Safety Assessment of Tromethamine as Used in Cosmetics

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All interested persons are provided 60 days from the above release date to comment on this Tentative Safety Assessment and to identify additional published data that should be included or provide unpublished data which can be made public and included. Information may be submitted without identifying the source or the trade name of the cosmetic product containing the ingredient. All unpublished data submitted to CIR will be discussed in open meetings, will be available at the CIR office for review by any interested party and may be cited in a peer-reviewed scientific journal. Please submit data, comments, or requests to the CIR Director, Dr. F. Alan Andersen.

The 2012 Cosmetic Ingredient Review Expert Panel members are: Chairman, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; Curtis D. Klaassen, Ph.D.; Daniel C. Liebler, Ph.D.; Ronald A Hill, Ph.D. James G. Marks, Jr., M.D.; Ronald C. Shank, Ph.D.; Thomas J. Slaga, Ph.D.; and Paul W. Snyder, D.V.M., Ph.D. The CIR Director is F. Alan Andersen, Ph.D. This report was prepared by Lillian C. Becker, Scientific Analyst/Writer.

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TABLE OF CONTENTS

INTRODUCTION

This is a scientific literature review of tromethamine (also referred to as Tris and THAM), an aliphatic compound that functions as a fragrance ingredient and a pH adjuster in cosmetics.

The Environmental Protection Agency's (EPA) High Production Volume Information System (HPVIS) has collected toxicity data on tromethamine (1,3-propanediol, 2-amino-2-(hydroxymethyl)-). Included in that information are safety and other data on aminomethylpropanol (AMP), suggesting that the EPA considers this chemical to be structurally close enough to tromethamine to consider those data. The Cosmetic Ingredient Review (CIR) issued an amended safety assessment in 2009, concluding that AMP is safe in the present practices of use and concentration, based on the then current use concentration (<1% in leave-on formulations, 2% in rinse-off formulations, and 7% in hair dyes that will be diluted during use) and on consideration of additional irritation and sensitization data.[1,2]

CHEMISTRY

Definition and Structure

Tromethamine (CAS No. 77-86-1) conforms to the structure in Figure 1. AMP has the structure shown in Figure 2.

Physical and Chemical Properties

Physical and chemical properties are presented in Table 1.

Tromethamine is a more effective buffer than bicarbonate at physiological conditions (pH 7.8; 37°C).[3]

The alkalinity of the pure base solution of tromethamine erodes ordinary glass and requires alkaline-resistant glass.[3]

The pH of tromethamine can be changed by adjusting its mix/equilibrium with tromethamine H+.[4] At near 100%, the pH of tromethamine is 10.0, at 25% the pH is 7.4, and at near 100% tromethamine H+ the pH is 6.0.

Tromethamine is reported to be stable when exposed to light and air but freezing should be avoided.[5,6]

Method of Manufacture

Tromethamine is prepared by the reduction of tris(hydroxymethyl)nitromethane.[7,8] Tromethamine may also be manufactured by additively reacting nitromethene with formaldehyde to yield tris(hydroxymethyl) nitromethane, which is then hydrogenated with the aid of Raney nickel catalyst.[6,9]

Impurities

When tromethamine is heated to decomposition, it emits toxic fumes composed of nitrogen oxide.[10]

USE

Cosmetic

Data on ingredient usage are provided to the Food and Drug Administration (FDA) Voluntary Cosmetic Registration Program (VCRP; Table 2).[11] A survey was conducted by the Personal Care Products Council (Council) of the maximum use concentrations for these ingredients.[12] Tromethamine is used in 480 leave-on products and 69 rinse-off products up to 4%. Products include eye makeup (up to 2%), fragrance preparations (up to 0.2%), and skin care preparations (up to 4%).

Non-Cosmetic

Tromethamine is used in the synthesis of surface-active agents, vulcanization accelerators, and pharmaceuticals. It is also reported to be used as and emulsifying agent for cosmetic creams and lotions, mineral oil and paraffin wax emulsions, leather dressings, textile specialties, polishes, cleaning compounds, so-called soluble oils. It is used as an absorbent for acidic gases and as a biological buffer.[13]

Tromethamine was reported to be used as a commercial emulsifier.[14]

Tromethamine has several medical uses. This is a representative sample:

- Orally administered tromethamine citrate syrup (1.5-9 mmol/kg) is used to treat renal acidosis, adjusted to maintain urinary pH, and for chemolysis of renal calculi.[15]
- Intravenously administered tromethamine (15 mmol/kg or 3.5L of 0.3 mol/L maximum) is used in the treatment of adult and infant respiratory distress syndromes, and in the management of increased intracranial pressure after trauma, over periods of several days.[3,16]
- Intravenously administered tromethamine is used to treat acidosis during pulmonary bypass and cardio surgery that requires hypothermic techniques.[17-20]
- Intravenously administered tromethamine is used to treat acidosis in burn victims.[21]
- Tromethamine (~60% of 0.15 mol/L) administration by peritoneal dialysis administered into the peritoneal cavity has been used for the treatment of intoxication with salicylates, barbiturates and methyl alcohol (methanol).[3,22,23]
- Tromethamine, mixed with hydrochloric acid (to a pH of 9.2) or acetate, sodium bicarbonate and disodium phosphate (to a pH of 8.1), is used for peritoneal dialysis to treat academia in humans and will cause alkalinization of the plasma.[3]

In veterinary medicine, tromethamine is an amine pH buffer prescribed for the prevention and correction of metabolic acidosis, usually as a 0.3 M solution (0.3 mEq/mL) in a 7.5% sodium bicarbonate (q.v.) solution.[24]

TOXICOKINETICS

Absorption, Distribution, Metabolism, and Excretion

Tromethamine is eliminated by the kidneys. Ionized tromethamine (chiefly as the bicarbonate salt) is rapidly and preferentially excreted in urine at a rate associated with the infusion rate. Urinary excretion continues over a period of 3 days; 75% or more appears in the urine after 8 hours. Other studies report 50%-75% of an i.v. dose was recovered in urine within 24 h. It has also been reported that recovery in healthy adults to be 64% and 77% after 2 and 3 days, respectively.[15,25-27] Excretion of tromethamine is accompanied by osmotic diuresis, since clinical doses of tromethamine considerably adds to osmolarity of glomerular filtrate.[6]

Tromethamine is primarily eliminated from plasma through renal filtration of its protonated form.[28] Tromethamine may accumulate in patients with renal insufficiency, and produce an 'osmolar gap' with pseudohyponatremia.

Tromethamine is a buffer that can bind hydrogen protons, and which is excreted via glomerular filtration.[3]

It is not known whether tromethamine is distributed in human milk.[25]

Dermal/Percutaneous

Dermal absorption was < 1% when radio-labeled tromethamine (0.1% and 10%; 100 µL) was administered to dermatomized, thawed human skin in Franz cells.[29] The receptor fluid was sampled at 2, 4, 6, 8, and 10 h. Recovery of the test material by washing was > 90%.

Oral

Oral administration of tromethamine (20 g) resulted in alkalinizes of the body fluids.[30]

In human subjects, daily administration of tromethamine citrate syrup (3 to 6 mmol/kg) produced urinary alkalization (pH increasing from a range of 5.6 - 6.8 to 7.2-7.3)[22]

Intravenous

When administered intravenously (i.v.) in a bolus or over a short-term, tromethamine rapidly distributes into the intracellular spaces and raises the pH of plasma.[16,26-28,31-35] Then the cells slowly take up the tromethamine; the rate of uptake increases when the pH is more alkaline. However, there is one study's conclusions contradict the findings of previous studies. A representative set of studies are presented here as well as the study with the opposite conclusion.

In rats of different ages (5 to 240 days old) the renal excretion of tromethamine was studied.[31] In older rats the renal excretion of tromethamine was slower than in rats of other age groups. Stimulation of diuresis by i.p. injection of mannitol, thiazide, or by oral water load resulted in an increase in THAM excretion in 5 and in 240 days old rats. The renal excretion of tromethamine was also increased by repeated administration of THAM in all age groups, except in new born rats.

When ^{14}C-tromethamine is administered i.v. to nephrectomized Sprague-Dawley rats (n = 21-26; with blood stabilized at pH 7.5, 7.4, 7.2), the following was found: 1) tromethamine diffuses very slowly into the intracellular spaces of various tissues; 2) the intracellular concentration of tromenamine increased faster with the higher pH; 3) the rate of increase of tromethamine was the same in spleen, heart, skeletal, muscle, and brain tissue; 4) tromethamine diffusion into liver cells is rapid, which is not so for spleen, heart, skeletal muscle, and brain tissue; and 5) the intracellular steady state was only reached in the liver.[35]

The rats were nephrectomized and catheterized (venous and arterial). After administration of the test material, some of the rats were killed and necropsied at 60, 180, 360, 720, and 1440 min. The experiment was repeated (n = 26) with the blood stabilized at 7.4. The authors concluded that the mechanism of tromethamine therapy is its elimination of H^+ ions from the extracellular space and the generation of bicarbonate that then penetrates the intracellular compartments.[35]

When ^{14}C-tromethamine (5 µc) was administered i.p. to nephrectomized Wistar rats (n = 6), the half-life in the plasma was 90 min.[32] The half-times to equilibrium for tromethamine distributed to heart and skeletal muscle were 2.7 and 5 h, respectively. Distribution to the brain and cerebrospinal fluid were very slow and did not obtain a constant tissue:plasma ratio in the brain at 24 h. The rats were killed and samples of blood, cerebrospinal fluid, skeletal muscle and cerebral cortex analyzed at 10, 20, 30, 40, 50, 60, 90, 120, 180, 240, 300, and 360 min after the test material was administered

In a second experiment, when administered i.p., the largest amount of ^{14}C-tromethamine collected in skeletal and heart muscle at 12 and 24 h. Accumulation was slower in brain tissue and cerebrospinal fluid.[32]

Rabbit (strain not provided; not provided) were intravenously injected with tromethamine (5-100 ml/kg; 0.3 M at pH 5.5 and 7.4) daily for 1 – 99 days.[34] Urinalysis revealed that the amount of THAM excreted in the urine reached a maximum at the end of infusion, and dropped rapidly after infusion stopped. Only a small quantity of chloride was excreted. With THAM pH 5.5, a larger amount of chloride than with Tris was excreted. At the end of the 7 hours, 44% of the infused THAM was found in the urine, while with Tris pH 5.5, 77% was found. Blood sampling showed that the glucose concentrations dropped during the infusions, but returned to normal or above normal following the end of the infusions (THAM-induced hypoglycemia persisted longer than the THAM-neutralized). Both treatments caused transient hypoglycemia. Blood analysis

on extracted blood (THAM added to blood droplets at varying levels) also determined that there was no deleterious effect on erythrocytes.

Tromethamine (121 mg/kg; 1 mmol/kg; pH 7.4) was mostly eliminated by the kidneys (82% was recovered in the urine at 24 h) when administered i.v. to healthy subjects (n = 6) and subjects with metabolic acidosis (n = 20).[28] Tromethamine did accumulated in the tissues, but equilibrium was slow.

In contrast to other conclusions, the authors concluded that tromethamine movement into intracellular space is too slow (compared to renal elimination kinetics) to influence intracellular pH by direct buffer action.[36] The distribution of ^{14}C labeled tromethamine was determined between intra- and extracellular space of nephrectomized Sprague-Dawley rats (n = 5) as a function of time at constant plasma pH of 7.4. An equilibrium in the distribution of tromethamine between external and internal cellular space was observed at 6-12 h after administration. The authors concluded that tromethamine permeates very slowly into intracellular space, in contrast to previous conclusions that is quickly diffuses into intracellular spaces to restore intracellular acidosis. Tromethamine passed from extracellular space in a multi-exponential fashion, indicating that it passes to different body tissues at variable rates. Tromethamine was in ionized form when transferring across cellular membranes.

Other Effects

Dogs (breed not specified) exhibited profuse diuresis during i.v. treatment with tromethamine.[26] Dogs (n = 5) were anesthetized and rendered apneic using succinylcholine chloride. Apnea was then induced by barbiturates. Under oxygen saturation, tromethamine (0.3 M; 1.1 ml/kg/min) was administered i.v.

Cytotoxicity

In cytotoxicity assays using multiple cell lines, the IC_{50} for THAM ranged from 129.07 - 404.37 µg/ml. In the 2,5-Diphenyl-3,-(4,5-dimethyl-2-thiazolyl) tetrazolium bromide (MTT) assay, after exposure for 24 h, the IC_{50}s were ~330 for 3T3 cells, ~160 for 3T6 cells, ~340 for HaCaT cells, ~180 for NCTC 2544 cells, ~340 for HeLa cells, and ~405 µg/ml for MCF-7 cells. In the neutral red uptake (NRU) assay, the IC_{50}s were ~295 for 3T3 cells, ~130 for 3T6 cells, ~160 for HaCaT cells, ~ 190 for NCTC 2544 cells, ~190 for HeLa cells, and ~ 315 µg/ml for MCF-7 cells.[37]

Miscellaneous Studies

Tromethamine, administered i.v., causes a fall in blood glucose levels in rats, rabbits, dogs, and humans.[33,34,38,38,39] Tromethamine lowered the blood sugar of dogs after the removal of the pancreas when given a few hours after pancreatectomy, but had little or no effect on the blood sugar of pancreatectomized dogs if insulin was withheld for 18 hours or longer before tromethamine was administered.

Hypoglycemic effect of tromethamine was due to the release of insulin and its activity.[39] Tromethamine-induced hypoglycemia is associated with a transient stimulation of insulin secretion in rats. A bolus injection of neutralized tromethamine (5 mmol/kg; pH 7.4), caused a transient increase of plasma insulin concentration (130 ± 20 µU/mL) but did not change the glucose concentration in male Wistar rats (n = 6). However, a continuous infusion of tromethamine (0.5 mmol/kg/min) for 90 min reduced the plasma glucose concentration (8.7 + 0.42 to 5.1 + 0.33 mmol/L) after 30 min. The plasma insulin concentration was elevated during the first 20 min (max +122 ± 21 µU/mL after l0 min). In streptozotocin-diabetic rats (administered 48 h prior to the experiments), an infusion of tromethamine changed neither glucose nor insulin concentration in plasma.

TOXICOLOGICAL STUDIES

Acute Toxicity

Oral – Non-Human

The oral LD_{50} for mice was reported to range from 3350 to 5500 mg/kg (Table 3). For rats, the LD_{50} was > 3000 mg/kg. The LC_{50} was between 1000 and 2000 mg/kg.

Dermal – Non-Human

The dermal LD_{50} of tromethamine for mice and rats was reported to be > 1000 mg/kg and > 2000 mg/kg for rabbits (Table 3).

Intraperitoneal

The intraperitoneal LD_{50} of tromethamine for mice was reported to be ~3350 mg/kg (Table 3).

Intravenous

The intravenous LD_{50} of tromethamine for mice was reported to be 16.5 mM/kg (Table 3). There were no mortalities reported at < 5000 mg/kg. The LD_{50} for rats was reported to range between 3.28 and 4.04 g/kg and up to ~6000 mg/kg. There were no treatment related mortalities in rabbits administered tromethamine up to 500 mg/kg. In dogs, the LC_{50} was reported to be >125 mg/kg.

Repeated Dose Toxicity

Oral – Non-Human

The lowest observed adverse effects level (LOAEL) for tromethamine for Sprague-Dawley rats (n not provided) was reported to be 2500 ppm when incorporated into feed (0, 25, 150, 250, 2500 ppm) for 3 months.[40]

There were signs of liver damage at necropsy when CD rats (n = 12) were orally administered tromethamine (300 mg/kg/d).[41]. Rats were orally administered tromethamine (100, 300, 1000 mg/kg/d) in feed. Males (n = not provided) were treated for between 14 and 37 days. Females (n = 12) were treated from 2 weeks prior to breeding, through gestation, and through 4 days of lactation.

At necropsy, increases in absolute and relative liver weights, a very slight degree of microvacuolization of periportal hepatocytes, with or without vacuolization of hepatocytes were observed in males. Females in all treatment groups had similar histopathological changes in the liver without the weight change.

Dermal – Non-Human

There were no clinical signs to rabbits (strain and n not provided) dermally administered tromethamine (100%) on clipped skin for 4 h for 5 days.[42]

Intravenous – Non-Human

There were no clinical signs or mortalities observed to mice (strain and n not provided) administered i.v. tromethamine (10, 50 mL/kg; 0.155 M; pH 5.5, 7.4) for 10 days.[34] Histological examination of the organs showed that there were no adverse effects from the treatment.

The no observed adverse effects level (NOAEL) for Sprague-Dawley rats (n = 6/sex) administered tromethamine i.v. (0.5 and 1.5 g/kg; 0.3 M) for 10 and 20 days was reported to be ~ 500 mg/kg.[43] Rats were allowed 24 h or 7 d for recovery. On day 11, a second high dose group were treated with an additional i.p. injection.

There were no mortalities in the 20-d low dose group. There was dry gangrene at injection sites in the 10- and 20-d low dose groups. In the 20-d groups, ~half of the rats had mild inflammation of various parts of the visceral peritoneum, or fat necrosis and hemorrhage of the serosa of various parts of the stomach, intestine, and peritoneum. Microscopic examination of tissues 24 h after injection i.p. showed 5/6 rats of the 20-d low dose group had chronic cellulites at injection sites, and peracute toxic nephrosis of the kidneys, but not in animals allowed the 7-day recovery period. In the 20-d high dose group, all rats necropsied at 24 h and 5/6 rats in the 7-day recovery group had similar findings.[43]

Other than necrotic effects at the injections site (ear) and transient body temperature changes, there were no adverse effects to New Zealand White rabbits (n= 4/sex) administered tromethamine (0.5 g/kg; 0.3 M) for up to 20 days.[43] Two rabbits/sex were necropsied within 24 h of the last dose. The rest had 20-days recovery before necropsy.

There were no effect on feed and water consumption and body temperature. Body weight fluctuated throughout study in all animals, including control, but not in any treatment-related pattern. Of the treated rabbits, 7/8 had inflammatory lesions of the external ear. The lesions varied from swelling and redness to dry gangrene and erosion.

Weekly blood samples were normal for: total serum proteins, albumin/globulin (A/G) ratio, serum bilirubin, cephalin flocculation, serum transaminase, red blood cell count, differential counts, hemoglobin, microhematocrit, and platelet counts. White blood cell counts in excess of 13,000 were seen in 5/8 rabbits receiving tromethamine. In all cases, elevated white blood cell counts coincided with dry gangrene in the external ear. Urinalysis was unremarkable.

At necropsy, 2/4 treated rabbits necropsied after recover had grossly visible infarcts in the kidneys; there were none in the control group. No gross lesions were observed in any other organ or tissue. In 7/8 test animals with gross lesions of the ear, there were microscopic lesions of chronic cellulites and necrosis at sites of injection in the subcutaneous tissues of the ear. Those with kidney lesions also had chronic interstitial nephritis. Infiltrations of lymphocytes were observed in tissue sections of the liver and kidney of 3 treated rabbits. The infiltrations were observed in animals in the recovery and non-recovery groups. Peracute toxic nephrosis was observed in 1 rabbit, which also presented urolithiasis.[43]

Treatment-related mortality began a few days after start of administration i.v. of tromethamine (100 ml 0.3 M at pH 5.5 and 7.4) to rabbits (strain not provided; n = 2-3).[34] Tromethamine was administered i.v. over 5 h daily for 19 d. Other groups were administered tromethamine (5 and100 ml 0.3 M/kg at pH 5.5 and 7.4) over 5 h; daily for 1 – 99 d.

The neutralized tromethamine was less toxic. Clinical signs included anorexia, bloody urine, hind leg paralysis, and irregular respiration. Observations at necropsy included abnormally red lungs, necrosis at the point of infusion, bleached liver, darkened spleen, bloated stomach, and lesions on the heart and kidney. Histology examination of the organs was negative.[34]

There were no treatment-related mortality or clinical signs to rabbits (strain not provided; n = 3) administered i.v. tromethamine (50 and 10 ml/kg 0.155 M; over 30 sec) once daily for 10 d.[34] Histological study of the organs was negative.

Intratracheal – Non-Human

Tromethamine (in an unknown mixture with 0.9% saline; 2 mL; vehicle control in an experiment for a drug) did not decrease survival or average body weight of male Syrian hamsters (n = 28-29) when administered over the lifetime of the

hamsters compared to hamsters in the no treatment group.[44] There were no differences in survival (88 ± 22 vs. 78 ± 25 weeks) and average body weight (116 ± 10 vs. 114 ± 6 g) between the vehicle and the no treatment groups.

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

Tromethamine had no effect on mating or conception, but did increase resorptions at 300 mg/kg/d.[41] There were signs of liver damage at necropsy. CD rats were orally administered tromethamine (100, 300, 1000 mg/kg/d) in feed. Males (n = not provided) were treated for at least 2 weeks before breeding up to 37 days. Females (n = 12) were treated from 2 weeks prior to breeding, through gestation, and through 4 days of lactation.

Tromethamine had no effect on mating performance or conception. At the high dose level, all pregnant females showed evidence of complete litter resorption; at 300 mg/kg/day, post-implantation loss was 70% (vs. 10% in controls). Effects associated with, or secondary to the post-implantation loss increase at 300 mg/kg/day included decreased litter size, increased pup body weight, and decreased gestation body weight and body weight gain. There were no treatment related effects on reproduction in the 100 mg/kg/day group.

GENOTOXICITY

In Vitro

There were no genotoxicity studies discovered or submitted.

CARCINOGENICITY

Studies

When administered intratracheally to male Syrian hamsters weekly for their entire lifespan, tromethamine (0.2 ml in 0.9% saline) did not induce tumors.[44]

IRRITATION AND SENSITIZATION

Irritation

Dermal – Non-Human

Intradermally injected tromethamine (0.1 mL) was severely irritating to rabbits (strain and n not provided) at a pH of 10.4 (0.2, 0.3 M) and at pH 7.4 (0.6, 1 M).[34] The causes of local necrosis around the infusion site were investigated using injected Trypan dye. The irritation caused by the solutions was evaluated by observing the amount of extravasated dye. The neutral tromethamine (pH 5.5) had reduced irritation/local necrosis. At pH 7.4, tromethamine was not irritating at lower doses (0.2, 0.3 M). The authors suggested that the pH of the tromethamine is the probable cause of the dermal irritation.

In a Draize test, rabbits (strain and n not provided) were dermally administered tromethamine, both in solution (25%, saturation; pH 10.8) and as a crystalline product, to intact and abraded skin.[45] There was no noticeable irritation produced by any state of the test material on intact skin. There was mild irritation by the crystals and saturated states on abraded skin. All signs of irritation were completely resolved in 48 h. The author concluded that tromethamine was a mild irritant under these conditions.

Tromethamine (40% in distilled water) was not irritating to rabbits (n = 6) in a Draize test.[46]

Sensitization

There were no sensitization studies discovered or submitted.

CLINICAL USE

Tromethamine (20 g in 3.3% glucose) was administered i.v. to male subjects (n = 4) with respiratory acidosis due to emphysema or carcinoma of the lung over 40 min.[30] Blood pH increased, O_2 tension decreased, and CO_2 tension remained unchanged (except for in 1 subject which decreased) over the administration time. Urinary pH increased within 20 min of the start of infusion with the exception of the same subject; the increase happened at 40 min.

Case Studies

A 30-year-old woman developed severe respiratory acidosis following cardiac surgery.[30] After she was administered tromethamine (120 g in water) by gastric tube over 24 h, the acidosis was resolved but she developed severe diarrhea. She also developed tetany which was controlled with calcium gluconate. Her arterial pH rose from 7.1 to 7.45 and she had no further acidosis. While she died from other complications, there were no adverse effects from the tromethamine observed at autopsy.

A 40-year-old man, who had a 9-rib thoracoplasty, presented with extensive pneumonia.[30] He was unconscious within 12 h with slow, gasping respirations. A tracheotomy and 100% oxygen were not helpful. O_2 saturation was 97%, CO_2 tensions was 160 mm Hg, and pH was 6.95. He was administered (30 g in water; 10%) over 1 h. Arterial blood was then at 92% saturation and CO_2 tension was 80 mg Hg with a pH of 7.2. Additional tromethamine (10 g) was administered after 5 h. O_2 saturation was 49%, CO_2 tension was 68 mm Hg, and the pH was 7.29.

SUMMARYOF AMP REPORTS

1990 Report

AMP and AMPD are substituted aliphatic alcohols. Both occur in solid and liquid forms.[2] AMP is miscible with water and soluble in alcohols, while AMPD is soluble in both water and alcohols. Both AMP and AMPD are used as emulsifying agents for cosmetic creams and lotions, and as neutralizing agents in hair sprays.

AMP is used in concentrations up to 10% and AMPD is used in concentrations up to 5%. All uses at concentrations above 1% involve neutralization of AMP or AMPD with fatty acids. In industry, AMP and AMPD are used in the synthesis of surface-active agents, vulcanization accelerators, and pharmaceuticals, and as emulsifying agents for a variety of products. AMP is also listed as an indirect food additive as a component of adhesives.

AMP appears to interfere with lipid catabolism and with choline utilization and synthesis in rats fed a choline-deficient diet. AMP also increased the incidence of hemorrhagic kidneys and the amount of hepatic lipid (except at higher doses of AMP, in which the latter was reversed) in choline-deficient rats. Aminoalcohols are incorporated into the phospholipids of rats; the degree of incorporation was related to the aminoalcohol's similarity to ethanolamine.

AMP caused a dose-dependent inhibition in vitro of the incorporation of [32P] into the phospholipids of swine pulmonary and coronary arteries, and of rabbit and human endometrial tissues.

In vitro, AMP altered the morphology of murine fibroblast plasma membranes, either by replacing certain types of phosphatidylethanolamine or by altering the asymmetric distribution.

Intraperitoneal injection of AMP resulted in urinary excretion of tritiated AMP in rats fed either choline-adequate or choline-deficient diets, with the rats fed the cholineadequate diet accumulating more of the unchanged AMP in the urine. Radioactivity also appeared in the serum within 30 minutes of i.p. injection, and then disappeared shortly thereafter. Rats fed the choline-adequate diet accumulated a greater amount of radioactivity in the serum. Uptake of radioactivity by various organs was greater for the rats fed the choline-adequate diet during the first 6 h postinjection, after which time the trend was reversed. In the liver, the uptake of radioactivity was greater at all times for the choline-deficient rats.

According to the classification system of Hodge and Sterner, "AMP is nontoxic to rats and albino mice, and slightly toxic to deer mice. In an acute oral toxicity study, AMP produced lesions in the liver, kidneys, spleen, and lungs at the LD_{50} dose. In another acute oral toxicity study in rats, AMP did not cause lesions in the kidneys and lungs of the test animals. In rhesus monkeys, the toxic effects of AMP were probably due to the alkalinity of the compound and irritation of gastrointestinal tract. In three acute oral toxicity studies of hair sprays or cosmetic formulations containing varying concentrations of AMP, the test material was nontoxic to rats. Results of another acute oral toxicity study of a hair spray containing AMP at a concentration which was also tested in the previous studies found the hair spray containing AMP to be toxic to albino rats.

According to the classification of Hodge and Sterner, "a hair spray containing AMPD was practically nontoxic to albino rats. Several acute inhalation studies were performed with cosmetic formulations containing AMP, as well as with AMP in alcohol and propellant. The study results indicated that AMP was nontoxic by inhalation. A hair spray containing AMPD was also nontoxic to rats. In dogs fed AMP, no gross lesions were found at necropsy. Microscopic lesions were found in the livers of all but one of the test animals, and the damage was dose dependent. Neither gross nor microscopic lesions were found in the livers of mice fed AMP in the diet for 8 weeks. Rats of another study had vacuolization of hepatocytes in all dose groups.

When rats were exposed to atmospheres of a hair spray containing AMP over a period of 2 weeks, no toxic effects resulted from the treatment. When AMP solutions, with pH's of 7 or 1 1 +, were administered to rats by stomach tube, it was found that any mortality was due to the alkalinity of the AMP solutions. In a subchronic oral toxicity study of AMP in beagle dogs, only the dogs of the high-dose group did not gain weight during the study. There were changes in some clinical chemistry parameters in the dogs of the high-dose group. Liver and liver/body weight ratios were increased, and tan and mottled livers were observed at necropsy in some dogs of the high-dose group. Microscopic lesions included vacuolization, lipid deposits, and bile duct hyperplasia in the livers of the dogs in the high-dose group, as well as in one dog of the low-dose group.

In a chronic inhalation study, rats were exposed to an aerosolized form of a pump hair spray containing AMP. The hair spray was not toxic under the exaggerated inhalation conditions of the test. Cynomolgus monkeys were exposed to hair sprays containing AMP under static and dynamic conditions in a subchronic inhalation toxicity study. The only compound related adverse effects were that the monkeys exposed under dynamic conditions did not gain weight during the study, and the monkeys exposed under either condition had lowered serum CO2 levels. In another study, cynomolgus monkeys exposed to a hair spray containing AMP showed some histopathologic changes in the pulmonary tissues. A slight to moderate increase was found in hepatocellular lipids in all test animals. Pulmonary alveolitis was noted in the high-dose monkeys. When both albino rats and Syrian Golden hamsters were exposed in a subchronic inhalation toxicity study to hair spray formulations containing AMPD, no significant compound-related adverse effects were observed.

In numerous primary irritation studies, cosmetic formulations containing varying concentrations of AMP were non- to minimally irritating to abraded and nonabraded rabbit skin. AMP in an ethanol vehicle was nonirritating to rabbit skin. Cosmetic formulations containing AMPD were also non- to minimally irritating to rabbit skin.

AMP was not an intradermal sensitizer in guinea pigs.

In eight studies, AMP in cosmetic formulations or in an aqueous vehicle was a minimal to mild ocular irritant. The degree of irritation was reduced by rinsing the eyes after exposure to the formulations. Cosmetic formulations containing AMPD were moderate ocular irritants.

AMP was not mutagenic, with and without metabolic activation, in S. *cerevisiae* strain D4, and in *S. typhimurium* strains TA1535, 1537, 1538,98, and 100. AMPD was not mutagenic, with and without metabolic activation, in *S. typhimurium* strains TA1535, 1537, 98, and 100.

In a clinical study, a cosmetic formulation containing AMP-95 was not a primary dermal irritant. In a primary irritancy test of a cosmetic formulation containing AMPD, scattered incidences of questionable responses were observed in two-thirds of the panelists. In addition, 2 of 15 panelists had slight redness at least once during the observation period. A cosmetic formula containing AMP-95 was not an allergic contact sensitizer when tested using a panel of 97 subjects. A cosmetic formulation containing AMPD was not a primary irritant, and it was neither a fatiguing agent nor a sensitizer. In another study, a cosmetic formulation containing AMPD was not a sensitizer.

2009 Amended Report

AMP and AMPD are substituted aliphatic alcohols used as cosmetic ingredients.[1] Isopropanolamine is another cosmetic ingredient and is a close structural analog to AMP. A CIR safety assessment of isopropanolamine found the ingredient safe as used as long as it was not used in products containing N-nitrosating agents.

AMP and AMPD occur in solid and liquid forms. AMP is miscible in water and soluble in alcohols, whereas AMPD is soluble in both water and alcohols.

Both AMP and AMPD function as pH adjusters in cosmetic products. AMPD is also a fragrance ingredient. AMP is used in concentrations up to 7%, and AMPD is used in concentrations up to 2%.

Several acute inhalation studies were performed with cosmetic formulations containing AMP as well as with AMP in alcohol and propellant. The study results indicated that AMP was nontoxic by inhalation. A hair spray containing 0.50% AMPD was also nontoxic to rats.

When rats were exposed to atmospheres of a hair spray con taining 0.58% AMP 1 hour per day, 5 days per week over a period of 2 weeks, no toxic effects resulted from the treatment. When AMJ 3 solutions with pHs of 7 or 11 + were administered to rats by stomach tube, it was found that any mortality was due to the alkalinity of the AMP solutions.

In a subchronic oral toxicity study of AMP in beagle dogs, only the high-dose group (62.5 mg/kg) did not gain weight during the study. There were changes in some clinical chemistry parameters in the dogs of the high-dose group. Liver and liver-to-body weight ratios were increased, and tan and mottled livers were observed at necropsy in some dogs of the high-dose group. Microscopic lesions included vacuolation, lipid deposits, and bile duct hyperplasia in the livers of the dogs in the high-dose group as well as in 1 dog of the low-dose (0.63 mg/kg) group.

Cynomolgus monkeys were exposed to hair sprays contain ing 0.40% AMP under static and dynamic conditions in a 90-day subchronic inhalation toxicity study. The only compound-related adverse effects were that the monkeys exposed under dynamic conditions did not gain weight during the study and the monkeys exposed under either condition had lowered serum CO 2 levels. In another 90-day study, cynomolgus monkeys exposed 1 h per day to a hair spray containing 0.2 1% AMP showed some histopathologic changes in the pulmonary tissues. A slight to moderate increase was found in hepatocellular lipids in all test animals. Pulmonary alveolitis was noted in the high-dose monkeys.

In a subchronic inhalation study, rats were exposed to an aerosolized form of a pump hair spray containing 0.21% AMP for 4 hours per day, 5 days per week. The hair spray was not toxic under the exaggerated inhalation conditions of the test. When both albino rats and Syrian Golden hamsters were exposed in a 13-week subchronic inhalation toxicity study to hair spray formulation containing 0.1350% AMPD for 4 hours per day, 5 days per week, no significant compound-related adverse effects were observed.

The NOEL in a chronic dietary toxicity study of AMP in beagle dogs was 110.0 ppm or greater.

In numerous primary irritation studies, cosmetic formulations containing varying concentrations of AMP were nonirritating to minimally irritating to abraded and nonabraded rabbit skin. AMP (0.25%) in an ethanol vehicle was nonirritating to rabbit skin. Cosmetic formulations containing AMPD were also nonirritating to minimally irritating to rabbit skin.

In an intradermal study, 0.1% AMP was not a sensitizer in guinea pigs. In a topical sensitization study, 5.9% AMP was not a sensitizer in guinea pigs.

An unspecified concentration of AMP was found to be a severe ocular irritant in rabbits. At concentrations ranging from 0.22% to 0.59%, AMP in cosmetic formulations or in an aqueous vehicle was a minimal to mild ocular irritant. The degree of irritation was reduced by rinsing the eyes after exposure to the formulations. A bovine corneal opacity and permeability test classified a waving gel containing 6.3% AMP as a mild ocular irritant. Cosmetic formulations containing 0.40% AMPD were moderate ocular irritants.

In an oral reproductive and developmental toxicity study of AMP hydrochloride salt in rats, the NOEL for general toxicity in males was 300 mg/kg/d. The NOEL for general toxicity in females could not be determined because of effects on the liver. Dose-related increases in embryo resorption were noted. The NOEL for fetuses was 100 mg/kg/d. A dermal

developmental toxicity study of 94.85% AMP in rats indicated a maternal NOAEL of 100 mg/kg/d and a NOEL for fetuses of 300 mg/kg/d.

AMP was not mutagenic, with and without metabolic activation, in *.S cerevisiae* strain D4, in *E. coli* strain WP2 uvrA, and in *S. typhimurium* strains TA 1535, 1537, 1538, 98, and 100. AMPD was not mutagenic, with and without metabolic activation, in *S. typhimurium* strains TA 1535, 1537, 98, and 100. AMP was also not mutagenic in a mouse lymphoma mutagenesis assay and in a mouse micronucleus assay.

In a clinical study, a cosmetic formulation containing AMP-95 was not a primary dermal irritant. In a primary irnitancy test of a cosmetic formulation containing AMPD, scattered incidences of questionable responses were observed in two thirds of the panelists. In addition, 2 of 15 panelists had slight redness at least once during the observation period.

A cosmetic formula containing 0.22% AMP-95 was not an allergic contact sensitizer when tested using a panel of 97 subjects. Sensitization did not occur in other RIPT studies of cosmetic formulations containing AMP ranging from 1.5% to 7.0%. A cosmetic formulation containing 0.073% AMPD was not a primary irritant, and it was neither a fatiguing agent nor a sensitizer. In another study, a cosmetic formulation containing 0.50% AMPD was not a sensitizer.

Two cases of airborne contact dermatitis were reported in patients who were exposed to AMP 100 in the production line of a cosmetic company.

SUMMARY

Tromethamine is an aliphatic compound that functions as a fragrance ingredient and a pH adjuster.

Tromethamine is used in 480 leave-on cosmetic products and 69 rinse-off products up to 4%.

Tromethamine has several medical uses, including treatment for acidosis under several circumstances.

The oral LD_{50} for mice was reported to range from 3350 to 5500 mg/kg. For rats, the LD_{50} was > 3000 mg/kg. The LC_{50} was between 1000 and 2000 mg/kg. The dermal LD_{50} of tromethamine for mice and rats was reported to be > 1000 mg/kg and > 2000 mg/kg for rabbits. The intraperitoneal LD_{50} of tromethamine for mice was reported to be ~3350 mg/kg.

The LOAEL for tromethamine for rats was reported to be 2500 ppm when incorporated into feed for 3 months. There were signs of liver damage at necropsy when rats were orally administered tromethamine at 300 mg/kg/d for 14 – 37 days.

There were no clinical signs to rabbits dermally administered tromethamine at 100% on clipped skin for 4 h for 5 days.

Intravenous toxicity of tromethamine was minimal at neutral pH. However, at more alkaline pH range, gangrene at the injection sites, tissue necrosis, inflammatory lesions, and visible infarcts in the kidneys, bleached liver, darkened spleen, and lesions on the heart were reported. Anorexia, bloody urine, and paralysis were also observed.

Intratracheal tromethamine in an unknown mixture with 0.9% saline did not decrease survival or average body weight of hamsters when administered over the lifetime of the hamsters.

There were no adverse effects on reproduction at 100 mg/kg/day to rats. However, there were increased resorptions, post implantation loss, and decreased litter size starting at 300 mg/kg/day.

Tromethamine at 0.2 ml did not induce tumors when administered intratracheally to hamsters weekly for their entire lifespan.

Intradermal injections of tromethamine were severely irritating at pH 10.4 but were only mildly irritating at pH 7.4. Tromethamine was mildly irritating at 25% with a pH of 10.8 and not irritating at 40% in distilled water.

DATA NEEDS

The Cosmetic Ingredient Review (CIR) recognizes the following data are not currently available and encourages interested parties to submit such data if available:

- Inhalation toxicity,
- Ocular irritation
- Sensitization, and
- Genotoxicity.

TABLES AND FIGURES

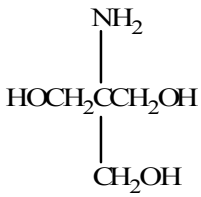

Figure 1. Tromethamine.

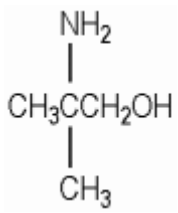

Figure 2. Aminomethylpropanol (AMP).

Table 1. Chemical and physical properties of tromethamine.

Property	Value	Reference
Physical Form	Crystalline powder	13
Color	White	47
Odor	Slight, characteristic	6
Molecular Weight g/mol	121.14	13
Vapor pressure mmHg@ 25ºC	2.20^{e-05}	47
Melting Point ºC	171-172	13
Boiling Point ºC	219-220	13
Solubility g/L water	550	47
ethylene glycol	0.0791	13
ethanol (95%)	0.022	13
acetone	0.020	13
Other Solubility g/L @ ºC & pH		
Diethyl ether	Insoluble	10
Chloroform	Practically insoluble	6
Benzene	Practically insoluble	6
Carbon tetrachloride	Practically insoluble	6
Disassociation constants (pKb) @ body temperature	7.8	6

Table 2. Frequency of use according to duration and exposure of tromethamine.[11,12]

Use type	Uses	Maximum Concentration (%)
Total/range	**549**	**0.00009-4**
Duration of use		
Leave-on	480	0.0002-4
Rinse-off	69	0.00009-4
Diluted for (bath) use	NR	NR
Exposure type		
Eye area	70	0.08-2
Incidental ingestion	1	0.002-0.3
Incidental Inhalation-sprays	10	0.02-0.5
Incidental inhalation-powders	NR	0.0002-0.05
Dermal contact	523	0.00009-4
Deodorant (underarm)	NR	NR
Hair-noncoloring	11	0.001-0.8
Hair-coloring	NR	NR
Nail	1	4
Mucous Membrane	12	0.00009-0.3
Baby	NR	NR

NR = Not Reported; Totals = Rinse-off + Leave-on + Diluted for bath Product Uses.
Note: Because each ingredient may be used in cosmetics with multiple exposure types, the sum of all exposure type uses may not equal the sum total uses.

Table 3. Toxicity data for tromethamine.

Species (n)	Dose(s)	Results	Reference
		Acute oral toxicity	
Mice, strain not provided (10)	2000, 3500, 5000, 7000, 10000 mg/kg by gavage	LD_{50} = 5500 mg/kg	48
Swiss mice (10)	1000, 2000, 3000 mg/kg as 5% and 20% solutions by gavage	LD_{50} >3000 mg/kg. No toxicity noted. Abundant urine output for some mice.	49
Mice, strain not provided (not provided)	2000, 2500, 3530, 5000, 7000 mg/kg by gavage	LD_{50} = ~3350 mg/kg	48
Wistar rat (10)	1000 and 3000 mg/kg by gastric tube as 20% solution	No toxicity noted. Abundant urine output was recorded for some rats.	49
Wistar rat (10)	1000, 2000, 3000 mg/kg by gavage as 5% and 20% solutions by gavage	LD_{50} >3000 mg/kg. No toxicity noted. Abundant urine output for some rats.	49
Rabbits, strain not provided (not provided)	Delivered neat by gavage	LC_{50} between 1.00 - 2.00 g/kg. Weakness and collapse. Coma preceded deaths. No CNS signs or convulsions. Toxicity was due to alkalinity; neutralization reduced toxicity.	42
		Acute dermal toxicity	
Mice, strain not provided (5)	500 or 1000 mg/kg as 5% solution by subcutaneous injection	500 mg/kg caused irritation at the injection site. 1000 mg/kg caused the formation of lesions. LD_{50} > 1000 mg/kg	49
Rat, strain not provided (5)	500 or 1000 mg/kg as 5% solution by subcutaneous injection	500 mg/kg caused irritation at the injection site. 1000 mg/kg caused the formation of lesions. LD_{50} > 1000 mg/kg	49

Rabbit, strain not provided (4)	1000, 1500, 2000 mg/kg under occlusion on shaved abdomen, with or without abrasion for 24 h. Observed for 14 d then necropsied.	At removal, intact and abraded sites were severely irritated and black in color. The sites became necrotic within 2-3 d and remained necrotic. Treated sites had severe eschar formation by day 14. Treated rabbits lost body weight over the observation period. Rabbits in all treated groups showed no signs of toxicity or abnormal pharmacological behavior. At necropsy, all organs were grossly normal. The treated skin sites in all rabbits were necrotic. LD_{50} > 2000 mg/kg and was a severe dermal irritant	40
Acute intraperitoneal toxicity			
Mice, strain not provided (10)	2000, 2500, 3250, 3600, 4000, mg/kg by intraperitoneal injection at 0.015 ml/g.	LD_{50} = ~3350 mg/kg.	50
Male CD-1 mice (4-11)	100 mg/kg after drug-induced hypothermia/ shock using lipopolysaccharide	Hypothermic response was reduced at 4, 24, and 48 h. No other effects were reported.	51
Acute intravenous toxicity			
Mice, strain not provided (10)	0.3 M. i.v. injection (pH 5.5, 10.4) with and without dextrose or sodium chloride and observed for 24 h.	LD_{50} = 16.5 mM/kg. Mice convulsed immediately before dying. Neutralizing the pH and the additives did not change toxicity.	34
Mice, strain not provided (10)	100, 200, 400, 500, 1000, 3000, 5000, 6000, 7000 mg/kg as 1% solution	No mortality at doses < 5000 mg/kg. 6000 mg/kg, 40% mortality; 7000 mg/kg, 100%. Muscle weakness accompanied by respiratory difficulty prior to death. LD_{50} = ~ 6100 mg/kg	49
Sprague-Dawley rat (3/sex)	2.0, 2.5, 3.0, 3.5 g/kg of 0.6M; 4.0 and 4.5 g/kg of 0.9M in saline injected over 1 min followed by 2-h observations then necropsy.	Most rats died during treatment or within 10 min of treatment. The rest survived the observation period. No gross lesions observed except for in the liver and kidneys. Peracute toxic nephrosis was observed in the kidneys; moderate degree of pyknosis of the nuclei of isolated segments of the renal tubular epithelium in 2 and 2.5 g/kg groups, and was dose dependent. In higher dose levels, the lesions were severe pyknosis of the nuclei of swollen renal tubular epithelial cells of carried segments of the cortex. The cytoplasm of the affected cells was coagulated, distinctly granular, and intensely eosinophilic. Lumens of the affected tubules were distended with eosinophilic, amorphous tissue debris and secretions. Lethargy was observed sporadically in rats at 3-4 g/kg dose groups. All had lesions of acute toxic hepatitis. The lesion was characterized by pyknosis of the nuclei of the hepatocytes and cloudy swelling of the cytoplasm of hepatocytes. However, the lesions did not constitute a consistent characteristic lesion as did the peracute toxic nephrosis. LD_{50} = 3.28 – 4.04 g/kg.	43
Rat, strain not provided (10)	100, 200, 400, 500, 1000, 3000, 5000, 6000, 7000 mg/kg as 1% and 2% solutions	No observations of toxicity at < 3000 mg/kg. 5000 mg/kg, 30% mortality; 6000 mg/kg, 60%; and 7000 mg/kg, 70%. LD_{50} = ~6000 mg/kg.	49
Male Wistar rats (6)	0.5mmol/kg/min @ pH 10.9 or 7.4	Both pH levels were well tolerated for 50-70 min; then metabolic alkalosis developed, then death. Plasma concentration increased linearly to 53.7 ± 9.09 mmol/L @ 60 min. No effects observed to BP, heart rate, ECG, and Na^+ and K^+ plasma or erythrocyte concentration. The authors stated that depressed ventilation was the cause of death. When infusion was stopped at 20 min, the rats recovered.	52
Rabbit, strain not provided (5)	250 and 500 mg/kg as 5% solution	No treatment-related mortality. Changes in respiratory rate and amplitude were observed.	49
Dog, breed not provided (5)	125 mg/kg as 5% solution	Alterations in respiratory rate and amplitude. LC_{50} > 125 mg/kg	49

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