Acetate. The rabbits, with weights ranging from 2.5 to 3.5 kg, were dosed with 20 ml/kg of the test substance, applied under an impervious plastic film to shaved skin. The dose was in contact with approximately 1/10 of the body surface. The film was removed after 24 h and the animals were observed for 14 days. The LD_{50} value was >20 ml/kg[26] (Table 4).

Butyl Acetate was administered dermally at a dosage of 5 g/kg to ten rabbits. One of the ten animals died on the eighth day of observation. On 1 day prior to its death, this animal had ptosis and was lethargic with flaccid muscle tone. On necropsy, one of the animals involved in the study (it was not stated whether it was the animal that had died during the test) had red areas on and bloating of the intestines and dark areas on the lungs. Slight to moderate erythema and edema was observed in two to six rabbits.[37] An LD_{50} value of 3 g/kg, in both guinea pigs and cats, was reported for Ethyl Acetate administered subcutaneously[28] (Table 4).

Irritation

Dermal

The primary skin irritation of Butyl Acetate was tested using rabbits. An application of 0.01 ml of the undiluted acetate was applied to the clipped skin of five albino rabbits. The reactions reported were the most severe seen and were scored within 24 h of the application. Butyl Acetate was scored as a Grade 1 irritant indicating "the least visible capillary injection" from the undiluted Butyl Acetate.[26]

A nail polish formulation containing 10% Ethyl Acetate was tested for dermal irritation in six female New Zealand White rabbits. The nail polish was applied under an open patch to the clipped skin of the rabbits for three consecutive 24 h periods. No positive responses were observed to any application in any of the six rabbits tested. The nail polish formulation was not a skin irritant.[41]

Ocular

The ocular irritation potential of a nail polish remover containing 16.5% Ethyl Acetate was evaluated using rabbits (number and strain not stated). The product (0.1 ml) was instilled into one eye of each animal. At 1 h postinstillation, slight corneal opacity and severe conjunctivitis were observed. Moderate corneal opacity and extreme iritis were noted on day 3. On day 8, corneal vascularization, slight corneal opacity, and moderate conjunctivitis were ob-

served. Corneal dullness, slight conjunctivitis, and 35% corneal vascularization were present at the conclusion of the study.[42]

The ocular irritancy of a nail polish containing 10% Ethyl Acetate and 25% Butyl Acetate was measured in a study using nine New Zealand White rabbits. The nail polish, 0.1 ml, was instilled into one eye of each rabbit, the untreated eye served as a control. In three of the rabbits the treated eye was rinsed with 20 ml of water 30 s after instillation of the polish. The reactions were scored 1, 2, 3, 4, and 7 days after the administration of the formulation. In the eyes of the rabbits that did not receive a water rinse, minimal corneal opacity was observed in three, moderate corneal opacity in two, and corneal stippling in six of the six rabbits tested. Minimal abnormalities were seen in the iris of five of six rabbits with nonrinsed eyes; the abnormalities had cleared by day 7 in all but one rabbit. Moderate to severe erythema and minimal to severe edema, as well as necrosis, were observed in the conjunctivas of rabbits not receiving a water rinse; most of the conjunctival irritation had cleared by day 7. In the three rabbits that received a water rinse, three had minimal corneal opacity and stippling that cleared after seven days. No abnormalities of the iris were seen in the rabbits that received a water rinse. Minimal to moderate erythema and edema were observed in three and two of the rabbits with rinsed eyes, respectively; these conjunctival effects had cleared by day 7.[43]

A nail polish formulation containing 10% Ethyl Acetate was tested for its ocular irritancy in nine New Zealand White rabbits. The nail polish (0.1 ml) was instilled into one eye of the rabbits and the reactions were observed and scored according to the Draize eye test procedures. In three of three rabbits that did not receive a water rinse, redness and chemosis were present at 72 h and 6 days; the reactions had cleared by day 9. In the rabbits that received a water rinse 4 s after instillation of the nail polish, two of three had slight redness after 72 h and 6 days, with the reactions clearing after 7 days. None of the three rabbits that received a water rinse 2 s after the instillation of the nail polish had any eye irritation. The nail polish formulation was classified as a mild eye irritant.[44]

Butyl Acetate was evaluated for its eye irritation potential in rabbits. The degree of corneal necrosis was reported from the instillation of various volumes and concentrations of the chemicals into the eyes of the animals. Butyl Acetate was reported as a grade 5 irritant, which indicates a "so-called severe burn" from 0.005 ml of the undiluted chemical. It was not stated whether the eyes received a water rinse after instillation of the test material.[26]

Sensitization

The mouse ear swelling test was developed as an alternative test for dermal sensitization. Butyl Acetate was tested for its sensitizing potential in both mice and guinea pigs. The study was conducted using groups of 10–15 female, 6–8-week-old CF-1 mice. On the first day of the study the animals' abdomens were clipped and tape stripped and two intradermal injections totaling 0.05 ml of Freund's Complete Adjuvant were administered. Undiluted Butyl Acetate, 100 μl, was applied to the shaved area on the first day and the following 3 days, with the skin being tape stripped before each application.

After a 7 day nontreatment period, a topical application of Butyl Acetate, 20 μl of a 50% solution in 70% ETOH, was made to the left ear of the mouse while the right ear received 20 μl of the vehicle alone. The ear thickness was measured 24 and 48 h after the challenge application. None of the mice were sensitized and the percentage swelling was reported as 103%, with a value of 100% indicating no swelling.[45]

A guinea pig maximization test[46] was also conducted on Butyl Acetate to validate the findings in the mouse ear swelling test. Fifteen Hartley strain guinea pigs were each given six intradermal injections on the first day of the study. Two injections of each of the following were given: a 50% solution of Butyl Acetate in ethanol, Freund's Complete Adjuvant, and Butyl Acetate in Freund's Complete Adjuvant. After a 7 day nontreatment period, a closed patch application of undiluted Butyl Acetate was applied to the intradermal (i.d.) site skin for 48 h. A challenge application of a closed patch to an untreated site was made 7 days after the induction patch and the challenge patch was left on for 24 h. None of the 15 animals tested were sensitized to Butyl Acetate.[45]

TERATOGENICITY

Maternal toxicity, reproductive performance, and developmental toxicology were evaluated in rats and rabbits following an inhalation exposure to 1500 ppm Butyl Acetate for 7 h/day. Sexually mature New Zealand White rabbit does weighing about 3 kg were used in the experiment and 30 does were assigned to the Butyl Acetate exposure group. The does were artificially inseminated; the morning after the insemination was regarded as day 1 of gestation. The rabbits were randomly assigned to one of the following exposure groups: filtered air (control), exposure to Butyl Acetate on days 7–19 gestation, and exposure to Butyl Acetate on days 1–19 of gestation. Food consumption was measured for 2 weeks prior to the initiation of the exposure and every 5 days during gestation. Body weights were measured in the morning every 5 days during gestation. The rabbits were killed and necropsied on day 30 of gestation and examined for toxic changes, including altered body weight, organ weights, and lesions. Reproductive measures included determination of the numbers of corpora lutea, implantation sites, resorptions, and dead and live fetuses. The live fetuses were weighed, measured, and examined to detect external, skeletal, and viseral morphologic anomalies. Although exposure of rabbits to 1500 ppm Butyl Acetate resulted in decreased food consumption, no related changes in body weights were observed. No effects were observed in the measures for fertility and reproductive status in rabbits exposed to Butyl Acetate. There was a significantly higher incidence of misaligned sternebrae and of retinal folds in the fetuses of rabbits inhaling Butyl Acetate during days 1–19 of gestation compared to those in the filtered air control group. No major malformations were observed in fetuses in any exposure group.[47]

Female young adult Sprague-Dawley CD rats were also exposed to an atmosphere containing 1500 ppm Butyl Acetate for 7 h each day of exposure.

The rats, weighing between 170 and 175 g, were classified into two pregestational exposure groups: 170 rats exposed to filtered air and 50 rats exposed to Butyl Acetate for 7 h a day, 5 days a week for 3 weeks. At the end of the third week of pregestational exposure the females were mated with male rats of the same strain that weighed between 200 and 225 g. The male rats did not receive any exposure to Butyl Acetate. The day during which sperm were first observed in the vagina was designated as day 1 of gestation. The rats that had not received the pregestational exposure were classified into the following exposure groups: filtered air (control) (Group 1), exposure to Butyl Acetate during days 7–16 of gestation (Group 2), and exposure to Butyl Acetate during days 1–16 of gestation (Group 3). The rats that had received the pregestational exposure were then exposed to Butyl Acetate during days 1–16 of gestation (group 4). The rats were killed and necropsied on day 21 of gestation. The parameters measured and procedures used were essentially the same as those described above. Food consumption was decreased in group 4 during their first week of pregestational exposure. These rats may have become conditioned to the exposure since their food consumption increased to rates above the rates of group 1 for subsequent time periods. Food consumption was lower in the animals exposed to Butyl Acetate than in the control animals and the consumption decreased significantly following the initiation of exposure. Body and liver weights were lower in the animals exposed to Butyl Acetate than in the control animals. The relative weight of the lungs and kidneys were higher in groups 2, 3, and 4 in comparison with group 1 and were the highest in the rats exposed to Butyl Acetate for 31 days (group 4). Mating performance, intrauterine mortality rate, and reproductive performance were not affected by the exposure of the rats to Butyl Acetate. The body weights and crown–rump lengths of the fetuses, as well as placental weights, were significantly lower in the rats exposed to Butyl Acetate than the rats exposed only to filtered air. Fetuses in groups 2, 3, and 4, (two, one, and three, respectively) had major malformations, which included multiple facial defects, eye defects, diaphragmatic hernias, and generalized brain dysmorphology. However, the incidence of these malformations was not significantly different from controls. The incidence of rib dysmorphology was increased in fetuses of rats exposed to Butyl Acetate. Reduced pelvic ossification was observed in the fetuses of groups 2 and 3. Compared to controls, a larger number of fetuses from rats exposed to Butyl Acetate had dilated ureters. The increase of rib dysmorphology might be an indicator of an effect of Butyl Acetate on development but the investigators hesitated "to define this as a teratogenic effect of *n*-butyl acetate, since a similar increase was not seen in the group of rats exposed during this period of gestation subsequent to a pregestational exposure." The authors concluded that Butyl Acetate was not teratogenic.[47]

MUTAGENICITY

Salmonella/microsome reverse mutation assays were carried out for both Ethyl and Butyl Acetate diluted in dimethylsulfoxide (DMSO) with maximum dosage of 5.0 and 10.0 mg/plate, respectively, according to the method of

TABLE 5. Mutagenicity of Ethyl and Butyl Acetate

Ingredient	*Study type, organism*	*Method notes*[a]	*Results and comments*	*Reference*
Ethyl Acetate	Ames test, *S. typhimurium*	Strains: TA92, TA94, TA100, TA1535, TA1537. +S9. Dosage: 5 mg/plate.	Negative	49
	Rec-assay, *B. subtilis*	Strains: H17 Rec + and M45 Rec −	Negative	51
	Mitotic aneuploidy, *S. cerevisiae*	Strain: D61.M. Dosage: 2.44%.	Positive, induced mitotic aneuploidy but no increase in frequency of point mutation or mitotic recombination	50
	Chromosomal aberrations	Chinese hamster fibroblasts. −S9. Dosage: 9.0 mg/ml.	Positive, total incidence of aberrations 11%	49
	Micronucleus assay, hamster bone marrow	Oral dosage: 2500 mg/kg. I.p. dosage: 473 mg/kg.	Negative	52
Butyl Acetate	Ames test, *S. typhimurium*	Strains: TA92, TA94, TA100, TA1535, TA1537. +S9. Dosage: 2.0 mg/ml.	Negative	49
	Ames test, *S. typhimurium* and *E. coli*	Strains: TA98, TA100, TA1535, TA1537, TA1538, and WP2uvrA. +S9 and −S9. Dosage ≤ 5000 μg/plate.	Negative	53
	Mitotic aneuploidy, *S. cerevisiae*	Strain: D61.M	Negative	50
	Chromosomal aberrations	Chinese hamster fibroblasts. −S9. Dosage: 2.0 mg/ml.	Negative	49

[a] +S9 = with metabolic activation, −S9 = without metabolic activation, other information regarding specific study methods.

Ames et al.[48] *Salmonella typhimurium* strains TA92, TA1535, TA100, TA1537, TA94, and TA98 with rat liver microsomal activation were used in the assays. Both Ethyl and Butyl Acetate were nonmutagenic. Chromosomal aberration tests were also carried out for Ethyl and Butyl Acetate using a Chinese hamster fibroblast cell line. Ethyl and Butyl Acetate were dissolved in DMSO, maximum dosages of 9.0 and 2.0 mg/ml, respectively, and no metabolic activation was used in the study. Ethyl Acetate was mutagenic, and the total incidence of cells with aberrations was 11%, a positive result being designated by a value of 10% or above. Butyl Acetate was nonmutagenic[49] (Table 5).

Ethyl Acetate induced mitotic chromosomal malsegregation in a D61.M strain of *Saccharomyces cerevisiae*. Butyl Acetate had a negative result. Ethyl Acetate, 2.44%, induced mitotic aneuploidy in the yeast, but did not increase the frequencies of point mutation or mitotic recombination. The authors suggested that the most likely target producing the malsegregation was the spindle apparatus[50] (Table 5).

Ethyl Acetate was tested, as a vehicle control, in a Rec-assay experimental procedure using *Bacillus subtilis* strains H17 Rec + and M45 Rec − without metabolic activation. Ethyl Acetate was nonmutagenic in both strains[51] (Table 5).

Male and female Chinese hamsters were used in a micronucleus test to determine the mutagenic potential of Ethyl Acetate. The animals were given a dose of approximately two-thirds of the LD_{50} of Ethyl Acetate suspended in corn oil. This corresponds to a dose of 2500 mg/kg administered orally and

473 mg/kg administered by i.p. injection. The animals were killed 12, 24, 48, and 72 h after a single administration of Ethyl Acetate. The micronucleus test was performed and the number of micronucleated erythrocytes was counted. Ethyl Acetate was nonmutagenic when administered either orally or by i.p. injection[52] (Table 5).

Ethyl Acetate stimulated the mutagenicity of sugar degradation compounds in *Salmonella typhimurium* and the depolymerization of DNA by the same sugar degradation compounds, but did not enhance the mutagenicity of other mutagenic compounds. The results demonstrated that the organic solvents, including Ethyl Acetate, enhanced the formation of oxygen radicals by the sugar degradation compounds, which led to the stimulation of their mutagenicity[54] (Table 5).

Butyl Acetate, diluted in DMSO, was tested according to the Ames procedure in five strains of *Salmonella typhimurium*: TA100, TA98, TA1535, TA1537, and TA1538, and in one strain of *Escherichia coli*: WP2uvrA. Butyl Acetate was nonmutagenic in tests conducted both with and without microsomal activation at dosages up to 5,000 μg/plate[53] (Table 5).

CARCINOGENICITY

Ethyl Acetate was tested for carcinogenicity by the pulmonary tumor response in strain A mice. Male and female A/He mice, weighing between 18 and 20 g, were used for the study. Two groups of 30 mice, 15 of each sex, received doses of 18 g/kg and 3.6 g/kg, the maximally tolerated single dose and a 1:5 dilution, respectively. The mice in each group received three weekly i.p. injections of Ethyl Acetate in tricaprylin for a total of 24 doses. The animals were killed 24 weeks after the first injection. The lungs were removed and fixed and the milky white nodules on the surface of the lungs counted and some submitted for histopathologic evaluation. The liver, kidneys, spleen, thymus, intestine, and endocrine and salivary glands were examined at necropsy for any abnormalities. Of the 30 male and female mice (first group) receiving 18.0 g/kg doses of Ethyl Acetate, 7 (20.3%) had lung tumors. Dosages of 3.60 g/kg induced lung tumors in 2 (6.6%) of 30 mice (second group). In the vehicle control group, 38 (23.7%) of 160 male and female mice had lung tumors. In the untreated control group, 20 (20.0%) of 100 mice had lung tumors. Ethyl Acetate did not produce an increase in lung tumors compared with controls.[55]

CLINICAL ASSESSMENT OF SAFETY

Dermal Irritation and Sensitization

The skin sensitization potential of a product containing 97% Ethyl Acetate was evaluated using the maximization test.[56] A total of 25 subjects (18–48 years old) were tested. The product (0.3 ml) was applied under an occlusive patch to the forearm (volar aspect) of each subject during five 48 h periods. After a 10-day nontreatment period, the product was reapplied (occlusive

patch) to a different site. Reactions were scored immediately after patch removal and 24 h later according to the scale 0 (no sensitization) to 3 (strong sensitization). The product was not a sensitizer[57] (Table 6).

The skin irritation and sensitization potentials of a nail polish remover containing 16.5% Ethyl Acetate were evaluated via a modification of the prophetic patch test. A total of 118 subjects (18–65 years old) were tested. The product (1 ml) was applied to the back of each subject with a brush applicator, and each site was covered with an open patch for 48 h. Sites were then rinsed and scored according to the scale 0 (no reaction) to 4+ (erythema, edema/induration, and blisters, with or without ulceration). Reactions were not observed in any of the subjects. The subjects were then instructed to use the nail polish remover for 4 weeks, after which each was patch tested (open patches) with the product according to the same procedure. Again, reactions to the product were not observed. The product was neither a skin irritant nor a sensitizer[58] (Table 6).

The sensitization potential of Ethyl Acetate was evaluated in another study. Ethyl Acetate, 10% in petrolatum, was applied under an occlusive patch to the same sites on the forearms of 25 male subjects (21–48 years old). The patches were applied to the skin, on 5 alternate days, for 48 h. The patch sites were pretreated for 24 h with a 5% aqueous sodium lauryl sulfate solution. After a nontreatment period of 10 days, occlusive challenge patches were applied to an untreated site for 48 h. The challenge patches were preceded by 1 h applications of 10% aqueous sodium lauryl sulfate under occlusive patches. Reactions to the challenge patches were scored after removal of the patch and again 24 h later. None of the subjects had any reaction to the challenge patch[59] (Table 6).

Fifty subjects were involved in a repeat insult patch test using an unspecified concentration of Butyl Acetate. Patches containing 0.5 ml of Butyl Acetate were applied for nine 24 h applications over a 3 week period. Challenge patches were applied 10–14 days after the final induction application. None of the subjects were sensitized[45] (Table 6).

Butyl Acetate was tested for its sensitizing potential; 26 of 35 subjects completed the test. Butyl Acetate, 4% in petrolatum, was applied under an occlusive patch to the forearm and was left in contact with the skin for five alternate-day 48 h periods. The patch sites were pretreated with a 5% aqueous sodium lauryl sulfate solution under an occlusive 24 h patch. After a nontreatment period of 10–14 days, two challenge patches were applied to the upper back for 48 h. One of the challenge patch sites was pretreated with a 30 min application of 5% aqueous sodium lauryl sulfate. The pretreatment with sodium lauryl sulfate produced a slight to moderate degree of irritation in this study. One subject had sweat retention reactions at the pretreated sites, but no other evidence of irritation or sensitization was observed[65] (Table 6).

A nail polish formulation containing 10% Ethyl Acetate and 25% Butyl Acetate was tested for its contact sensitizing potential. The polish was applied, under occlusive patches, to the same site on 11 male and 14 female subjects, ages 18–40. The patches were in contact with the skin for five 48 h periods. The patch site was pretreated for 24 h with a 2.5% solution of sodium lauryl sulfate. Following a 10 day nontreatment period, a challenge patch was

TABLE 6. Clinical Assessment of Safety: Dermal Irritation and Sensitization of Ethyl and Butyl Acetate

Ingredient	*Type of test/method notes*	*No. of subjects*	*Results/comments*	*Reference*
Ethyl Acetate (97% in product)	RIPT[a]	25	No sensitization	57
Ethyl Acetate (16.5% in nail polish remover)	Modified prophetic patch test	118	No irritation or sensitization	58
Ethyl Acetate (10% in petrolatum)	RIPT/SLS[b] pretreatment	25	No sensitization	59
Ethyl Acetate (10% in nail polish)	RIPT	218	Three positive reactions, not a "clinically significant" irritant or sensitizer	60
Ethyl Acetate (6.5% in nail color)	Photopatch test	30	Not phototoxic, not photoallergenic	61
Ethyl Acetate (10%) Butyl Acetate (25%) in nail polish	RIPT/SLS pretreatment	25	No sensitization	62
Ethyl Acetate (97% in product)	Cumulative irritation/21 consecutive 23 h patches	10	No cumulative skin irritation	63
Ethyl Acetate (10%) Butyl Acetate (25%) in nail polish	Cumulative irritation/21 consecutive 23 h patches	13	Slight irritation, score of 106/630	64
Butyl Acetate (4% in petrolatum)	RIPT/SLS pretreatment	26	One subject with sweat retention, no irritation or sensitization	65
Butyl Acetate	Nine 24 h induction patches, 10–14 days nontreatment before challenge	50	No sensitization	45
Butyl Acetate (25.5% in nail enamel)	RIPT/SLS pretreatment	25	No sensitization	66
Butyl Acetate (25.5% in nail enamel)	Cumulative irritation/21 consecutive 23 h patches	10	No cumulative skin irritation	67
Butyl Acetate (25.5% in nail enamel)	Clinical use study/three applications per week for 3 weeks	55	No adverse reactions	68

[a]RIPT = repeat insult patch test
[b]SLS = sodium lauryl sulfate

applied after a pretreatment of 1 h with 5–10% sodium lauryl sulfate. The challenge patch was in contact with the skin for 24 h and the reactions were read after the removal of the patch and again 24 h later. None of the subjects had any positive reactions to the challenge patch and the nail polish was nonsensitizing[62] (Table 6).

A maximization test was conducted to determine the sensitizing potential of a nail enamel formulation containing 25.5% Butyl Acetate. The formulation, 0.3 ml, was applied to the skin of 25 subjects, 10 men and 15 women. Occlusive patches were applied for five 48 h periods following a 24 h pretreatment with 1.5% sodium lauryl sulfate. No irritation was observed during the induction period. Following 10 days of nontreatment a challenge patch was applied after a 1 h pretreatment with 5% sodium lauryl sulfate. No reactions in any of the 25 subjects, ages 18–57, were observed either upon removal of the challenge patch or 24 h later. The enamel was judged unlikely to present "a danger of contact-sensitization during normal, intended use"[66] (Table 6).

The cumulative skin irritation potential of a product containing 97% Ethyl Acetate was evaluated using 10 subjects (40–60+ years old). Twenty-one daily consecutive applications of the product (0.1 ml) were made to the back of each subject under closed patches. Each patch was removed after 23 h and the product was rinsed from the skin. Sites were scored 1 h after patch removal according to the scale 0 (no irritation) to 7 (strong reaction spreading beyond test site). There was essentially no evidence of cumulative skin irritation[63] (Table 6).

In another study, the cumulative irritation potential of a nail polish containing 10% Ethyl Acetate and 25% Butyl Acetate was investigated in a study using 2 male and 11 female subjects. The polish, 0.4 ml, was applied to a gauze patch 30 min before application in order to permit evaporation of its volatile ingredients. The patches were applied to the backs of the panelists for 21 consecutive 23 h periods. The patches were removed by the subjects and the reactions scored 1 h after their removal. The nail polish was given a composite total score of 138 of a 819 maximum value and a total calculated score for 10 panelists of 106.2 of a 630 maximum. The formulation was judged to be slightly irritating[64] (Table 6).

A nail enamel formulation containing 25.5% Butyl Acetate was tested in a cumulative irritation test using one male and nine female subjects. A closed patch containing 0.3 ml of the enamel was applied to the skin of the subjects for 21 consecutive 23 h periods. The patches of the enamel were prepared 30 min before their application to allow the volatile ingredients to evaporate. The reactions were scored each day 1 h after the patches were removed. The enamel formulation received a cumulative irritation score of 38 of a 630 maximum. The enamel was a mild material[67] (Table 6).

A nail color containing 6.5% Ethyl Acetate was tested for its irritation and sensitization potential in 218 subjects. Occlusive patches containing the nail color were applied to the backs of the subjects on Mondays, Wednesdays, and Fridays for 3 consecutive weeks. Reactions to the patches were scored before the next patch application. After a 2 week nontreatment period, two consecutive 48 h challenge patches were applied to a previously untreated site on the

back of the subjects. Reaction to the challenge patches were scored after each one was removed. Of the 218 subjects tested, 3 had positive responses. These responses were classified as insignificant and were "irritant in nature possibly due to residual solvent on patches while under occlusion." The nail color was not a clinically significant irritant or sensitizer[60] (Table 6).

A clinical use study was conducted to determine the irritancy potential of a nail enamel containing 25.5% Butyl Acetate, under consumer use conditions. The formulation was tested by 55 female subjects. Approximately half of the subjects were sensitive to a lactic acid facial sting test. The subjects were instructed to remove and reapply the nail enamel at least three times per week. Dermatologic examinations of the nail, cuticles, and hands were performed before the study began and after 3 weeks of product use. Changes related to the product in the face, neck, and eyelids were also examined. No instances of any adverse reaction to the product were reported. The nail enamel was "considered to be acceptable from a safety standpoint"[68] (Table 6).

In a 40 month study involving 11 dermatologists, the North American Contact Dermatitis Group (NACDG) listed Butyl Acetate as a dermatitis-causing ingredient identified by patch test. Butyl Acetate was reported as having one cutaneous reaction in 149 patients patch tested, according to NACDG and the International Contact Dermatitis Group procedures, with individual cosmetic ingredients.[69]

Photosensitization

Ethyl Acetate, 6.5% in a nail color, was tested for its photoallergenic potential in 30 subjects ages 18–65. A xenon arc solar simulator was used as the light source and emitted a continuous spectrum in the ultraviolet A (UVA) and B (UVB) range (290–400 nm). Each subject was tested for his or her minimal erythemal dose (MED) before the study began. Occlusive patches containing the nail color were applied to each subject for 24 h. After the patches were removed the sites were scored and irradiated with three times each subject's MED. The reactions were read 48 h after the irradiation and patches were again applied. This procedure was repeated for a total of six applications. An occlusive challenge patch was applied to a previously untreated site after a 10 day nontreatment period. The challenge patch was removed after 24 h and the site was irradiated, using a Schott WG345 filter over the light source, for 3 min. The reactions were observed 15 min, and 24, 48, and 72 h after the irradiation. Two control sites on each subject were treated by the same procedure but without irradiation for one and without the product application for the other. None of the subjects had any reaction during the study, and the nail color produced no phototoxicity or photoallergy[61] (Table 6).

SUMMARY

Ethyl Acetate is the ester of ethyl alcohol and acetic acid. It is a clear liquid miscible with water, alcohols, acetone, chloroform, and ether. Butyl Acetate is

the ester of butyl alcohol and acetic acid. It is a clear liquid miscible with ethanol, ether, and hydrocarbons.

Ethyl and Butyl Acetate are used as solvents in nail polish, nail polish removers, basecoats, and other manicuring preparations. In 1987, Ethyl Acetate was used in 559 cosmetic products and Butyl Acetate was used in 566 cosmetic products. The majority of the uses were in the > 5–10% range for Ethyl Acetate and in the > 10–25% range for Butyl Acetate.

Ethyl Acetate was rapidly hydrolyzed to ethyl alcohol in rat, rabbit, and human blood. It was also rapidly metabolized by rats and humans in vivo.

Both Ethyl and Butyl Acetate were relatively nontoxic when administered orally, dermally, or by i.p. injection or inhalation to rabbits, rats, mice, and guinea pigs.

Butyl Acetate was a mild irritant to rabbit skin. A nail polish remover containing 16.5% Ethyl Acetate induced slight to moderate ocular irritation when instilled into the eyes of rabbits; eyes were not rinsed after instillation. Also, a nail polish formulation containing 10% Ethyl Acetate and 25% Butyl Acetate was a moderate to severe irritant in unrinsed rabbit eyes and a mild irritant in rinsed rabbit eyes. Butyl Acetate caused a "severe burn" when instilled into rabbit eyes.

Butyl Acetate was not a sensitizer either in mice or guinea pigs. Butyl Acetate was not teratogenic when inhaled by rats and rabbits at a concentration of 1500 ppm.

Ethyl Acetate was nonmutagenic when tested by the Ames procedure, Rec-assay, and micronucleus assay. It did induce mitotic aneuploidy in yeast and chromosomal aberrations in Chinese hamster fibroblasts.

Negative results were reported for Butyl Acetate when tested for its mutagenic potential via the Ames procedure. It did not induce either mitotic aneuploidy in yeast or chromosomal aberrations in Chinese hamster fibroblasts.

Ethyl Acetate did not increase the number of lung tumors in mice when administered by i.p. injections in a 24 week carcinogenicity study.

Neither skin irritation nor sensitization was observed in human subjects tested with a nail polish remover containing 16.5% Ethyl Acetate or other products containing 97.0% Ethyl Acetate. When tested at concentrations of up to 10% in petrolatum and cosmetic formulations, Ethyl Acetate was a slight irritant and was not a sensitizer to humans. Butyl Acetate, tested at an unspecified concentration and up to 25.5% in cosmetic formulations, was at most a mild irritant and was not a sensitizer to humans. Ethyl Acetate, at 6.5% in a nail color, did not produce signs of phototoxicity or photoallergenicity in a clinical study.

CONCLUSION

On the basis of data presented in this report, the CIR Expert Panel concludes that Ethyl Acetate and Butyl Acetate are safe as cosmetic ingredients in the present practices of use and concentration.

ACKNOWLEDGMENT

Nancy J. Toy, Scientific Analyst and Writer, prepared the literature review and technical analysis used by the Expert Panel in developing this report.

REFERENCES

1. ESTRIN, N.F., CROSLEY, P.A., and HAYNES, C.R. (EDITORS). (1982). *Cosmetic, Toiletry and Fragrance Association [CTFA] Cosmetic Ingredient Dictionary*, 3rd ed., Washington, DC: CTFA, Inc.
2. WINDHOLZ, M. (EDITOR). (1983). *The Merck Index*, 10th ed. Rahway, NJ: Merck.
3. NATIONAL INSTITUTE FOR OCCUPATIONAL SAFETY AND HEALTH (NIOSH). (1981–82). *Registry of Toxic Effects of Chemical Substances*. H.E. Christenson and E.J. Fairchild. Washington, DC: NIOSH. NTIS/PB83-107.
4. ESTRIN, N.F., HAYNES, C.R., and WHELAN, J.M. (EDITORS). (1982). *CTFA Compendium of Cosmetic Ingredient Composition. Specifications/Spectra*. Washington, DC: CTFA, Inc.
5. GRASSELLI, J.G. (1975). *Atlas of Spectral Data and Physical Constants for Organic Compounds*. Cleveland: CRC Press.
6. LEE, G.L., and WALDRON, T. (1976). The determination of ethyl acetate vapours in air. Ann. Occup. Hyg. **19**(2), 129–33.
7. YOUNG, P., and PARKER, A. (1984). Vapors, odors, and toxic gases from landfills. ASTM Spec. Tech. Publ. ISS Hazard. Ind. Waste Manage. Test. **851**, 24–41.
8. RAMSEY, J.D., and FLANAGAN, R.J. (1982). Detection and identification of volatile organic compounds in blood by headspace gas chromatography as an aid to the diagnosis of solvent abuse. J. Chromatogr. **240**(2), 423–44.
9. WILKINSON, J.B., and MOORE, R.J. (EDITORS). (1982). *Harry's Cosmeticology*, 7th ed. New York: Chemical Publishing Co.
10. FOOD AND DRUG ADMINISTRATION (FDA). (1987). Cosmetic product formulation data: Ingredients used in each product category. Computer printout. Washington, DC.
11. CODE OF FEDERAL REGULATIONS (CFR). (Revised as of April 1, 1984). Title 21 Parts 73.1, 172.515, 175.105, 175.320, 177.1200, 182.60, and 720.4. Washington, DC: U.S. Government Printing Office.
12. SETH, P.K., and SRIVASTAVA, S.P. (1974). Biochemical changes induced by ethyl acetate in blood and liver of rat. Bull. Environ. Contam. Toxicol. **12**(5), 612–6.
13. PEROCCO, P., BOLOGNESI, S., and ALBERGHINI, W. (1983). Toxic activity of 17 industrial solvents and halogenated compounds on human lymphocytes cultured in vitro. Toxicol. Lett. (Amst.). **16**(1-2), 69–76.
14. BORENFREUND, E., and SHOPSIS, C. (1985). Toxicity monitored with a correlated set of cell-culture assays. Xenobiotica **15**(8-9), 705–11.
15. HOLMBERG, B., and MALMFORS, T. (1974). Cytotoxicity of some organic solvents. Environ. Res. **7**(2), 183–92.
16. NAKANO, J., and KESSINGER, J.M. (1972). Cardiovascular effects of ethanol, its congeners and synthetic bourbon in dogs. Eur. J. Pharmacol. **17**, 195–201.
17. NAKANO, J., MORRE, S.E., and KESSINGER, C.L. (1973). Myocardial depressant action of ethyl acetate. J. Pharm. Pharmacol. **25**(12), 1018–20.
18. JACOBI, O. (1968). Effect of various substances on the insensible perspiration of the nail. Arch. Biochim. Cosmetol. **11**, 7–16.
19. GALLAHER, E.J., and LOOMIS, T.A. (1975). Metabolism of ethyl acetate in the rat: Hydrolysis to ethyl alcohol in vitro and in vivo. Toxicol. Appl. Pharmacol. **34**(2), 309–13.
20. SCHRIKKER, A.C., DE VRIES, W.R., ZWART, A., and LUIJENDIJK, S.C. (1985). Uptake of highly soluble gases in the epithelium of the conducting airways. Pflugers Arch. **405**(4), 389–94.
21. NOMIYAMA, K., and NOMIYAMA, H. (1974a). Respiratory elimination of organic solvents in man. Benzene, toluene, n-hexane, trichloroethylene, acetone, ethyl acetate and ethyl alcohol. Int. Arch. Arbeitsmed. **32**(1), 85–91.
22. NOMIYAMA, K., and NOMIYAMA, H. (1974b). Respiratory retention, uptake and excretion of organic solvents in man. Benzene, toluene, n-hexane, trichloroethylene, acetone, ethyl acetate, and ethyl alcohol. Int. Arch. Arbeitsmed. **32**(1-2), 75–83.

23. ESPOSITO, G., FAELLI, A., and CAPRARO, V. (1976). Effect of ethyl acetate on the transport of sodium and glucose in the hamster small intestine in vitro. Biochim. Biophys. Acta **426**(3), 489–98.
24. DUGARD, P.H., and SCOTT, R.C. (1986). A method of predicting percutaneous absorption rates from vehicle to vehicle: An experimental assessment. Int. J. Pharmacet. **28**(2-3), 219–27.
25. MUNCH, J.C. (1972). Aliphatic alcohols and alkyl esters: Narcotic and lethal potencies to tadpoles and to rabbits. Ind. Med. **41**(4), 31–3.
26. SMYTH, H.F., Jr., CARPENTER, C.P., WEIL, C.S., and POZZANI, U.C. (1954). Range-finding toxicity data; list V. Arch. Ind. Hyg. Occup. Med. **10**, 61–8.
27. SMYTH, H.F. Jr., WEIL, C.S., WEST, J.S., and CARPENTER, C.P. (1969). Exploration of joint toxic action: Twenty-seven industrial chemicals intubated in rats in all possible pairs. Toxicol. Appl. Pharmacol. **14**(2), 340–7.
28. SANDMEYER, E.E., and KIRWIN, C.J. (1981). Esters. In: *Patty's Industrial Hygiene and Toxicology*. Vol. 2A, 3rd Rev. Ed. Clayton, G.D., and Clayton, F.E. (eds.). New York: John Wiley and Sons.
29. DE CEAURRIZ, J.C., MICILLINO, J.C., BONNET, P., and GUENIER, J.P. (1981). Sensory irritation caused by various industrial airborne chemicals. Toxicol. Lett. (Amst.) **9**(2), 137–44.
30. KANE, L.E., DOMBROSKE, R., and ALARIE, Y. (1980). Evaluation of sensory irritation from some common industrial solvents. Am. Ind. Hyg. Assoc. J. **41**(6), 451–5.
31. STILLMEADOW, INC. (1984). Submission of unpublished data by CTFA. Acute oral toxicity study of a product containing 97% ethyl acetate.*
32. COSMETIC, TOILETRY, AND FRAGRANCE ASSOCIATION (CTFA). (1987). Submission of unpublished data by CTFA. Actue oral toxicity of a nail polish remover containing 16.5% ethyl acetate.*
33. CTFA. (January 30, 1980). Submission of unpublished data by CTFA. Acute oral toxicity test.*
34. CTFA. (April 20, 1976). Submission of unpublished data by CTFA. Acute oral toxicity study.*
35. BROWNING, E. (1965). *Toxicity and Metabolism of Industrial Solvents*. London: Elsevier Publishing Co.
36. DIVINCENZO, G.D., and KRASAVAGE, W.J. (1974). Serum ornithine carbamyl transferase as a liver response test for exposure to organic solvents. Am. Ind. Hyg. Assoc. J. **35**, 21–9.
37. MORENO, O.M. (1976). Report to Research Institute on Fragrance Materials (RIFM). 19 October.
38. KOJIMA, T., TAKEOKA, T., YASHIKI, M., and KOBAYASHI, H. (1977). Toxicological study of toluene poisoning by inhalation: Effect of inhalation of toluene and ethyl acetate mixed vapor on death. Nippon Hoigaku Zasshi **31**(5), 280–90 (Abstract).
39. AMERICAN CONFERENCE OF GOVERNMENTAL INDUSTRIAL HYGIENISTS (ACGIH). (1986). Threshold limit values and biological exposure indices for 1986–1987. Cincinnati, OH: ACGIH.
40. ALARIE, Y. (1981). Dose–response analysis in animal studies: Prediction of human responses. Environ. Health Perspect. **42**, 9–13.
41. CTFA. (January 17, 1980). Submission of unpublished data by CTFA. Dermal irritation test.*
42. CTFA. (1987). Submission of unpublished data by CTFA. Ocular irritation study of a nail polish remover containing 16.5% ethyl acetate.*
43. CTFA. (April 15, 1976). Submission of unpublished data by CTFA. Rabbit eye irritation study.*
44. CTFA. (January 17, 1980). Submission of unpublished data by CTFA. Ocular irritation test.*
45. GAD, S.C., DUNN, B.J., DOBBS, D.W., REILLY, C., and WALSH, R.D. (1986). Development and validation of an alternative dermal sensitization test: The mouse ear swelling test (MEST). Toxicol. Appl. Pharmacol. **84**(1), 93–114.
46. MAGNUSSON, B. and KLIGMAN, A.M. (1969). The identification of contact allergens by animal assay. The guinea pig maximization test. J. Invest. Dermatol. **52**(3), 268–76.
47. HACKETT, P.L., BROWN, M.G., BUSCHBOM, R.L., CLARK, M.L., and MILLER, R.A. (1983). Teratogenic study of ethylene and propylene oxide and n-butyl acetate. Gov. Rep. Announc. and Ind. Iss. 26. NTIS/PB83-258038.
48. AMES, B.N., MCCANN, J., and YAMASAKI, E. (1975). Methods for detecting carcinogens and mutagens with the Salmonella/mammalian-microsome mutagenicity test. Mutat. Res. **31**, 347–64.
49. ISHIDATE, M.J., SOFUNI, T., YOSHIKAWA, K., HAYASHI, M., NOHMI, T., SAWADA, M., and MATSUOKA, A. (1984). Primary mutagenicity screening of food additives currently used in Japan. Food. Chem. Toxicol. **22**(8), 623–36.

*Available for review: Director, Cosmetic Ingredient Review, 1110 Vermont Ave., N.W., Suite 810, Washington, D.C. 20005

50. ZIMMERMANN, F.K., MAYER, V.W., SCHEEL, I., and RESNICK, M.A. (1985). Acetone, methyl ethyl ketone, ethyl acetate, acetonitrile and other polar aprotic solvents are strong inducers of aneuploidy in *Saccharomyces cerevisiae*. Mutat. Res. **149**(3), 339–51.
51. SHIRASU, Y., MORIYA, M., KATO, K., FURUHASHI, A., and KADA, T. (1976). Mutagenicity screening of pesticides in the microbial system. Mutat. Res. **40**, 19–30.
52. BASLER, A. (1986). Aneuploidy-inducing chemicals in yeast evaluated by the micronucleus test. Mutat. Res. **174**(1), 11–3.
53. SHIMIZU, H., SUZUKI, Y., TAKEMURA, N., GOTO, S., and MATSUSHITA, H. (1985). The results of microbial mutation test for forty-three industrial chemicals. Jpn. J. Ind. Health **27**(6), 400–19.
54. YAMAGUCHI, T. (1985). Stimulating effects of organic solvents on the mutagenicities of sugar-degradation compounds. Agric. Biol. Chem. **49**(12), 3363–8.
55. STONER, G.D., SHIMKIN, M.B., KNIAZEFF, A.J., and WEISBURGER, J.H. (1973). Test for carcinogenicity of food additives and chemotherapeutic agents by the pulmonary tumor response in strain A mice. Cancer Res. **33**(12), 3069–85.
56. KLIGMAN, A.M., and EPSTEIN, W. (1975). Updating the maximization test for identifying contact allergens. Contact Derm. **1**, 231–9.
57. IVY RESEARCH LABORATORIES, INC. (1983). Submission of unpublished data by CTFA. The determination of the contact-sensitizing potential of a product containing 97% ethyl acetate by means of the Maximization Test.*
58. EPINETTE, W.W. (1987). Submission of unpublished data by CTFA. Prophetic patch and in-use testing of a nail polish remover containing 16.5% ethyl acetate.*
59. KLIGMAN, A.M. (1972). Report to RIFM. 2 May.
60. CTFA. (January, 1980). Submission of unpublished data by CTFA. Clinical repeated insult patch test.*
61. CTFA. (January, 1980). Submission of unpublished data by CTFA. Clinical photopatch test.*
62. CTFA. (June 2, 1976). Submission of unpublished data by CTFA. Maximization test.*
63. HILL TOP RESEARCH, INC. (1984). Submission of unpublished data by CTFA. Report of a human cumulative skin irritation test of a product containing 97% ethyl acetate.*
64. CTFA. (May 20, 1976). Submission of unpublished data by CTFA. Cumulative irritation test.*
65. EPSTEIN, W.L. (1976). Report to RIFM. 8 October.
66. CTFA. (May 10, 1984). Submission of unpublished data by CTFA. Maximization test.*
67. CTFA. (January 17, 1984). Submission of unpublished data by CTFA. Cumulative irritation test.*
68. CTFA. (March 6, 1984). Submission of unpublished data by CTFA. Clinical use test.*
69. NORTH AMERICAN CONTACT DERMITITIS GROUP (NACDG). (1982). Prospective study of cosmetic reactions: 1977–1980. J. Am. Acad. Dermatol. **6**, 909–17.