GREEN

Safety Assessment of Tromethamine as Used in Cosmetics

CIR EXPERT PANEL MEETING

MARCH 18-19, 2013

Cosmetic Ingredient Review
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February 22, 2013

MEMORANDUM

To: CIR Expert Panel and Liaisons

From: Lillian C. Becker, M.S.
Scientific Analyst and Writer

Subject: Draft Report for Tromethamine as used in cosmetics

Attached is the Draft Report for Tromethamine as used in cosmetics. A scientific literature review was issued for public comment in November, 2012.

Comments were received from the Personal Care Products Council's CIR Science and Support Committee and have been addressed.

The CIR Science and Support Committee and Dow Chemical suggested that we do not use aminomethyl propanol in an analogue approach (as was done by EPA), but to use aminoethyl propanediol (AEPD) and aminomethyl propanediol (AMPD) instead as analogues for which data were available. We agreed and made the change.

Data from the Dow Chemical Company's REACH submission (including data on AEPD and AMPD) have been incorporated into the report. The summaries from the CIR safety assessment and amended safety assessment of AMPD are included in the report and the entire safety assessment is included in the data section for the Panel's information.

The Panel should review the draft report and decide 1) if it is appropriate to consider AEPD and AMPD data in evaluating tromethamine and 2) if there are sufficient data to come to a conclusion. If there is sufficient data, the Panel is to issue a tentative report and develop a discussion. If not, then an insufficient data announcement, with a list of data needs, is to be issued.

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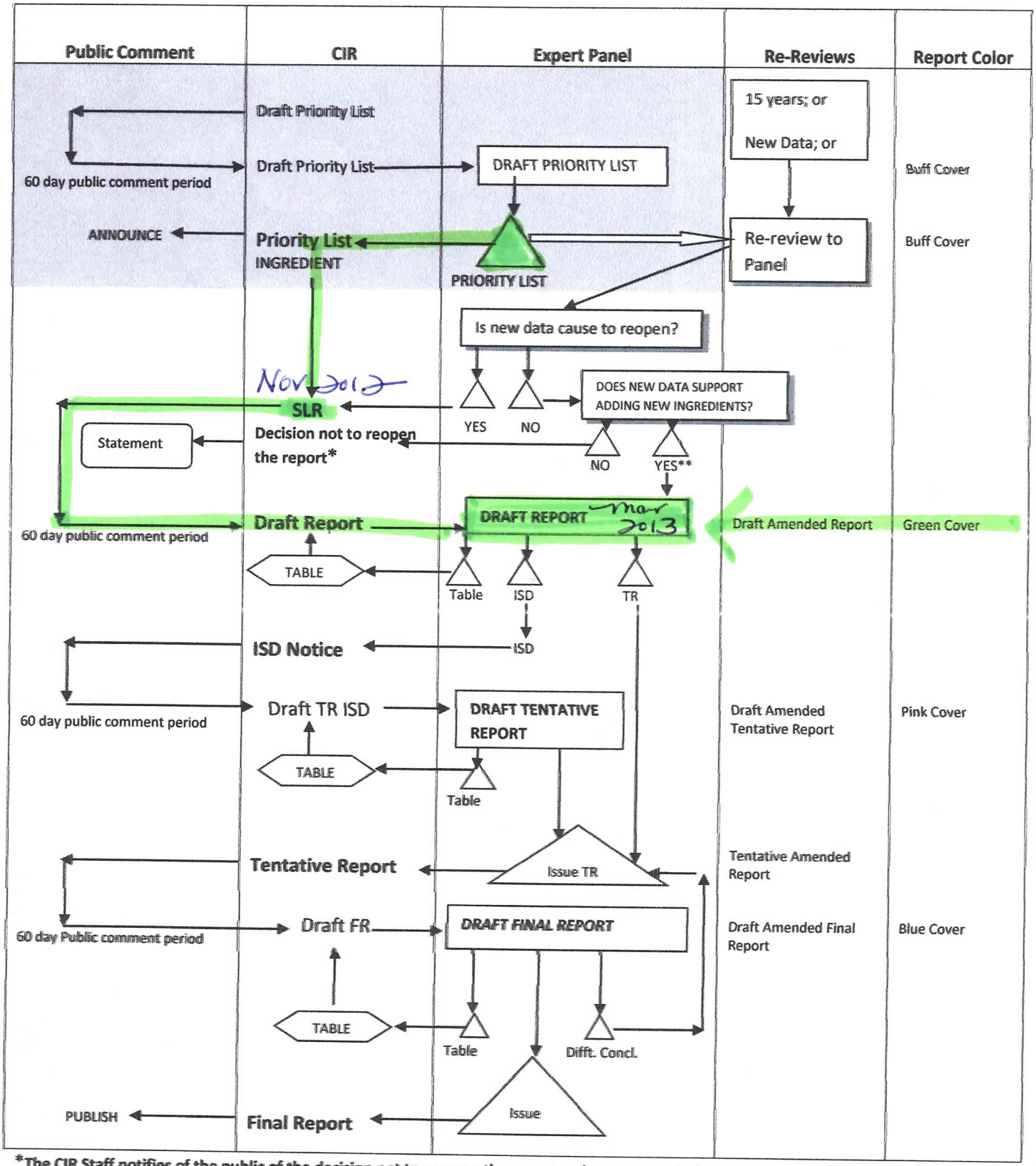
Tromothamine – Mar 2013

SAFETY ASSESSMENT FLOW CHART



*The CIR Staff notifies of the public of the decision not to re-open the report and prepares a draft statement for review by the Panel. After Panel review, the statement is issued to the Public.

**If Draft Amended Report (DAR) is available, the Panel may choose to review; if not, CIR staff prepares DAR for Panel Review.

△ Expert Panel Decision

▭ Document for Panel Review

⇨ Option for Re-review

History of Tromethamine

November, 2012 – SLR was posted for public comment.

March, 2013 – Panel reviews draft report for the first time.

Tromethamine Data Profile for March, 2013. Writer - Lillian Becker																		
	ADME			Acute toxicity			Repeated dose toxicity			Irritation			Sensitization					
	Dermal Penetration	Log K_{ow}	Use	Oral	Dermal	Inhale	Oral	Dermal	Inhale	Ocular Irritation	Dermal Irr. Animal	Dermal Irr Human	Sensitization Animal	Sensitization Human	Repro/Devel toxicity	Genotoxicity	Carcinogenicity	Phototoxicity
Tromethamine	X		X	X	X		X	X		X	X	X			X	X	X	
AMPD													X			X		
AEPD													X	X				

Search Strategy for Tromethamine

Scifinder – “tromethamine” and CAS no.

Google - “tromethamine” and CAS no.

EPA HPV Database – CAS no.

Safety Assessment of Tromethamine as Used in Cosmetics

Status:	Draft Report for Panel Review
Release Date:	February 22, 2013
Panel Meeting Date:	March 18-19, 2013

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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INTRODUCTION

Tromethamine (also referred to as Tris and THAM) is an aliphatic compound that functions as a fragrance ingredient and a pH adjuster in cosmetics.

Because minimal data were available for tromethamine itself, the Cosmetic Ingredient Review (CIR) considered data on realted chemicals. 2-amino-2-methyl-1,3-propanediol (AMPD) and 2-amino-2-ethyl-1,3-propandiol (AEPD) were justified in a REACH registration as analogues for tromethamine.[1,2] According to the registration, these two substances have similar physico-chemical properties to tromethamine. They reportedly also have similar environmental fate, environmental toxicological, and mammalian toxicological properties.

CIR issued a safety assessment of AMPD in 1990, concluding that AMPD is safe in the present practices of use up to 1%.[3] This conclusion was amended in 2009 with a safe as used conclusion.[4] The summary of the amended safety assessment is provided below. Other data on AMPD and AEPD also are included in this safety assessment.

CHEMISTRY

Definition and Structure

Tromethamine (CAS No. 77-86-1) conforms to the structure in Figure 1. AMPD and AEPD are shown in Figure 2.

Physical and Chemical Properties

Physical and chemical properties are presented in Table 1.

Tromethamine is reported to be stable when exposed to light and air but is unstable with freezing.[7,8]

Method of Manufacture

Tromethamine is prepared by the reduction of tris(hydroxymethyl)nitromethane.[9,10] Tromethamine may also be manufactured by additively reacting nitromethane with formaldehyde to yield tris(hydroxymethyl) nitromethane, which is then hydrogenated with the aid of Raney nickel catalyst.[8,11]

Impurities

When tromethamine is heated to decomposition, it emits toxic fumes composed of nitrogen oxide.[12]

USE

Cosmetic

Data on ingredient usage are provided to the Food and Drug Administration (FDA) Voluntary Cosmetic Registration Program (VCRP; Table 2).[13] A survey was conducted by the Personal Care Products Council (Council) of the maximum use concentrations for these ingredients.[14] 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.[15]

Tromethamine is regulated in Europe under Annex III entry 61 Monoalkylamines, Monalkanolamines, and Their Salts which states that: secondary amine content is limited to 0.5%, no use in nitrosating systems, nitrosamine content is limited to 0.5% (raw materials), and that tromethamine must be stored in nitrite-free containers.[16]

Tromethamine was reported to be used as a commercial emulsifier.[17]

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.[18]
- Intravenously administered tromethamine (15 mmol/kg or 3.5 L 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.[5,19]
- Intravenously administered tromethamine is used to treat acidosis during pulmonary bypass and cardio surgery that requires hypothermic techniques.[20-23]
- Intravenously administered tromethamine is used to treat acidosis in burn victims.[24]
- 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).[5,25,26]
- 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 alkalization of

the plasma.[5]

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.[27]

TOXICOKINETICS

Absorption, Distribution, Metabolism, and Excretion

Tromethamine is eliminated by the kidneys. Ionized tromethamine (chiefly as the bicarbonate salt or tromethamine citrate) 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.[18,28-30] Excretion of tromethamine is accompanied by osmotic diuresis, since clinical doses of tromethamine considerably adds to osmolarity of glomerular filtrate.[8]

Tromethamine is primarily eliminated from plasma through renal filtration of its protonated form.[31] 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.[5]

It is not known whether tromethamine is distributed in human milk.[28]

Dermal/Percutaneous

Dermal absorption was < 1% when radio-labeled tromethamine (0.1% and 10%; 100 µL) was administered to dermatomed, thawed human skin in Franz cells.[32] 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 alkalization of the body fluids.[33]

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)[25]

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.[19,29-31,34-38] 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.[34] 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 tromethamine 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.[38]

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.[38]

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.[35] 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. to rats, 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.[35]

Rabbit (strain and n 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.[37] 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 tromethamine pH 5.5, a larger amount of chloride than with tromethamine was excreted. At the end of the 7 hours, 44% of the infused tromethamine was found in the urine, while with tromethamine 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 (tromethamine-induced hypoglycemia persisted longer than the tromethamine-neutralized). Both treatments caused transient hypoglycemia. Blood analysis on extracted blood (tromethamine 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).[31] Tromethamine did accumulated in the tissues, but equilibrium was slow.

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.[39] 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 it quickly diffuses into intracellular spaces to restore intracellular acidosis. The authors also noted that tromethamine passed from extracellular space in a multi-exponential fashion, indicating that it passes to different body tissues at variable rates. And that 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.[29] 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 tromethamine 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 µg/ml for 3T3 cells, ~160 µg/ml for 3T6 cells, ~340 µg/ml for HaCaT cells, ~180 µg/ml for NCTC 2544 cells, ~340 µg/ml for HeLa cells, and ~405 µg/ml for MCF-7 cells. In the neutral red uptake (NRU) assay, the IC_{50}s were ~295 µg/ml for 3T3 cells, ~130 µg/ml for 3T6 cells, ~160 µg/ml for HaCaT cells, ~ 190 µg/ml for NCTC 2544 cells, ~190 µg/ml for HeLa cells, and ~ 315 µg/ml for MCF-7 cells.[40]

Blood Effects

Tromethamine administered i.v. caused a fall in blood glucose levels in rats, rabbits, dogs, and humans.[36,41,42] 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.[42] 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 > 5000 mg/kg. The LC_{50} was between 1000 and 2000 mg/kg.[43-45]

Dermal – Non-Human

The dermal LD_{50} of tromethamine for rats was reported to be > 5000 mg/kg for rats and > 2000 mg/kg for rabbits (Table 3).[46,47]

Subcutaneous – Non-Human

The subcutaneous LD_{50} was reported to be > 1000 mg/kg for mice and rats.[44]

Intraperitoneal

The intraperitoneal LD_{50} of tromethamine for mice was reported to be ~3350 mg/kg (Table 3).[48,49]

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.[37,44,50,51]

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.[46] Specific adverse effects were not provided.

The NOAEL for local toxicity was 100 mg/kg/d and ≥ 1000 mg/kg/d for systemic toxicity for Crl:CD(D) rats (n = 10) orally administered tromethamine (100, 300, 1000 mg/kg/d adjusted to pH 9) by gavage in a reproduction study.[47] Males (n = not provided) were treated for at least 2 weeks before breeding up to 29 days. Females (n = 12) were treated from 2 weeks prior to breeding, through gestation, and through 4 days of lactation for up to 54 days. There were no systemic effects but there was irritation to the forstomach.

When tromethamine (2500 mg/kg) was orally administered to rats (n = 38; strain not provided) for 15 days, there were no mortalities or clinical signs observed.[47]

When tromethamine (250-4000 mg/kg) was orally administered to rats (n = 36; strain not provided) for 31 days, there were no mortalities or clinical signs observed except for moderate diarrhea in the highest dose.[47]

Dogs (n – 12/dose; strain not specified) orally administered tromethamine (250, 1000, 4000 mg/kg) for 30 days had no mortalities.[47] Dogs in the mid dose group had occasional loose stool and vomiting. Dogs in the high dose group had frequent loose stool and vomiting. Urinalysis showed reduced urinary potassium in the mid and high dose groups. The authors considered the NOAEL to be 4000 mg/kg because none of the effects were considered permanent.

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.[45]

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.[37] Histological examination of the organs showed that there were no adverse effects from the treatment.

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.[51] 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.[51]

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).[37] 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.[37]

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.[37] Histological study of the organs was negative.

Rabbits (n = 5) administered tromethamine (1500, 3000 mg/kg; 0.2 mL/kg/min in Ringer's solution; 0.34 M) by

catheter for 21 days had two mortalities (days 6 and 12) in the high dose group.[47] Clinical signs included rapid, shallow breathing during infusion.

Catheterized dogs (n = 5) administered i.v. tromethamine (1500, 3000 mg/kg: 0.34 M in Ringer's solution; 0.5 mL/kg/min) for 21 days exhibited sporadic convulsions and vomiting.[47] One dog in the high dose group died during treatment. Three dogs in the low dose group had increased retention of bromosulfophthalein (BSP). Infracts (multiple abscesses) of the liver was observed in three dogs in the low dose group. Colonies of bacteria, acute inflammatory exudate, and hypertrophy of the Kuppfer cells were observed in the same livers.

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.[51] Rats were allowed 24 h or 7 d for recovery. On day 11, a second high dose group were treated with an additional tromethamine using 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.[51]

Intraperitoneal – Non-Human

Tromethamine (30 mL/kg: 0.075 M) administered i.p. to dogs (n = 3) under anesthesia for days caused no clinical signs during treatment.[47] One dog died on day 3, but was attributed to heartworms. There were no histopathological signs attributed to the test substance.

Intratracheal – Non-Human

Tromethamine (in an unknown mixture with 0.9% saline; 2 mL; vehicle control in an experiment) 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.[52] 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

The NOAEL for reproduction and teratogenicity for tromethamine using rats was ≥ 1000 mg/kg/d. [47] Crl:CD(D) rats (n = 10) were orally administered tromethamine (100, 300, 1000 mg/kg/d adjusted to pH 9) by gavage. Males (n = not provided) were treated for at least 2 weeks before breeding up to 29 days. Females (n = 12) were treated from 2 weeks prior to breeding, through gestation, and through 4 days of lactation for up to 54 days.

Tromethamine had no effect on mating performance or conception. There were no effects to mating index, fertility index, gestation period, deliver index, and number of live pups. There were no effects observed to the F1 pup at birth.

GENOTOXICITY

In Vitro

Tromethamine (1 mg/mL; pH 7.4) was toxic but not mutagenic to *Escherichia coli* (CHY832) in an RK assay.[53] The *E. coli* were killed at 42°C but not at 30°C.

AEPD

In an in vitro mammalian chromosome aberration test using Chinese hamster lung (CHL/IU) cells, AEPD (75, 150, 300, 600, 1200 µg/mL in saline) was not genotoxic with and without metabolic activation when exposed for 24 and 48 h.[47]

AEPD (156, 313, 625, 1250, 2500, 5000 µg/plate in water) was not mutagenic to *S. typhimurium* (strains TA98, TA100, TA1535, TA1537) and *E. coli* (strain WP2 uvr A), with or without metabolic activation.[47]

AEPD (12, 38, 119, 337, 1192 µg/mL with metabolic activation; 15, 44, 132, 397, 1192 µg/mL without) was not mutagenic to Chinese hamster ovary (CHO) cells in an in vitro mammalian cell gene mutation test with or without metabolic activation.[47] AEPD was cytotoxic at 1192 µg/mL.

When the above study was repeated, AEPD (12, 38, 119, 337, 1192 µg/mL with metabolic activation; 15, 44, 132, 397, 1192 µg/mL without) was not mutagenic to CHO cells in an in vitro mammalian cell gene mutation test with or without metabolic activation.[47] AEPD was cytotoxic at 1192 µg/mL.

CARCINOGENICITY

Studies

When administered intratracheally to male Syrian hamsters weekly for their entire lifespan, tromethamine (2 ml in 0.9% saline) did not induce tumors.[52]

IRRITATION AND SENSITIZATION

Irritation

Dermal – Non-Human

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.[54] 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.[55]

In a dermal irritation test using New Zealand White rabbits (n = 3 males), tromethamine (0.5 g in enough water to make a paste) was not irritating when administered to shaved skin under semi-occlusion[47] Test sites were observed at 1, 24, 48 and 72 h.

Dermal – Human

A cosmetic product containing tromethamine (3.1%; neat) was not irritating when administered in a patch test (n = 11).[56]

Intradermal – 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).[37] 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.

Ocular

Tromethamine (0.1 g; finely ground) was not an ocular irritant when administered to New Zealand White rabbits (n = 3.[47] The eyes were observed at 1, 24, 48, and 72 h with a hand slit lamp. Fluorescein was used at 24 h. There was slight/ moderate redness and chemosis at 1 h; the irritation effects cleared by 24 or 72 h. No damage to the iris or cornea was observed.

Tromethamine (100%) was not an ocular irritant when administered to rabbits (strain and n not provided).[47]

Sensitization

There were no sensitization studies discovered or submitted for tromethamine.

Non-Human

AEPD

AEPD (0.05% - 0.5%; 0.5 ml) was not a sensitizer to male Hartley guinea pigs (n = 10) in Buehler sensitization assay.[47] Some of the guinea pigs showed mild erythema during the initial 5 applications at 0.5% of the induction period, so the concentration was reduced to 0.05% the last 5 applications.

In a Draize test, AEPD (0.05% - 1% in saline; 0.5 ml) was not a sensitizer to male Hartley guinea pigs (n = 10).[47] Some of the guinea pigs showed mild erythema during the initial 5 applications at 0.5% of the induction period, so the concentration was reduced to 0.05% the last 5 applications.

In a Draize test, intradermally injected AEPD (0.05% - 1% in saline; 0.05 mL) was not a sensitizer to male Harley guinea pigs (n = 10). Some of the guinea pigs showed mild erythema during the initial 5 applications of the induction period, so the concentration was reduced to 0.05% for the last 5 applications.

AMED

In a peptide reactivity assay for screening contact allergens, AMED (4 nM) is not expected to cause skin sensitization.[47]

Human

AMPD

In a patch test of dermatitis patients (n = 233) with past or present exposure to metalworking fluids, AEPD (85% aqueous) produced a positive reaction in only one subject.[57] This subject currently had work-related hand dermatitis and had been in the metalworking industry for 2 -20 years.

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.[33] 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.[33] 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 treatment observed at autopsy.

A 40-year-old man, who had a 9-rib thoracoplasty, presented with extensive pneumonia.[33] 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 tromethamine (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. No adverse effects from the tromethamine treatment were reported.

SUMMARY OF DATA FROM THE AMPD SAFETY ASSESSMENT

AMPD is a substituted aliphatic alcohols used as cosmetic ingredients.[4]

AMPD occurs in solid and liquid forms. AMPD is soluble in both water and alcohols.

AMPD function as a pH adjuster in cosmetic products. AMPD is also a fragrance ingredient. AMPD is used in concentrations up to 2%.

A hair spray containing 0.50% AMPD was nontoxic to rats.

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.

Cosmetic formulations containing 0.40% AMPD were moderate ocular irritants.

AMPD was not mutagenic, with and without metabolic activation, in *S. typhimurium* strains TA 1535, 1537, 98, and 100.

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 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.

SUMMARY

Tromethamine is an aliphatic compound that functions as a fragrance ingredient and a pH adjuster. Toxicity data for AMPD and AEPD were considered.

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

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

Tromethamine is eliminated by the kidneys. There was little dermal absorption.

Tromethamine was cytotoxic to multiple cell types in the range of 129 – 405 µg/ml.

Tromethamine administered i.v. caused a fall in blood glucose levels in rats, rabbits, dogs, and humans.

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 local NOAEL for orally administered tromethamine was 300 mg/kg/d for 14 – 37 days. Tromethamine at 1000 mg/kg caused loose stool and vomiting in dogs.

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 hamsters.

There were no adverse effects on reproduction at 1000 mg/kg/day to rats.

Tromethamine was toxic, but not mutagenic, to *E. coli* in an RK assay. AEPD was not mutagenic in a chromosome

aberrations tests.

Tromethamine at 2 ml did not induce tumors when administered intratracheally to hamsters weekly for their entire lifespan.

Tromethamine was a mild irritant to rabbits at 25% with a pH of 10.8. At 40%, pH unknown, was not irritating.

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. Tromethamine in a paste with water was not irritating to the shaved skin of rabbits.

A cosmetic product containing 3.1% tromethamine was not irritating a patch test.

Tromethamine was not an ocular irritant to rabbits at 100%.

AEPD was not a sensitizer to guinea pigs up to 1%.

There was only one positive reaction among 233 subjects with past or present exposure to metalworking fluids to AEPD at 85%.

Tromethamine at 20 g administered i.v. was not toxic to subjects being treated for respiratory acidosis.

DISCUSSION

To be developed.

CONCLUSION

To be developed.

TABLES AND FIGURES

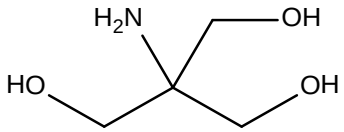
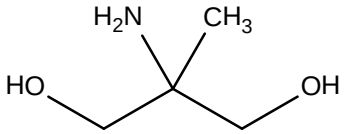
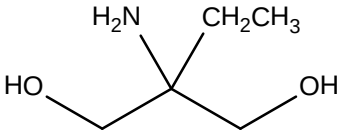


Figure 1. Tromethamine.



2-amino-2-methyl-1,3-propanediol (AMPD)



2-amino-2-ethyl-1,3-propandiol (AEPD)

Figure 2. Analogs for tromethamine

Table 1. Chemical and physical properties of tromethamine.

Property	Value	Reference
Physical Form	Crystalline powder	15
Color	White	58
Odor	Slight, characteristic	8
Molecular Weight g/mol	121.14	15
Vapor pressure mmHg@ 25°C	2.20^{e-05}	58
Melting Point °C	171-172	15
Boiling Point °C	219-220	15
Solubility g/L water	550	58
	678-689	47
ethylene glycol	0.0791	15
ethanol (95%)	0.022	15
acetone	0.020	15
Other Solubility g/L @ °C & pH		
Diethyl ether	Insoluble	12
Chloroform	Practically insoluble	8
Benzene	Practically insoluble	8
Carbon tetrachloride	Practically insoluble	8
Disassociation constants (pKb) @ body temperature	7.8	8
Partition coefficient (octanol/water) @ 20 °C	2.31	47

Table 2. Frequency of use according to duration and exposure of tromethamine.[13,14]

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. Acute 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	[43]
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.	[44]
Mice, strain not provided (not provided)	2000, 2500, 3530, 5000, 7000 mg/kg by gavage	LD_{50} = ~3350 mg/kg	[43]
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.	[44]
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.	[44]
Wistar rat, female (3)	5000 mg/kg in water by oral gavage; 3 doses with 2-day intervals	LD_{50} > 5000 mg/kg. No deaths or clinical signs.	[47]
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.	[45]
		Acute dermal toxicity	
Wistar rats (3)	5000 mg/kg for 24 h under semiocclusion on shaved skin	No mortalities or clinical signs.	[47]

Table 3. Acute toxicity data for tromethamine.

Species (n)	Dose(s)	Results	Reference
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	46
		Subcutaneous 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	44
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	44
		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.	48
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.	49
		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.	37
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	44
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.	51
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.	44
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.	50
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.	44
Dog, breed not provided (5)	125 mg/kg as 5% solution	Alterations in respiratory rate and amplitude. LC_{50} > 125 mg/kg	44

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Cosmetic Ingredient Review
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February 22, 2013

MEMORANDUM

To: CIR Expert Panel and Liaisons

From: Lillian C. Becker, M.S.
Scientific Analyst and Writer

Subject: Data for consideration for the Draft Report for Tromethamine as used in cosmetics

Attached is the data for consideration for the Draft Report for Tromethamine as used in cosmetics.

These data include:

1. A summary of a patch test of a cosmetic product containing 3.1% tromethamine;
2. FDA VCRP data;
3. The 1990 CIR safety assessment of aminomethyl propanediol (AMPD);
4. The 2009 amended safety assessment of AMPD;
5. Letter from Dow Chemical asking CIR to use AMPD and AEPD instead of AMP as analogs of tromethamine and the justification submitted to REACH for using these chemicals; and
6. Dow Chemical's submission to REACH with summaries of data on tromethamine, AMPD, and AEPD.
7. Council's concentration of use data.

The summaries of these two reports are included in the Tromethamine safety assessment. The reports are included for the Panel's information.

1101 17th St., NW, Suite 412 Washington, DC 20036-4702
Phone 202.331.0651 FAX 202.331.0088 http://www.cir-safety.org

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

TO: F. Alan Andersen, Ph.D.
Director - COSMETIC INGREDIENT REVIEW (CIR)

FROM: Halyna Breslawec, Ph.D.
Industry Liaison to the CIR Expert Panel

DATE: December 7, 2012

SUBJECT: Information on a Product Containing Tromethamine

Anonymous. 2012. Summary: Evaluation of the acute cutaneous tolerance of a cosmetic product (mask for hands containing 3.1% Tromethamine) on adult subjects: Single patch test method under dermatological control.

1101 17th Street, N.W., Suite 300 | Washington, D.C. 20036-4702 | 202.331.1770 | 202.331.1969 (fax) | www.personalcarecouncil.org

RESUME DE L'ETUDE / *SUMMARY*

<table>
<tr><td>Titre de l'étude / Study title</td><td colspan="3">ETUDE DE LA TOLERANCE CUTANEE AIGUE D'UN PRODUIT COSMETIQUE CHEZ LE VOLONTAIRE ADULTE : PATCH-TEST SIMPLE SOUS CONTROLE DERMATOLOGIQUE
EVALUATION OF THE ACUTE CUTANEOUS TOLERANCE OF A COSMETIC PRODUCT ON ADULT SUBJECTS : SINGLE PATCH-TEST METHOD UNDER DERMATOLOGICAL CONTROL</td></tr>
<tr><td rowspan="2">Produit / Product</td><td colspan="3">Référence / Reference:
MASK FOR HANDS WITH 3.1% OF TROMETHAMINE</td></tr>
<tr><td colspan="2">Forme galénique / Galenic form:
Emulsion épaisse blanche / White thick emulsion</td><td>Concentration / Concentration:
Pur / Pure applied undiluted</td></tr>
<tr><td>Patch</td><td colspan="2">Type de patch / Patch type:
Occlusif / Occlusive</td><td>Durée du patch / Patch duration:
48 heures / 48 hours</td></tr>
<tr><td>Date(s) de l'étude / Study dates</td><td colspan="3">Du 13 au 16 mars 2012 / From March 13 to 16, 2012.</td></tr>
<tr><td>Objectif / Objective</td><td colspan="3">Déterminer le potentiel irritant primaire d'un produit cosmétique après application unique sous patch-test / To determine the acute irritating potential of a cosmetic product after single application under patch-test.</td></tr>
<tr><td>Plan expérimental / Study design</td><td colspan="3">Etude monocentrique en simple aveugle / Monocentric and simple blind study.</td></tr>
<tr><td>Critère(s) d'évaluation / Assessment criteria</td><td colspan="2">• Scorage érythème et œdème / assessment of erythema and edema
• Calcul du MCII / MCII calculation</td><td>Zone</td><td>Dos (zone scapulaire) / scapular part of the back</td></tr>
<tr><td></td><td></td><td>Cinétique / Kinetics</td><td>30 minutes et 24 heures après le retrait des patchs / 30 minutes and 24 hours after patch removal</td></tr>
<tr><td rowspan="3">Population étudiée / Study population</td><td colspan="3">Nombre de volontaires analysés / Number of analyzed subjects: 11.</td></tr>
<tr><td colspan="3">Age moyen / Average age: 39 ans (entre 20 et 63 ans) / 39 years (between 20 and 63)</td></tr>
<tr><td colspan="3"><u>Critères principaux d'inclusion</u> / <u>Main inclusion criteria</u>
• Age > 18 ans / Age > 18 years old.
• Phototype I à III / Phototype I to III.</td></tr>
<tr><td>Résultats / Results</td><td colspan="3">Valeur d'IICM / MCII value
0,09
Conclusion
Non irritant / Non irritating</td></tr>
</table>

Date: 19/03/2012

FDA VCRP Use
TROMETHAMINE

03A - Eyebrow Pencil	TROMETHAMINE	1
03B - Eyeliner	TROMETHAMINE	14
03C - Eye Shadow	TROMETHAMINE	2
03D - Eye Lotion	TROMETHAMINE	23
03E - Eye Makeup Remover	TROMETHAMINE	2
03F - Mascara	TROMETHAMINE	13
03G - Other Eye Makeup Preparations	TROMETHAMINE	15
04B - Perfumes	TROMETHAMINE	2
04E - Other Fragrance Preparation	TROMETHAMINE	2
05A - Hair Conditioner	TROMETHAMINE	1
05F - Shampoos (non-coloring)	TROMETHAMINE	2
05G - Tonics, Dressings, and Other Hair Grooming Aids	TROMETHAMINE	7
05H - Wave Sets	TROMETHAMINE	1
07A - Blushers (all types)	TROMETHAMINE	2
07C - Foundations	TROMETHAMINE	9
07D - Leg and Body Paints	TROMETHAMINE	1
07E - Lipstick	TROMETHAMINE	1
07F - Makeup Bases	TROMETHAMINE	8
07I - Other Makeup Preparations	TROMETHAMINE	7
08C - Nail Creams and Lotions	TROMETHAMINE	1
10A - Bath Soaps and Detergents	TROMETHAMINE	8
10E - Other Personal Cleanliness Products	TROMETHAMINE	3
11A - Aftershave Lotion	TROMETHAMINE	11
11D - Preshave Lotions (all types)	TROMETHAMINE	1
11E - Shaving Cream	TROMETHAMINE	1
11G - Other Shaving Preparation Products	TROMETHAMINE	2
12A - Cleansing	TROMETHAMINE	41
12C - Face and Neck (exc shave)	TROMETHAMINE	71
12D - Body and Hand (exc shave)	TROMETHAMINE	67
12F - Moisturizing	TROMETHAMINE	172
12G - Night	TROMETHAMINE	25
12H - Paste Masks (mud packs)	TROMETHAMINE	7
12I - Skin Fresheners	TROMETHAMINE	1
12J - Other Skin Care Preps	TROMETHAMINE	19
13A - Suntan Gels, Creams, and Liquids	TROMETHAMINE	1
13B - Indoor Tanning Preparations	TROMETHAMINE	3
13C - Other Suntan Preparations	TROMETHAMINE	2
		549

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6

Final Report on the Safety Assessment of Aminomethylpropanol and Aminomethylpropanediol

AMP and AMPD are substituted aliphatic alcohols. AMP is used in cosmetic products at concentrations up to 10%, AMPD is used at concentrations up to 5%. AMP and AMPD when buffered, and orally administered, are practically nontoxic to rats and mice.[1]

In primary irritation studies, AMP and formulations containing AMP were, at most, minimally irritating to abraded and nonabraded rabbit skin. Cosmetic formulations containing AMPD were only minimally irritating to rabbit skin. AMP was not an intradermal sensitizer in guinea pigs. Cosmetic formulations containing AMPD and/or AMP were minimal to moderate ocular irritants.

AMP and AMPD were nonmutagenic, both with and without metabolic activation, in *Salmonella typhimurium* strains.

In clinical studies, AMP was neither a primary dermal irritant nor a contact sensitizer. AMPD was neither a primary irritant, fatiguing agent, nor sensitizer when tested in humans.

AMP and AMPD are highly alkaline in pure form, they are buffered in cosmetic formulations, and, therefore, the adverse reactions seen with the undiluted chemical would not be expected with the cosmetic product. The highest level of both AMP and AMPD for which test data were available was 1.0%, therefore the safe use of these two compounds should be limited to this test value. Neither ingredient should be used in cosmetic products containing nitrosating agents.

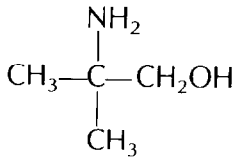
INTRODUCTION

The following report is a literature review on the chemistry, use, and toxicology of Aminomethylpropanol (AMP) and Aminomethylpropanediol (AMPD).

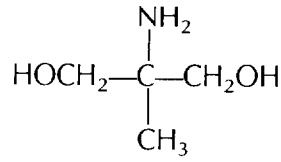
CHEMISTRY

Definition and Structure

Aminomethylpropanol (AMP) and Aminomethylpropanediol (AMPD) are substituted aliphatic alcohols with the following structures:[2]



AMP



AMPD

AMP (CAS No. 124-68-5) is also known as 2-amino-2-methyl-1-propanol[2] and isobutanolamine,[3] and commercially as AMP-Regular[2] and AMP-95 (a dilution of AMP-Regular, with 5% water),[4] while AMPD (CAS No. 115-69-5) is also known as 2-amino-2-methyl-1,3-propanediol,[2] aminobutylene glycol, and butanediolamine.[3]

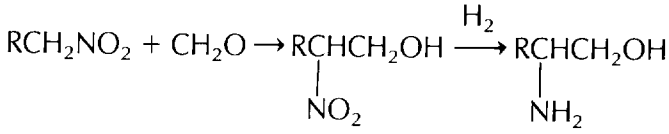
Properties

AMP is either a colorless liquid or a white crystalline solid,[3] though it may appear as a paste since its melting point is slightly above room temperature.[4] As a solid, AMP is odorless, while in liquid form it possesses a slight amine odor.[4] The molecular weight of AMP is 89.14, its melting range is 30–31°C and its boiling point is 165°C.[5] The flash point of AMP is 153°C.[6] At low temperatures, AMP has a low vapor pressure.[4] AMP has a density of 0.934 at 20°/20°C.[5] AMP is miscible with water, soluble in alcohols,[5] slightly soluble in aromatic hydrocarbons, and insoluble in aliphatic hydrocarbons.[4] The pH of a 0.1 M solution of AMP is 11.3.[3,5]

AMPD is either a white crystalline solid,[3] yellowish crystals,[7] or a clear liquid.[6] It has a molecular weight of 105.14, with melting and boiling ranges of 109–111°C and 151–152°C, respectively.[5] AMPD is soluble in water and alcohols,[5,7] and is insoluble in mineral oil.[7] The pH of a 0.1 M solution of AMPD is 10.8.[3,5]

Method of Manufacture

Both AMP and AMPD may be made by the condensation of the corresponding nitroparaffins with formaldehyde. After condensation, reduction is carried out to the β-aminoalkanols:[4]



The desired end product may then be isolated by distillation.[4]

Analytical Methods

Infrared (IR), nuclear magnetic resonance (NMR), and mass spectra (MS) have been published for AMP.[8] The IR spectrum of commercial AMP closely matches the standard spectrum, with no evidence of foreign materials.[9] For AMPD, the infrared spectrum has been published.[8] Alkanolamines, both AMP and AMPD, can be determined in hair spray formulations, after acetylation, by gas-liquid chromatography.[10]

Impurities

No definitive report on impurities, including *N*-nitrosamines, was available.

Chemical Reactions

AMP can react with copper, brass, and aluminum, but will not react with steel or iron. With mineral acids, AMP can form ammonium salts; these salts are easily hydrolyzed by water, and will dissociate upon heating. Soaps containing AMP are important industrially as emulsifying agents.[4]

Aminohydroxy compounds react with the methyl ester of an organic acid in the presence of an alkaline catalyst, and at low temperatures and pressures to form amides. When this reaction is carried out at higher temperatures, oxazolines are produced. The aminohydroxy compounds react with acid anhydrides to form imides. The substituted ethyleneimine is formed by the reaction of AMP with excess mineral acids at temperatures above 75°C, followed by reaction with a caustic agent. Oxazolidines are formed by reaction of the aminohydroxy compounds with aldehydes.[11]

USE

Cosmetic

Both AMP and AMPD are listed in the *Merck Index*[5] as emulsifying agents for cosmetic creams and lotions and, according to the *CTFA Cosmetic Ingredient Handbook*,[12] they are used as pH adjusters. In hair sprays, they are used as neutralizing agents to regulate the solubility, flexibility, and tackiness of the resin, usually in amounts of 2–15% of the resin.[10] They are used in gelling products where a polymer is required to function as a thickener, and they may also be used to form soaps in waterless hand cleaners, liquid hand soaps, shampoos, shave creams, and other creams and lotions.[13]

Data submitted to the Food and Drug Administration (FDA) in 1987 by cosmetic firms participating in the voluntary registration program indicated that AMP was used in a total of 161 cosmetic products (Table 1). Product types containing AMP included hair sprays, wave sets, other noncoloring hair preparations, hair dyes and colors (requiring a caution statement and patch test), face preparations, and eye area products, skin care preparations, and miscellaneous products. AMP was listed in a total of seven categories, with the greatest use in the categories of aerosol fixative hair sprays (83 products) and other hair preparations (38 products). AMP is used in concentrations ranging from ≤0.1% to >5–10% (3 products).[14]

AMPD was used in a total of 18 products (Table 2). Product types containing AMPD included aerosol fixative hair sprays, wave sets, and other noncoloring hair preparations, and makeup preparations including eye area makeup; a total of three product categories. The greatest use of AMPD was in aerosol fixative hair sprays (10 products). AMPD was used in concentrations of ≤0.1% (7 products), >0.1–1% (9 products), and >1–5% (2 products).[14]

The FDA cosmetic product formulation computer printout[15] is compiled through voluntary filing of such data in accordance with Title 21 part 720.4 of the Code of Federal Regulations.[16] 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

TABLE 1. Product Formulation Data for Aminomethyl Propanol[14]

Product category	Total no. of formulations in category	Total no. containing ingredient	No. of product formulations within each concentration range (%)				
			>5–10	>1–5	≥1	>0.1–1	≤0.1
Hair sprays (aerosol fixatives)	174	83	—	2[a]	—	71	10
Wave sets	155	16	—	1[a]	—	14	1
Other hair preparations (noncoloring)	1185	38	—	1[a]	—	34	3
Hair dyes and colors (all types requiring caution statement and patch test)	915	6	3[a]	3[a]	—	—	—
Face preparations and eye area products	1013	5	—	—	5	—	—
Skin care preparations	1896	6	—	—	—	5	1
Miscellaneous products	291	7	—	—	7	—	—
1987 Totals		161	3	7	12	124	15

[a]According to voluntarily registered formulations, all uses above 1% involve neutralization of AMP with fatty acids of acidic polymer resins. Other uses also likely to involve neutralization.

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 provides 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 two- to tenfold error in the assumed ingredient concentration.

Products containing AMP or AMPD may come into contact with the skin, eyes, and mucous membranes. Either ingredient may be inhaled when an aerosol hair spray is used. Contact with the ingredient may be temporary or prolonged. Products containing either ingredient may be used repeatedly over a period of time.

AMP and AMPD are unlikely to exist as the free bases in cosmetic products, but rather as salts as the result of neutralization of acidic components of the cosmetic formulation.

TABLE 2. Product Formulation Data for Aminomethyl Propanediol[14]

Product category	Total no. of formulations in category	Total no. containing ingredient	No. of product formulations within each concentration range (%)		
			>1–5	>0.1–1	≤0.1
Hair sprays (aerosol fixatives)	265	10	—	4	6
Wave sets and other hair preparations (noncoloring)	337	5	—	4	1
Makeup preparations (including eye)	550	3	2[a]	1	—
1987 Totals		18	2	9	7

[a]According to voluntarily registered formulations, AMPD neutralized by reacting with excess of fatty acids. Other uses also likely to involve neutralization.

Noncosmetic

In industry, AMP and AMPD have a variety of uses. They are used in the synthesis of surface-active agents, vulcanization accelerators, and pharmaceuticals. They are also used as emulsifying agents in leather dressings, cleaning compounds and polishes, and as absorbents for acidic gases.[5] AMPD may be used to stabilize emulsions, though stability depends on the concentration of AMPD, the length of storage, and temperature.[17]

AMP also is used as an emulsifying agent in insecticides. Small amounts of AMP are used as pigment dispersers in water-based paints; in paints, AMP helps to stabilize pH, viscosity, and odor of the product. The salts of AMP and acidic polymers are used to make resins water soluble, and AMP salts are also used as catalysts for textile resins, coating resins, and adhesives. In boiler water, AMP provides protection for copper and steel, and absorbs CO_2 efficiently.[4] In cutting fluids, AMP is useful as an antimicrobial agent, especially against molds.[18] AMP is also listed as an indirect food additive for use, without restrictions, as a component of adhesives.[16]

GENERAL BIOLOGY

Biochemical Effects

In rats which were fed a choline-deficient diet, oxidative phosphorylation in the rat-liver mitochondria was uncoupled.[19] The explanation for the incidence of uncoupling was an increase in free fatty acids in the liver. When AMP was injected intraperitoneally in rats fed a choline-deficient diet, the uncoupling effect was reversed. The authors concluded that this reversal was due to the interference of AMP with the formation of free fatty acids from lipids. It was noted that the addition of AMP increased the fat content of the liver above that of rats fed a choline-deficient diet, suggesting that while choline deficiency inhibited "fat removal," AMP inhibited fat catabolism, resulting in an increase in the fat content of the liver. AMP appeared to interfere not only with lipid catabolism, but also with choline utilization and synthesis. The authors also cited evidence that AMP, or a metabolite of AMP, might become incorporated into phospholipids, and concluded that this may be the mechanism of action of AMP.

In another study in which rats were fed a choline-deficient diet, AMP (incorporated into the diet in micromolar amounts) increased the incidence of hemorrhagic kidneys and the amount of fat in the liver; the renal damage was dose dependent.[20] Ocular hemorrhages were noted in rats consuming the higher doses of AMP (actual amount not stated). Also at higher AMP doses, the amount of hepatic fat decreased; this was associated with a corresponding decrease in daily feed intake and body weight gain. The authors interpreted their results in light of evidence from other studies that indicated that AMP inhibited the incorporation of ethanolamine and dimethylethanolamine into rat hepatic phospholipids, and that because AMP was incorporated into the phospholipids, the amount of ethanolamine in the phospholipids available for conversion into choline was decreased. The authors also cited research that indicated that the *N*-methyl derivatives of ethanolamine could reduce the "antilipotropic effects" of AMP, with betaine, methionine, and ethanol having a similar, but weaker, effect.

Yue et al.[21] reported that the effect of aminoalcohols on choline uptake by mitochondria was relatively small compared to the effect on phosphate uptake. In

addition, the aminoalcohols had only a small effect on O_2 consumption, suggesting that mitochondrial membrane permeability changes were not the principal mechanism of inhibition by the aminoalcohols.

Bridges and Ricketts[22] stated that the degree to which aminoalcohols were incorporated into the phospholipids corresponded to the degree of similarity between the aminoalcohol and ethanolamine. For the most part, the aminoalcohols were incorporated into the phospholipids unchanged. The mechanism of incorporation was not clear; the authors postulated that either calcium-mediated direct exchange (which is known to occur in vertebrates) or a magnesium-dependent pathway described by Kennedy and Weiss,[23] or a balance between the two, may be responsible for the incorporation and final distribution of unnatural aminoalcohols in phospholipids.

DiPrisco and Strecker[24] found that AMPD hydrochloride, like phosphate, could change the inhibitory effects of thyroxine and other compounds from competitive to noncompetitive with respect to crystalline beef liver glutamate dehydrogenase.

In an *in vitro* study of the incorporation of [^{32}P]phosphate into the phospholipids of swine coronary and pulmonary arteries, Morin[25] found that AMP at a concentration of 0.1 mmol/ml caused an almost complete inhibition of the incorporation of [^{32}P] into all phospholipids studied. Inhibition was dose dependent. The inhibition by AMP of phosphatidylcholine was greater than for the other phospholipids studied, and inhibition of the incorporation of [^{32}P] into the phospholipids was more pronounced in the coronary arteries than in the pulmonary arteries at all AMP concentrations studied. The addition of choline in excess of AMP caused partial to complete reversal of the inhibition. In addition to inhibiting the net synthesis of phosphatidylcholine, AMP also inhibited, though to a lesser extent, the synthesis of sphingomyelin, phosphatidylserine, and phosphatidylethanolamine.

AMP also inhibited the incorporation of [^{32}P]phosphate into the phospholipids of rabbit and human endometrial tissues *in vitro*.[26] The results obtained in this study were similar to those obtained with the coronary and pulmonary arteries of swine.

In a study of plasma membranes of murine fibroblasts, Schroeder[27] found that ethanolamine analogues, including AMP, caused alterations in the morphology of the plasma membrane, as well as alterations in thymidine transport and hormone-stimulated adenylate cyclase activity. The analogs did not simply replace the phosphatidylethanolamine; either certain types of phosphatidylethanolamine were replaced or their asymmetric distribution was changed. The conclusion was that the ethanolamine analogs created "a more symmetric distribution of acyl chains in aminophospholipids across the surface membrane bilayer" than did choline analogs. The ethanolamine analogs increased the percent aminophospholipids found in the outer monolayer without increasing their total amount; the number of negatively charged phospholipids in the outer monolayer was also increased. The conclusion reached by the author was that "choline and ethanolamine analogs may alter or regulate the aminophospholipid asymmetry of LM [choline-requiring mouse fibroblast] cell plasma membranes."[27]

D-Serine-induced renal tubular necrosis in rats was studied by Kaltenbach et al.[28] Compounds which were structurally related to D-serine were reviewed to analyze a possible mechanism of action of D-serine on the rat kidney. Among the compounds tested were AMP and AMPD. Neither were nephrotoxic; in fact, AMP reversed the toxic effects induced by 2-amino-1-propanol (this compound's toxic effects were inconsistent).

Kaltenbach et al.[29] while reviewing the protective effects of various chemicals against D-serine- and D-2,3-diaminopropionic acid-induced renal tubular necrosis in rats, found that AMP did not have a protective effect against either of these compounds.

In a study on the effects of lysosomotropic amines on protein degradation and synthesis in rat hepatocytes,[30] it was found that both AMP and AMPD inhibited degradation and synthesis, although inhibition of synthesis could be somewhat reversed with the addition of amino acids and pyruvate. This latter observation suggested that the apparent inhibition of protein synthesis by these compounds was a result of their inhibitory effects on protein degradation.

Distribution and Excretion

Yue et al.[31] studied the fate of [^{3}H]AMP when injected intraperitoneally in young male Sprague-Dawley rats on choline-adequate and choline-deficient diets. The rats receiving the choline-deficient diets started the diet 24 h before the injection of [^{3}H]AMP, and continued on this diet until they were sacrificed at 30 min, 1, 2, 3, 6, 24, or 96 h postinjection. The rats fed the choline-adequate diet (*ad libitum*) followed the same protocol. Thirty minutes after the i.p. injection, tritium appeared in the serum, with radioactivity disappearing shortly after its initial uptake. Radioactivity in the serum was consistently lower in the rats fed the choline-deficient diet, with the exception of the 6-h value, at which time the activity was approximately equal for both dietary groups.

Radioactivity in the urine followed the same pattern as that in the serum, with the rats on the choline-adequate diet excreting a greater amount of radioactivity in their urine than the rats on the choline-deficient diet. Paper chromatography results suggested that the radioactivity in the urine was the [^{3}H]AMP, which had been excreted unchanged, as indicated by samples of [^{3}H]AMP which were chromatographed concurrently.

From 0.5–6 h, the uptake of radioactivity by the brain, skeletal muscle, heart muscle, intestine, and spleen was greater in the rats fed the choline-adequate diet. At 6 h, this trend was completely reversed and remained so until the end of the study. In the liver, uptake of radioactivity was greater in the choline-deficient group throughout the study. By 96 h, the radioactivity in the liver of both groups had decreased considerably, but that in the liver of the choline-deficient group remained higher than that in the choline-adequate diet group.

The distribution of the radioactive AMP in the phospholipids of the liver was also examined. At 0.5 h, the amount of free radioactive AMP in hepatic mitochondria from rats fed the choline-adequate diet was approximately 72%, the remaining 28% being incorporated into phospholipids. In the choline deficient rats, about 29% of the AMP was free, the remaining 71% was present in the phospholipids. This same trend was seen in hepatic microsomes, with the exception that a greater amount of the AMP (81%) in rats fed the choline-deficient diet was incorporated into phospholipids. There was no indication that the AMP had been phosphorylated. At all times, the livers of the choline-deficient rats had a higher amount of tritium in all subcellular fractions. The cytosol of both the liver and kidneys contained the most tritium. In the choline-adequate diet rats, the radioactivity in the kidneys and liver decreased with time while the opposite was true for rats fed the choline-deficient diet; radioactivity increased in the hepatic subcellular fractions and remained constant in the renal subcellular

fractions. In the choline-deficient rats, this change was especially striking in the liver microsomal fraction. The authors also noted that the radioactivity found in the cytosol was not free AMP since no free AMP was identified after 30 min, and that the radioactive AMP was redistributed among several phospholipid fractions. This latter observation indicated that incorporation of AMP into phospholipids may occur with other derivative forms of AMP than just with the phosphatidyl derivative.

ANIMAL TOXICOLOGY

Acute Toxicity

Oral

A reported approximate LD_{50} for AMPD in the deer mouse was 0.140 g/kg,[32] and in albino mice all animals survived a dose of 5.0 g/kg.[13] For fasted young adult male rats, the LD_{50} of AMP was 2.90 ± 0.14 g/kg.[33] The oral LD_{50} values in Cox strain albino mice for both AMP and AMP-95 were estimated at 2.15 ± 0.2 g/kg and 2.4 ± 0.089 g/kg, respectively.[34]

The acute toxicity of AMP in both rats and monkeys was studied.[35] Four groups of ten Long-Evans rats, with equal numbers of each sex, were fed a diet of lab chow and water *ad libitum*. The rats received AMP by gavage daily for 5 days at the following doses: 0, 0.5, 1.0, and 2.5 g/kg. All rats were observed for 15 days, at which time all survivors were necropsied. At the 1.0 g/kg dose, two of the five female test animals died, one on day 6 and one on day 11. At the 2.5 g/kg dose, none of the rats survived beyond the third day of feeding. In a more detailed report on this same study, it was noted that the liver and kidneys of the test animals were examined both grossly and microscopically, while the lungs were examined only for gross changes.[36] None of the changes noted were attributed to the treatment with AMP; all changes were indicative of spontaneous diseases of rats, and these changes occurred equally among control and test animals.

Two rhesus monkeys were allowed to acclimate to the laboratory for 1 week, during which time baseline values for clinical chemistry, hematology, and urinalysis were established.[35] One monkey received an AMP dose of 0.5 g/kg in distilled water and the second monkey received an AMP dose of 1.0 g/kg. Dosing continued daily for 5 days or until the high-dose monkey died. The surviving monkey was observed for a total of 14 days and was sacrificed and necropsied on the 15th day. The high-dose monkey died 2 h after the third dose of AMP. The cause of death was gastrointestinal hemorrhage. The liver appeared normal. Both monkeys lost weight during the study. The low-dose monkey had no significant hematologic changes, except for a slight increase in the while blood cell count. The low-dose monkey had significant changes in some of the clinical chemistry values: calcium decreased from 9.8 to 3.0 mg/dl; blood urea nitrogen (BUN) and creatinine were increased, and the activities of serum glutamate pyruvate transaminase (SGPT), creatine phosphokinase (CPK), and ornithine carbamyl transferase (OCT) were all increased. The toxic effects of AMP were attributed to its effects on the gastrointestinal tract, and this could be due to the alkalinity of the AMP solutions (pH > 11).

An acute toxicity study of a hair spray containing 0.25% AMP was performed using ten albino rats, five of each sex.[37] After fasting overnight, the animals received a dose

by gavage of 5.0 ml/kg of the undiluted hair spray, and then were observed for 14 days thereafter, during which they were allowed feed and water *ad libitum*. Most of the rats had either slightly decreased activity or decreased activity up to 3 h after administration of the test material, and all appeared normal from the 6 h point until the end of the study. All of the survivors gained weight during the study. At necropsy, no abnormalities were observed in the survivors or in the rat which died during the study.

Another study following the same protocol was performed with a hair spray containing 0.58% AMP.[38] All rats survived the 14-day observation period. The rats had severely decreased activity an hour after administration of the test material. Their activity remained decreased at a lessened intensity through the 6-h observation point, and then returned to normal for the remainder of the study. All animals gained weight during the study, and no gross abnormalities were noted at necropsy.

In another study following the same protocol, a hair spray containing 0.59% AMP was tested for oral toxicity in albino rats.[39] All of the rats died before the end of the study, seven of the ten rats on or before day 2. All rats had some degree of decreased activity for the first 24 h. Three rats which survived through day 2 appeared normal on the second day, but had recurring slightly decreased activity on day 3. These three rats all died within the first week. The following observations were noted at necropsy: the three rats which died within 1 h had severely reddened pyloric mucosae, the two rats which died at 24 h had moderately reddened pyloric and duodenal mucosae, the two rats which died on day 2 had severely reddened pyloric and duodenal mucosae, the rat which died on day 5 had necrosis of the pyloric mucosa, the rat which died on day 6 had consolidation of the superior and inferior lobes of the right lung, and the rat which died at 1 week had moderately reddened pyloric and duodenal mucosa and gas-filled stomach and intestines. The hair spray containing 0.59% AMP was toxic to rats by the oral route under the conditions of the test.

A fourth test following the same protocol was performed with three cosmetic formulations containing 0.58, 0.59, and 0.58% AMP, respectively.[40] No animals in the three test groups died during the study, and all rats gained weight. All of the test animals had varying degrees of decreased activity; in no case did the decreased activity last beyond 24 h. No gross abnormalities were observed at necropsy, and the three formulations containing 0.58, 0.59, and 0.58% AMP were not toxic to rats by the oral route under the conditions of the study.

An aerosol spray containing 0.40% AMPD was tested for acute oral toxicity using Charles River albino rats.[41] The rats were divided into groups of four, equally divided by sex; there were four dosage groups. The rats received the test material undiluted at the following doses: 10.2, 15.4, 23.1, and 34.6 g/kg. The pH of the test material was 8.7. The animals were observed for 14 days following the administration of the test material, at which time all surviving animals were sacrificed and necropsied (animals that died during the study were also necropsied). None of the rats of the low-dose group, and one rat of the 15.4 g/kg group, died during the study. All rats in the two high-dose groups died, those in the 23.1 g/kg group within the first week, and those in the high-dose group from 45 min to 3 h after administration of the test material. The 7- and 14-day LD_{50} doses were both 17.0 ± 1.7 g/kg.

Inhalation

A group of ten Wistar rats, equally divided by sex, was exposed for 1 h to an atmosphere containing 200 mg/L of a hair spray containing AMP at a concentration of

0.59%.[42] The test animals were observed for 2 weeks following the exposure. All but one rat survived the duration of the study, all survivors gained weight during the study, and all, including the rat which died, appeared normal during the observation period. At necropsy, the left lung of the rat which died (day 3) was adhered to the dorsolateral body wall; none of the other rats had any abnormalities.

In a second study following the above protocol, three cosmetic formulations containing 0.58, 0.59, and 0.58% AMP, respectively (groups 1, 2, and 3), were tested.[43] All rats survived the 2-week observation period, and all but one gained weight (one rat maintained a steady weight). The rats of the first two groups appeared normal throughout the observation period, while those of the third group had slightly decreased activity at hour 1 and were normal thereafter. The only abnormality noted upon necropsy was in one rat of group 2; all lobes of the right lung were consolidated and had adhered to the ventral body wall. The formulations containing 0.58, 0.59, and 0.58% AMP were not toxic by inhalation to rats under the conditions of the study.

A group of 20 Sprague-Dawley rats, ten of each sex, were exposed for 1 h to an atmosphere containing 168.2 mg/L of a spray containing AMP at a concentration of 0.26%.[44] Except for the hour during which they were exposed to the test material, the rats were allowed feed and water *ad libitum*. After exposure to the test material, the rats were sponged off, dried, and placed in clean cages. The rats were observed during the exposure, and half of the rats of each sex were sacrificed 24 h later, and the remainder of the test animals were observed for 14 days.

During exposure to the test material all rats had decreased activity, and had labored respiration, squinting, and ataxia. The decreased activity, labored/slow respiration, and squinting continued after the exposure, and in addition, the rats had depressed righting and placement reflexes. One female rat had tremors and prostration upon removal from the test chamber, and another female also had intermittent tremors. All rats, with the exception of one male rat with a slight nasal discharge, appeared normal at 24 h. One male rat was wheezing on days 2, 3, and 14, and a second male rat appeared depressed on days 4 and 5. All of the remaining rats appeared normal through the remainder of the observation period. One female rat in the control group was wheezing on days 13 and 14; all other control rats appeared normal.

There were no differences in weights and weight gains between the control and test animals. The kidney weight and kidney/body weight ratio were significantly higher for the treated rats. No treatment-related lesions were observed at necropsy. One hour of exposure to an atmosphere containing 168.2 mg/L of a spray containing 0.26% AMP caused no significant histopathological changes in rats.

No deaths occurred when rats were exposed for 1 h to atmospheres containing 200 mg/L of an aerosol containing AMP at concentrations of 0.25% or 2.5% in alcohol and propellant.[34]

An acute inhalation toxicity study of a hair spray containing 0.50% AMPD was performed using ten male Sprague-Dawley rats.[45] The rats were exposed for 1 h to an aerosol atmosphere containing approximately 200 mg/L of the hair spray formulation. The animals were observed during exposure and for 14 days thereafter. The rats were weighed before the study and on days 7 and 14. At the end of the study, the rats were necropsied; the trachea, lungs, liver, and kidneys were examined microscopically. All rats survived the duration of the study; body weights and weight gains were normal. The animals had no pharmacotoxic signs during or after exposure to the test material. There was no evidence of toxicity with respect to organ weights and gross lesions. The results of the microscopic study was unavailable.

Short-Term Toxicity

Oral

Eight beagle dogs were used in a study of the toxic effects of AMP over a 28-day period.[46] AMP was administered in the diet at concentrations of 600, 1800, 5400, and 16,200 ppm to two dogs, one of each sex for each dose. Once weekly the dogs were weighed and feed consumption was recorded. Hematologic studies and urinalyses were performed once before the administration of AMP and at week 4 during the study. Both of the dogs of the 1800 and 16,200 ppm groups, as well as the female dog of the 5400 ppm group, had frequent soft stools or diarrhea. Both dogs of the highest dose group had marked weight loss and anorexia, and at week 2, both had dry noses and mouths. The male dog of the 5400 ppm group had similar but less severe reactions. All dogs survived the duration of feeding.

Results of the urinalyses were normal throughout the study. The hematologic changes 4 weeks into the study included elevated hemoglobin, packed cell volume, and erythrocyte count for the female high-dose dog. The male dogs of the 5400 and 16,200 ppm groups had slight neutropenia. For all dogs, except those of the 600 ppm group, SGPT and alkaline phosphatase activities were moderately to markedly increased; and for the dogs of the 5400 and 16,200 ppm groups serum glutamic oxaloacetic transaminase (SGOT) activity was slightly to moderately increased.

No gross lesions due to the AMP were found at necropsy. Microscopic lesions in the liver included hepatocytic vacuolation, necrosis of hepatocytes, pigment deposits, centrolobular inflammatory infiltrate, and fibrosis and atrophy of centrolobular parenchymal tissue; this was observed in all dogs except the male exposed to 600 ppm. The damage to the liver, as well as a decrease in liver weight, was dose dependent.

In another study, AMP was fed in the diet to Charles River CD-1 mice for 8 weeks.[47] Concentrations were 200, 400, 800, 1600, and 3200 ppm; 10 mice of each sex were in each diet group. A control group was also included. The mice were observed daily, and weights and feed consumption were recorded weekly. At the end of the study, all mice appeared normal. No gross or microscopic lesions were found in the liver of the test animals (all of the 3200 ppm mice and 4 mice from each other dosage group were examined).

A similar study was undertaken with Charles River CD rats.[48] The test protocol was the same as in the mouse study[47] except that the dietary concentrations were 1000, 2000, 4000, 8000, and 16,000 ppm. The rats of the 16,000 ppm group were emaciated, and had rough hair coats, small skin lesions, and loss of hair. Two female rats of the highest dose group died before the end of the study. Alopecia and focal skin erosions were observed in the rats of the 16,000 ppm group, and these were considered compound-induced. Microscopically, hepatocyte vacuolation was noted in rats of all dose groups (all rats of 16,000 and 8000 ppm groups and 4 from each other dose group were examined), and this change was considered compound-induced.

Inhalation

An inhalation study was performed with a hair spray containing AMP at a concentration of 0.58%.[49] A group of 16 Wistar rats, eight of each sex, was exposed to an atmosphere containing 200 mg/L of the hair spray for 1 h/day, 5 days per week for 2 weeks. Four rats were sacrificed at the end of the first week, another four at the end of the second week, and the remainder after a one-week recovery period. All rats were examined for gross pathological changes, and the respiratory tissues were preserved for

possible microscopic examination. None of the rats died as a result of exposure to the test material. All rats had slightly decreased activity 1 h after exposure, had returned to normal by 3 h, and once again had slightly decreased activity at 24 h. The rats continued to have slightly decreased activity until day 14, at which time the rats which were to undergo a 1 week recovery period all appeared normal. No gross changes were noted at necropsy, and weight gains were comparable between the test animals and the control group.

Subchronic Toxicity

Oral

In a 90-day study, AMP solutions, at pH's of 11+ or 7, were administered to rats by stomach tube.[50] At each pH, the AMP solution was administered at doses of 0.5, 0.75, 1.1, or 1.7 g/kg/day; the dosage groups consisted of 20 rats, divided equally by sex. The rats were observed daily, and body weights and feed consumption were recorded weekly. All rats that died during the study were necropsied, and those that survived to the end of the study were sacrificed and necropsied after samples were taken for hematologic, urologic, and clinical chemistry measurements. Results of the study indicated that mortality caused by AMP was due to the AMP solutions with a pH of 11 or greater. The noted behavior changes were hyperventilation, hyperirritability, and hyperactivity; these were most often noted in the pH 11+ group. All surviving rats gained weight and consumed feed in a normal manner, though the test rats did appear to drink more water. In the pH 11+ group rats, packed cell volumes, hemoglobin values, and erythrocyte counts were markedly decreased for the males of the 1.1 g/kg dose group; this was due to blood loss by these rats. The pH 11+ group rats receiving doses of 0.5 and 0.75 g/kg also had slight, though significant, decreases in erythrocyte counts. In the pH 7 group rats, some occurrences of increased SGPT and OCT activities were noted, and the males of the 1.7 g/kg group had significant decreases in packed cell volume and hemoglobin. Urinalyses were performed only on the rats from the pH 11+ group; some samples contained protein. No gross lesions were found at necropsy.

Tissues were taken from the rats of the 90-day study and from control rats of both pH groups and from nine male and eight female rats of the pH 7, 1.7 g/kg group and from one female of the pH 11+, 1.7 g/kg group.[51] The 1.7 g/kg oral dose of an AMP solution at a pH of 7 did not cause any significant changes in male or female rats "under the conditions of the experiment." In the tissues of the pH 11+, 1.7 g/kg group female rat, the only abnormality noted was a few papillary protrusions of epithelium at the junction between the squamous and glandular portions of the stomach.

For three months, groups of four male and four female beagle dogs were fed diets containing 0.63, 15.0, or 62.5 mg AMP/kg.[52] The AMP was used as AMP-hydrochloride, pH 7.0. The physical conditions and feed consumptions of the dogs were monitored, and urinalyses, hematology, and clinical chemistries were obtained at the start of the study and at 1 and 3 months. At the end of the study, some tissues were examined microscopically (not indicated in this limited report). Except for the high-dose group, body weight gains were normal during the study. The high-dose group also had increased activities of SGOT, SGPT, and alkaline phosphatase at months 1 and 3. The liver weights and liver/body weight ratios were slightly higher in the dogs of the high- and mid-dose groups at necropsy. In addition, two females and one male of the high-dose group had tan and mottled livers. At microscopic examination, vacuoliza-

tion, lipid deposits, and bile duct hyperplasia were found in the livers of all of the high-dose dogs and in one of the mid-dose dogs. No other organs appeared to be affected. Results of the clinical chemistry of the dogs in the mid-dose groups did not correlate with the histopathological findings for those groups. No other comments were made about the effects in dogs of 90 days of dietary consumption of AMP.

Inhalation

In a 13-week inhalation study, a group of CD-Crl: CS(SD)BR Charles River albino rats, 11 of each sex, was exposed to an aerosolized form of a pump hair spray containing 0.44% AMP for 4 h/day, 5 days/week for a total of 67 exposures.[53] The exposure concentration was 0.23 mg/m^3, an amount which was calculated to be a 100-fold increase over normal human exposure. The animals were observed daily, weighed weekly, and blood and urine samples were obtained on weeks 7 and 13. The animals were sacrificed after the 67th exposure, gross observations were made, and various tissues and organs were removed for weighing and microscopic study.

All animals survived the duration of the study, and none had an intolerance for the aerosol atmosphere. There was a significant decrease in body weight gains for female rats during weeks 1–3, but this was considered within normal limits for the species in this laboratory.

Statistically significant hematologic changes included increased packed cell volume and erythrocyte counts for males at weeks 7 and 13, increased hemoglobin values for males at week 7, and increased packed cell volume for females at week 7. Though these differences were significant with respect to the controls, they were still within the normal range established by the laboratory for the strain of rat used.

Male rats had a statistically significant increase in serum glucose at week 7, and females had a significant decrease in BUN at week 13. These differences were not considered toxicologically significant when included with the other study criteria.

No abnormalities were noted in the results of the urinalyses, and no lesions were found at necropsy. Female rats had a significant decrease in uterine and lung weights; there were also significant increases in heart- and liver-to-body weight ratios for the females.

No treatment-related microscopic changes were found in the evaluated tissues; frequency and severity of noted changes were equivalent for both the treated and control rats. The microscopic changes observed in the lungs and upper respiratory tract of both the treated and control rats were attributed to chronic murine pneumonia, and were unrelated to treatment. The pump hair spray formulation containing 0.44% AMP was safe under the exaggerated inhalation conditions of the test.

A 90-day inhalation toxicity study of two pump sprays, each containing 0.40% AMP, was performed using cynomolgus monkeys.[54] The test animals were divided into groups consisting of three males and six females each. One group (group 2) was exposed to the test material under static conditions by automatic dispensation of one pump sprayer every 7.5 s/10 min period/day, for a total of 800 sprays/day. The monkeys of group 3 were exposed to the test material following the same spraying regimen but under dynamic conditions (in an air flow of 622 L/min) for the first 25 days, followed by static exposure for the remaining 64 days. The other two groups of monkeys were the control group and a group exposed to a different test material. The monkeys were fed after the daily exposure, and water was available *ad libitum*. All monkeys had negative reactions to a tuberculosis test and had clear chest x-rays prior to the start of the study.

The monkeys were weighed prior to the start of the study and weekly thereafter. They were observed daily during and after exposure for signs of behavioral abnormalities or toxicity. Prior to the start of the study and after the 89 days of the study, the following respiratory function parameters were assessed: distribution of ventilation, diffusion capacity, mechanics of respiration, mid-maximum expiratory flow, and spontaneous anesthetized tidal volume and respiratory rate. These tests were accomplished by anesthetizing the monkeys and placing them on a whole-body respirator. Hematology and clinical chemistry values were performed on blood samples from each monkey prior to the start of the study, and at 30 and 89 days. After 89 days of exposure, the monkeys were necropsied; organ weights were obtained and various organs were preserved for microscopic examination.

The monkeys of group 2 were exposed to a mean gravimetric concentration of 6.63 ± 1.50 μg/L, and the monkeys of group 3 were exposed to a mean gravimetric concentration of 6.06 ± 1.99 μg/L during the study. All animals survived the study and no exposure-related clinical signs were noted. Only the monkeys in group 3 failed to gain weight during the study (body weights were slightly but significantly lower for weeks 3–12). Monkeys in group 3 required a significantly higher number of breaths and cumulative tidal volume to washout to 2% nitrogen and had a significantly higher pulmonary flow resistance. These data "did not indicate any increase in these parameters in Group 3, merely lesser decreases between pre- and postexposure as compared to Group1 [controls]." There were no significant hematological differences noted. The test animals had lower BUN values and higher SGPT activities than the controls at both week 4 and week 13. These differences were not considered significant, since the values were still within the normal range for the species and because there was no microscopic evidence of damage to the affected organs. An increase in serum CO_2 was noted for all test groups, but since there was no evidence of hyperventilation, the cause was believed to be due to ingestion of the acidic resin, causing a nonrespiratory acidosis. Group 3 monkeys had an increased liver/body weight ratio which resulted from a higher mean liver weight coupled with lower average body weights. No compound-related alterations were found upon histopathological evaluation of the tissues in the monkeys of groups 2 and 3. Though the monkeys in group 3 did not gain weight during the study and though both groups 2 and 3 had lower serum CO_2 levels, no other compound-related adverse effects were evident after 89 days of exposure to atmospheres containing either 6.06 or 6.63 μg/L of hair spray containing 0.40% AMP.

In another 90-day study, groups of eight cynomolgus monkeys, divided equally by sex, were exposed for 1 h per day to a hair spray formulation containing 0.21% AMP.[55] Groups were exposed to high and low concentrations of the hair spray, as well as of the vehicle control. In addition, there was a room air control group. The 90-day high and low mean values for the hair spray concentrations were 27.0 ± 3.1 μg/L and 2.73 ± 0.56 μg/L, respectively. No treatment-related effects were noted in body weights, weight gains, organ weights, organ/body weight ratios, organ/brain weight ratios, hematology, clinical chemistry, neurologic and ophthalmic parameters, or at necropsy. Histopathologic examination of the pulmonary tissues indicated increased numbers of free macrophages and macrophage aggregates in the alveolar spaces, as well as foci of interstitially located particle-laden alveolar macrophages. No signs of inflammation or interstitial fibrosis were evident. In addition, pulmonary alveolitis was noted in the high-dose hair spray group, and a slight to moderate increase in hepatocellular lipid was noted in all test animals.

A 13-week inhalation toxicity study in female Chr/CD Charles River albino rats and in female outbred Syrian golden hamsters was performed with two hair spray formulations containing 0.1350% AMPD.[56] One hair spray formulation also contained 3.00% ethylene maleic anhydride copolymer, 50%; this formulation was referred to as the hair spray, while the second formulation, without the resin, was labelled the hair spray vehicle.

Dosage groups consisted of 16 animals of each species. All animals were allowed feed and water *ad libitum*. The following concentrations were used: 10 mg/m^3 hair spray, 100 mg/m^3 hair spray, 100 mg/m^3 vehicle, and controls. Animals were exposed to the formulations 4 h/day, 5 days/week, for 13 weeks. The aerosol concentrations in the inhalation chambers were monitored hourly and adjusted as necessary; the temperature, pressure, and humidity were also closely monitored. After 32 exposure days, five animals of each species of each group were sacrificed. The remaining test animals were sacrificed starting 3 days after the last day of exposure. Blood analyses was performed on all of the test animals. Gross and microscopic examinations were also performed.

During the study, five animals either died or were sacrificed when moribund (one rat and one hamster of the low-dose hair spray group, one hamster of the high-dose hair spray group, and one animal of each species of the vehicle group). None of the deaths were the result of the aerosol treatment.

Exposure of the test animals to the aerosols was well tolerated; other than the reactions listed below, no adverse reactions were observed. The low- and high-dose hair spray group hamsters had a decreased body weight gain; these values were statistically significant for the hamsters of the high-dose group. The high-dose hair spray hamsters also had lower, but not statistically significant, body weights at the end of the study.

There were scattered incidences of statistically significant differences in various parameters of the hematology and clinical chemistry, but no dose- or exposure-dependent trends were noted and, thus, these differences were not considered toxicologically significant. The same was true for the gross observations made at necropsy.

The organ weights and histopathological findings did not include any comments on the animals exposed to the hair spray vehicle. Exposure of Chr/CD Charles River rats and Syrian golden hamsters to atmospheres containing 144 mg/m^3 of a hair spray vehicle containing AMPD at a concentration of 0.1350% was not harmful.

Dermal Irritation and Sensitization

Irritation

A group of six rabbits was tested for primary skin irritation to AMP at a concentration of 0.25% in ethanol.[57] The test was a single insult, occlusive patch test modified to include abraded and nonabraded skin. The rabbits' skin was graded for erythema and edema 24 and 72 h after patch removal. Neither the abraded nor the nonabraded skin of any of the rabbits had a reaction during the study. The 0.25% AMP in ethanol was not irritating to rabbit skin.

In a limited summary, it was noted that AMP at concentrations of 0.25% and 2.5% in aqueous and alcoholic vehicles caused no irritation in single insult occluded patch tests in rabbits.[34]

The primary irritation indices (PII) for two formulations containing 0.26% AMP were 1.13 and 1.31 (maximum possible score = 8), respectively.[58] The test formulation, 0.5 ml, was applied under an occlusive patch to the intact and abraded skin of two rabbits of each sex. The patch was removed 24 h later. The sites were graded at 25 h (1 h after patch removal) and 72 h. Each group of rabbits was tested with one formulation. With the first formulation, all of the rabbits had erythema at both sites at both gradings, with slight desquamation at the 72 h grading. One rabbit also had edema at the abraded site; this reaction had subsided by 72 h. The reactions of the rabbits tested with the second formulation were essentially the same. All rabbits had erythema at both gradings, with slight desquamation at 72 h. One rabbit had edema at the abraded site at 25 h, but was negative at 72 h. The reactions to the second formulation were slightly more severe than those to the first formulation, accounting for the differences in the PII's. The formulations containing 0.26% AMP were considered mildly irritating to intact and abraded rabbit skin.

The following four tests were each performed on cosmetic formulations containing AMP.[59–62] In each test the formulation, 0.5 ml, was applied under an occlusive patch to the abraded and nonabraded skin of six rabbits of mixed sex. After 24 h, the patch was removed. The test sites were graded upon patch removal and at 72 h. The results of these tests follow. A hair spray containing 0.25% AMP caused no irritation to either the intact or abraded skin of rabbits; the PII was 0.0.[60] The PII of a hair spray containing 0.58% AMP was 0.38.[59] At the 24-h grading, three of the rabbits had erythema at both the intact and abraded sites, while the other three rabbits had erythema at the abraded sites only. All of the reactions had cleared by 72 h. The PII for a hair spray containing 0.59% AMP was 0.35.[61] Four of the six rabbits had erythema at both the intact and abraded skin sites at the 24-h grading. All reactions at 72 h were negative. The hair spray containing 0.59% AMP was not a primary dermal irritant.

Three products containing 0.58, 0.59, and 0.58% AMP had PII's of 0.75, 1.40, and 0.35, respectively.[62] With the first formulation, five of the six rabbits had erythema at both the abraded and intact sites at 24 h; the irritation persisted through the 72-h grading period, with one rabbit having edema in addition to the erythema at both sites. With the second formulation (0.59% AMP), four rabbits had both erythema and edema at 24 h. A fifth rabbit had erythema alone, which had subsided by 72 h. Of the other four rabbits with reactions, one had no reaction at 72 h, one had erythema only, one had increased erythema and continued edema, and the last had increased erythema and edema. All of the reactions noted occurred at both the intact and abraded sites. With the third formulation (0.58% AMP), one rabbit had erythema at the abraded site, and two rabbits had erythema and edema at both sites at the 24-h grading. All of the reactions had subsided by 72 h. None of the formulations were primary dermal irritants under the conditions of the test.

In another study, an unspecified cosmetic formulation containing AMP-95 (95% AMP solution) at a concentration of 0.22% was tested for primary skin irritation potential in a group of nine rabbits using the single insult, occlusive patch test procedure.[63] The skin reactions were graded 2 and 24 h after patch removal. Three rabbits had erythema 2 h after patch removal; of these three, one had undiminished erythema 24 h after patch removal. In addition, a fourth rabbit had erythema at the 24-h grading. The group PII for the formulation containing AMP-95 at a concentration of 0.22% was 0.56 (maximum 8.00), indicating that the formulation was minimally irritating.

A "foam hair groom" containing 0.715% AMPD was tested in four New Zealand albino rabbits for primary dermal irritancy.[64] The undiluted test material, 0.5 ml, was applied under an occlusive patch to the intact and abraded skin of each rabbit, where it remained for 24 h. The sites were graded 1 h after patch removal and at 72 h. No adverse reactions were noted; the hair groom containing 0.715% AMPD was nonirritating when applied to intact and abraded rabbit skin.

A hair spray formulation containing 0.50% AMPD was tested following the protocol outlined in the previous paragraph.[65] Two rabbits had erythema and edema at the intact skin site; the reaction had cleared by 72 h. One rabbit had erythema persisting through 72 h at the intact site. The fourth rabbit had no reaction at the intact skin site. At the abraded skin sites, three rabbits had erythema and edema, with the erythema persisting through 72 h while the edema subsided in all but one of the rabbits. The fourth had continuing erythema and no edema. The PII for the hair spray was 1.38.

Sensitization

The intradermal sensitization potential of AMP was studied in guinea pigs.[66] Three groups of ten male guinea pigs each were used in the study: negative control (saline), positive control (0.3% dinitrochlorobenzene), and test group (AMP). The backs and flanks of the guinea pigs were shaved, and 0.05 ml of the appropriate solution was injected intradermally. The injection sites were graded 24 h later. At 48 h, 0.1 ml of the appropriate solution was injected, and the injections were repeated two to three times a week for ten injections. Two weeks after the last injection, the animals received challenge injections at a previously untreated site. The challenge injections for the test and control groups consisted of 0.1 ml each of 0.01% and 0.05% solutions of AMP. The challenge sites were chemically depilated 24 h after the injection; grading of the sites was performed 3 h later, and again at 48 h.

The first two injections of the induction phase, using 0.5% and 1% AMP solutions, respectively, caused necrotic lesions, and so the remainder of the induction injections were made with a 0.1% solution. One guinea pig of the test group had a slight reaction at the 24-h grading of the 0.05% AMP challenge site. This reaction had cleared by 48 h. No reactions were noted in the test group at the second challenge. At the second challenge with AMP solutions, four guinea pigs of the saline control group had reactions to the 0.05% AMP and one had a reaction to the 0.01% AMP. All of these reactions had cleared by 48 h. The positive control animals had the expected reactions. AMP was not an intradermal sensitizer in guinea pigs.

Ocular Irritation

In a limited summary, it was stated that AMP at a concentration of 0.25% in an aqueous vehicle caused slight transient irritation when instilled in the eyes of rabbits with and without rinsing.[34] The irritation had cleared by days 2 and 4, respectively.

Five New Zealand White rabbits received in the left eye a single spray of a formulation (pH 8.3) containing 0.26% AMP.[67] The spray was directed from a distance of 6 inches from the eye; the right eye served as a control. Observations of the eyes were made at 1 and 24 h, and at 3, 4, and 7 days postinstillation. At 1 h, two rabbits had slight conjuntivitis and dull corneas, both clearing by 24 h. A third rabbit had slight conjunctivitis at 1 h, also clearing by 24 h. The fourth rabbit had slight conjunctivitis

persisting through 24 h and clearing by day 3. The fifth rabbit had no reaction. All rabbits had negative fluorescein stains 7 days after instillation of the test material. The spray containing 0.26% AMP was minimally irritating when not rinsed from sprayed rabbit eyes.

Twelve New Zealand White rabbits received a single 1-s spray, from a distance of 4 inches, of a hair spray containing 0.25% AMP.[68] The eyes of six of the rabbits were rinsed 30 s after the spraying. The animals were observed for 3 days. Two of the six rabbits of the no-rinse group had signs of irritation. One had slight iritis and conjunctivitis on day 1, with the conjunctivitis continuing through day 2 and clearing by day 3. The second rabbit had slight corneal opacity, iritis, and conjunctivitis; the corneal opacity had cleared by day 2 and the remainder of the irritation had cleared by day 3. Three rabbits of the rinsed group had slight conjunctivitis on day 1 which was cleared by day 2.

A second test following the protocol described in the previous paragraph was performed with a hair spray containing 0.58% AMP.[69] Of the rabbits that did not have their eyes rinsed, three had slight conjunctivitis on day 1; the conjunctivitis had cleared by day 2 in two of the rabbits, and by day 3 in the third. The remaining three rabbits of the group had no reactions. Of the rabbits receiving a rinse, one had slight corneal opacity and conjunctivitis on day 1; the opacity had cleared by day 2, and the conjunctivitis by day 3. None of the other rabbits of the rinsed group had adverse reactions.

A hair spray contining 0.59% AMP was instilled into the eyes of 12 New Zealand White rabbits.[70] The volume of the material tested was 0.1 ml. Six of the rabbits received no eye rinse for the first 24 h, while the eyes of the other six were rinsed 30 s after instillation of the test material. The rabbits were observed for 3 days after the instillation. Of the rabbits that received no eye rinses, one had scattered areas of opacity over most of the cornea, as well as slight redness and chemosis on day 1. On day 2, this rabbit had obvious translucent areas over a small part of the cornea, and by day 3, the eye appeared normal. The remainder of the rabbits in the test group had no ocular reaction. Of the rabbits which received eye rinses, one rabbit had scattered areas of opacity over a small portion of the cornea and moderate chemosis, both of which had cleared by day 2. None of the other rabbits had adverse reactions. The hair spray containing 0.59% AMP was considered a mild ocular irritant to rabbits under the conditions of the test. Rinsing reduced the extent of the irritation.

A cosmetic formulation containing 0.22% AMP-95 was tested in six rabbits for eye irritation potential.[71] The test material was not rinsed from the eyes of the rabbits, and the reactions were graded on days 1–4, and on day 7 after instillation. Three different rabbits had conjunctivitis, one each on days 1–3. No reactions were observed on days 4 and 7. The formulation containing 0.22% AMP-95 had a mild eye irritation potential according to the Draize classification system.

A hair spray containing 0.40% AMPD was sprayed into the left eye of each of five New Zealand White rabbits for a duration of 1 s.[72] The right eyes served as controls. The eyes were observed for signs of irritation at 1 and 24 h, and on days 2, 3, 4, and 7. All of the rabbits had severe iritis and slight conjunctivitis at 1 h, and in four of the rabbits this was reduced at 24 h, and cleared by day 2. In the fifth rabbit, the severe iritis continued, along with the slight conjunctivitis, through day 2, and was cleared by day 3.

A foam hair groom, 0.1 ml, containing 0.715% AMPD was instilled into the left eye of each of ten New Zealand White rabbits.[73] Half of the rabbits had their eyes rinsed 4

s after instillation of the test material. Ocular reactions were graded at 1 and 24 h, and on days 2, 3, 4, and 7. Sodium fluorescein examinations were performed on day 7, as well as at other times during the study as necessary. One rabbit of the nonrinsed group had moderate conjunctivitis at 1 h, clearing by 24 h. Two rabbits had moderate conjunctivitis which diminished steadily and was cleared by day 3. One rabbit had moderate iritis and conjunctivitis at 1 h; the iritis had cleared by 24 h, and the conjunctivitis by day 2. The fifth rabbit had moderate iritis at 1 h, clearing by 24 h. In addition, this rabbit had moderate conjunctivitis at 1 h; this reaction gradually diminished through day 4 and was clear by day 7. Two of the rabbits of the rinsed eye group had moderate iritis and conjunctivitis, with the iritis clearing by 24 h and the conjunctivitis diminishing at 24 h and clearing by day 2. Two rabbits had moderate conjunctivitis at 1 h, clearing by 24 h. The fifth rabbit had moderate conjunctivitis, which had diminished at 24 h and cleared by day 2. The rabbits of both test groups had negative fluorescein dye examinations on day 7.

MUTAGENICITY

A plate assay mutagenicity test, with and without metabolic activation, was performed using AMP and *Saccharomyces cerevisiae* strain D4 and *Salmonella typhimurium* strains TA1535, 1537, 1538, 98, and 100.[74] Positive activation and nonactivation controls were used; the controls were positive for either frame-shift or base-pair substitution mutations. The AMP was tested over a range of concentrations from 0.01 μl to 5 μl; the high dose produced some toxic effects, and the low dose was below a toxic level. The results indicated that AMP was not mutagenic, with and without metabolic activation, under the conditions of the test.

AMPD was tested for mutagenic potential using *Salmonella typhimurium* strains TA1535, 1537, 98, and 100.[75] Testing in these strains represents frame-shift and base-pair substitution type mutations. AMPD was tested at concentrations of 100, 333, 1000, 3330, and 5000 μg/plate, with and without metabolic activation. The test was performed twice. There were no dose-related increases in the number of revertants in either study over the concentration range tested, and AMPD was not considered mutagenic under the conditions of the study.

CLINICAL ASSESSMENT OF SAFETY

Dermal Irritation and Sensitization

Irritation

The skin irritation potential of a cosmetic formulation containing 0.22% AMP-95 was examined using a single insult occlusive patch test on 15 panelists.[76] One panelist had an equivocal reaction (±), resulting in a group PII of 0.03. The cosmetic formulation containing 0.22% AMP-95 had a negligible primary skin irritation potential.

A hair spray containing 0.40% AMPD was tested for primary irritancy in 15 human subjects.[77] The patches were applied to the arms of the panelists. The test was referred to as a "5 hour–4 day test" with the test beginning on Monday and the readings being

made on the mornings of Tuesday, Wednesday, Thursday, and Friday. Four panelists had no reactions. There were scattered instances of questionable responses in nine panelists, with seven having one questionable response and the remainder having two questionable responses. In addition, one panelist had slight redness on day 4, and one panelist had slight redness on days 2–4.

Sensitization

A conditioning hair mousse containing 0.22% AMP-95 was tested for allergic contact sensitization potential in 97 panelists.[78] None of the panelists (86 females and 11 males) had skin conditions or medical histories that would interfere with the purpose of the study.

Ten formulations were tested simultaneously; five patches were placed on either side of the upper back, next to the midline. Only if there was a severe reaction was a patch removed. Approximately 0.1 ml of the test material was applied to the patch. The patched were applied every Monday, Wednesday, and Friday for 3 weeks. The patches were removed by the panelists 24 h after application, and the patch sites were graded prior to the application of the new patch. The final induction patch sites were graded prior to the challenge phase of the study. The challenge sites were graded 24 and 48 h after patch removal.

Thirteen panelists had reactions during the induction phase of the test. Of these 13, nine had single reactions, two had two reactions each (insults 4 and 7, 1 and 8), one had three reactions (insults 1, 6, and 7), and one had four reactions (insults 2, 3, 8, and 9). All of the reactions that occurred during the induction phase were recorded as barely perceptible. In addition, another panelist had a barely perceptible reaction at the 24-h grading of the challenge phase; the results of the 72-h grading were not available. The conditioning hair mousse containing 0.22% AMP-95 did not have allergic contact sensitization potential.

A cosmetic formulation containing 0.073% AMPD was tested for sensitization potential in a group of 30 human test subjects using a repeated insult open patch test.[79] The test material was applied to the arm daily 4 days/week for 2 weeks, alternating arms daily. In addition, an occlusive patch was applied on the first day of the test. After the 2-week application period, there was a 2-week nontreatment period. After this 2-week period, the test subjects received a reapplication of the formulation to the test site along with an occlusive patch at an adjacent site. The original patch, challenge patch, and open challenge test sites were read at 24, 48, and 96 h. No reactions were observed in any of the test subjects. The formulation containing 0.073% AMPD was neither a primary irritant, nor a fatiguing agent, nor a sensitizer, and the formulation was safe under the conditions of the study.

A modified repeated insult patch test of a cosmetic formulation containing 0.50% AMPD was performed on a panel of 39 women and 20 men.[80] The test material, 0.5 ml, was applied to a semiopen patch on the arm of each panelist every Monday, Tuesday, Wednesday, and Thursday for two weeks. The patch sites were graded approximately 24 h after application. In addition, a closed patch was applied to each panelist on the first day of the study and on the day of challenge. No patches were applied for 2 weeks after the induction phase. On the Monday following the nontreatment period, challenge patches were applied to the original test site and an adjacent site; the second closed patch was also applied at this time. The challenge sites were graded 1, 2, and 4 days after application. Slight erythema was noted at one adjacent

application site at each of the grading times, but it was not clear whether these reactions occurred in the same panelist. The formulation containing 0.50% AMPD was not a sensitizer under the conditions of the test.

SUMMARY

AMP and AMPD are substituted aliphatic alcohols. Both occur in solid and liquid forms. 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 [^{32}P] 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 choline-adequate 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,[1] 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,[1] 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 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 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 CO_2 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.

DISCUSSION

Though AMP and AMPD are highly alkaline in pure form, the Panel notes that these chemicals are buffered in cosmetic formulations, and, therefore, the adverse reactions seen with the undiluted chemical would not be expected with the cosmetic product. Of greater concern is the possible presence of impurities in AMP and AMPD, especially oxazolidine or other secondary amines, which are vulnerable to *N*-nitrosation. If there is a possibility of these chemicals being present in the cosmetic-grade AMP and AMPD, then the Panel recommends that AMP and AMPD not be included in cosmetic formulations containing nitrosating agents.

AMP and AMPD were not mutagenic in the Ames assay using five and four tester strains, respectively.

The Panel is aware that AMP and AMPD are used at concentrations greater than 1%, but because available test data do not exceed 1%, the Panel recommends that AMP and AMPD are safe as cosmetic ingredients at concentrations not exceeding 1%.

CONCLUSION

On the basis of the available animal and clinical data presented in this report, the CIR Expert Panel concludes that at concentrations not exceeding 1%, Aminomethylpropanol and Aminomethylpropanediol are safe for use in cosmetics.

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Final Amended Report on Safety Assessment on Aminomethyl Propanol and Aminomethyl Propanediol

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Abstract

Aminomethyl propanol and aminomethyl propanediol are substituted aliphatic alcohols that function as pH adjusters in cosmetic products at concentrations less than 10%; additionally, aminomethyl propanediol is a fragrance. Extensive oral toxicity data are reviewed, with fewer inhalation toxicity data. Dermal toxicity data are presented that demonstrate, for example, that a mascara with 1.92% aminomethyl propanediol does not cause dermal irritation or allergic contact sensitization, suggesting that the maximum reported use concentration of 2% in mascara would be safe. Although these ingredients are primary amines that are not substrates for N-nitrosation, they may contain secondary amines as impurities in finished products that may undergo N-nitrosation. These ingredients should not be included in cosmetic formulations containing N-nitrosating agents. The Cosmetic Ingredient Review Expert Panel concludes that aminomethyl propanol and aminomethyl propanediol are safe as cosmetic ingredients in the practices of use and concentrations as described in this safety assessment.

Keywords

aminomethyl propanol, aminomethyl propanediol, cosmetics, safety

A safety assessment for aminomethyl propanol (AMP) and aminomethyl propanediol (AMPD) was published by the Cosmetic Ingredient Review (CIR) in 1990.[1] At that time, the CIR Expert Panel concluded that "at concentrations not exceeding 1%, aminomethyl propanol and aminomethyl propanediol are safe for use in cosmetics." New data were provided suggesting the safety of these ingredients at concentrations higher than 1%. This report is a compilation of new data and data from the original safety assessment on AMP and AMPD that are relevant to the assessment of these chemicals as used in cosmetics.

In 1987, a safety assessment for isopropanolamine, a close analog of AMP, was published with the conclusion from the CIR Expert Panel that this ingredient is safe as used in the practices of use and concentration but should not be used in products containing N-nitrosating agents.[2] This conclusion was confirmed during a subsequent review of new published literature.[3]

Chemistry

Definition and Structure

AMP (CAS 124-68-5) is defined in the *International Cosmetic Ingredient Dictionary and Handbook* as a substituted aliphatic alcohol that conforms to the formula in Figure 1.[4]

AMPD (CAS 115-69-5) is defined in the *International Cosmetic Ingredient Dictionary and Handbook* as a substituted aliphatic diol that conforms to the formula in Figure 2.[4]

Synonyms and trade names for these ingredients can be found in Table 1. Both AMP and AMPD are in the general chemical groups of alkanolamines (also, alcohol amines).

Properties

Chemical and physical properties for AMP and AMPD are described in Table 2.

Method of Manufacture

Both AMP and AMPD can be synthesized by the condensation of the corresponding nitroparaffins with formaldehyde and reduction to the β-aminoalkanols.[5] The reduction to the alkanolamine is accomplished by hydrogenation in the presence of a Raney nickel catalyst.

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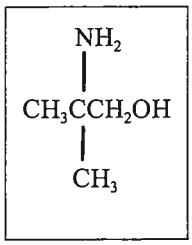

Figure 1. Structure of aminomethyl propanol.

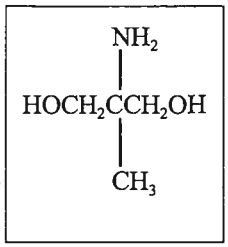

Figure 2. Structure of aminomethyl propanediol.

Table 1. Synonyms and Trade Names for Aminomethyl Propanol (AMP) and Aminomethyl Propanediol (AMPD)[95]

Ingredient	Synonyms	Trade Name	Trade Name Mixtures
Aminomethyl propanol	2-Aminoisobutanol	AMP-95	Cerasynt IP
	2-Amino-2-methyl-1-propanol	AMP-Regular	FZ-3148
	2-Hydroxymethyl-2-propylamine		FZ-3158
	Isobutanolamine		Hair gloss polymer A
	1-Propanol, 2-amino-2-methyl-		
Aminomethyl propanediol	2-Amino-2-methylpropane-1,3-diol	AMPD	
	2-Amino-2-methyl-1,3-propanediol		
	AMPD		
	1,3-Dihydroxy-2-methyl-2-propylamine		
	1,3-Propanediol, 2-amino-2-methyl-		

Table 2. Chemical and Physical Properties of Aminomethyl Propanol (AMP) and Aminomethyl Propanediol (AMPD)[5,20,96,97]

	AMP	AMPD
Physical description	Colorless liquid or crystals; crystals are odorless, but liquid has slight amine odor.	Colorless liquid or crystals; crystals are odorless, but liquid has slight amine odor.
Molecular weight	89.14	105.14
Empirical formula	$C_4H_{11}NO$	$C_4H_{11}NO_2$
Melting point, °C	30-31	109-111
Boiling point, °C	165 (760 mm Hg)	151-152 (10 mm Hg)
Flash point, °C	67	—
Vapor density	3.04	3.63
Specific gravity	0.934 at 20/20 °C	—
pH in 0.1 M solution	11.3	10.8
Solubility	Miscible in water, soluble in alcohols, slightly soluble in aromatic hydrocarbons, and insoluble in aliphatic hydrocarbons	Soluble in water and alcohols, slightly soluble in aromatic hydrocarbons, and insoluble in aliphatic hydrocarbons and mineral oil

Analytical Methods

Infrared (IR), nuclear magnetic resonance (NMR), and mass spectra (MS) have been published for AMP.[6] The IR spectrum of commercial AMP closely matches the standard spectrum, with no evidence of foreign materials.[7] For AMPD, the infrared spectrum has been published.[6] Alkanolamines such as AMP and AMPD can be determined in hair spray formulations, after acetylation, by gas-liquid chromatography.[8]

Impurities

Angus Chemical Company reported that AMP may have up to 6.8% of secondary amine impurity. AMPD has impurity levels below 0.5%.[9] Analysis of AMP by this company found no nitrosamines at the limit of detection, 50 ppb. Ultra PC grades of AMP and AMPD meet European Union Cosmetics Directive standards that require a minimum purity of 99%, a secondary amine content no greater than 0.5%, and a nitrosamines content no greater than 50 ppb.

Chemical Reactions

Alkanolamines can react with copper, brass, and aluminum but not with steel or iron.[5]

Alkanolamines react with the methyl ester of an organic acid in the presence of an alkaline catalyst, and at low temperatures and pressures they form amides. When this reaction is carried out at higher temperatures, oxazolines are produced. The alkanolamines react with acid anhydrides to form imides. The

substituted ethyleneimine is formed by the reaction of AMP with excess mineral acids at temperatures above 75°C, followed by reaction with a caustic agent. Oxazolidines are formed by reaction of the alkanolamines with aldehydes.[10]

Use

Cosmetic Uses

AMP functions as a pH adjuster in cosmetic products.[4] AMPD functions as a pH adjuster and is also a fragrance ingredient.[4] As reported to the US Food and Drug Administration (FDA) by industry, AMP is used in a total of 853 cosmetic products, primarily aerosol hair sprays and hair dyes, whereas AMPD is used in a total of 47 cosmetic products, most frequently in mascara.[11] Table 3 presents the product formulation data for AMP and AMPD. Based on a survey conducted by the Cosmetic, Toiletry and Fragrance Association (CTFA), the highest use concentration reported for AMP is 7% in hair dyes and colors and the highest use concentration for AMPD is 2% in mascara.[12]

Products containing AMP or AMPD may come into contact with the skin, eyes, and mucous membranes. Contact with the ingredient may be temporary or prolonged. Products containing either ingredient may be used repeatedly over a period of time.

AMP and AMPD are unlikely to exist as the free bases in cosmetic products but rather as salts as the result of neutralization of acidic components of the cosmetic formulation.[13]

AMP and AMPD are not included among the substances listed as prohibited, restricted, or provisionally allowed in the use of cosmetic products marketed in Japan.[14,15] In the European Union, monoalkanylamines, monoalkanolamines, and their salts are listed under Annex III, Part 1 of the Cosmetics Directive with the following restrictions: maximum secondary amine content in finished products and raw materials of 0.5%, must not be used with nitrosating systems, minimum purity of 99%, maximum nitrosamine content of 50 μg/kg, and must be kept in nitrite-free containers.[16]

Aerosol use. AMP and AMPD also are used in hair sprays, which may be inhaled. Jensen and O'Brien[17] reviewed the potential adverse effects of inhaled aerosols, which depend on the specific chemical species, the concentration, the duration of the exposure, and the site of deposition within the respiratory system.

The aerosol properties associated with the location of deposition in the respiratory system are particle size and density. The parameter most closely associated with this regional deposition is the aerodynamic diameter, $\mathbf{d_a}$, defined as the diameter of a sphere of unit density possessing the same terminal setting velocity as the particle in question. These authors reported a mean aerodynamic diameter of 4.25 $\pm$ 1.5 μm for respirable particles that could result in lung exposure.[17]

Bower[18] reported diameters of anhydrous hair spray particles of 60 to 80 μm and pump hair sprays with particle diameters of 80 μm or greater. Johnsen[19] reported that the mean particle diameter is around 38 μm in a typical aerosol spray. He stated that in practice, aerosols should have at least 99% of particle diameters in the 10 to 110 μm range.

Noncosmetic Uses

AMP and AMPD have a variety of uses in the synthesis of surface-active agents, vulcanization accelerators, and pharmaceuticals. They are also used as emulsifying agents in mineral oil and paraffin wax emulsions, leather dressings, textiles, cleaning compounds, polishes, and soluble oils and as absorbents for acidic gases.[20] AMPD can be used to stabilize emulsions, although stability depends on the concentration of AMPD, the length of storage, and temperature.[21]

Alkanolamines can be used in pigment dispersion, resin solubilizers, catalysts, boiler water treatment, formaldehyde scavenging, applications in oil and gas production, biomedical applications, and synthetic applications.[5] In cutting fluids, AMP is useful as an antimicrobial agent.[22] AMP is also listed as an indirect food additive for use, without restrictions, as a component of adhesives.[23]

General Biology

Absorption, Distribution, Metabolism, and Excretion

Skin absorption. Musial and Kubis[24] evaluated the interaction of AMP and AMPD along with several other alcoholamines with model skin sebum for potential use in topical treatments and prevention of acne. This study found that AMP and AMPD penetrated the artificial sebum and that reaction product accumulated above the sebum layer (sebum thickness not defined). The depth of penetration increased as a function of time and plateaued after 48 hours at approximately 2.7 mm for AMP and at approximately 3.6 mm for AMPD. After 72 hours, the penetration of AMP and AMPD was about 3.1 mm and 4.1 mm, respectively.

Metabolism. AMP is incorporated into phospholipids.[25] AMP inhibits incorporation of ethanolamine and diethanolamine in phospholipids. This, in turn, may limit the conversion of ethanolamine (in the phospholipids) to choline.[26] No metabolism data were available for AMPD.

Distribution and excretion. Yue et al[27] studied the fate of [^{3}H]AMP (dose not reported) injected intraperitoneally in young male Sprague-Dawley rats on choline-adequate and choline-deficient diets. The rats receiving the choline-deficient diets started the diet 24 hours before the injection of [^{3}H]AMP and continued on this diet until they were killed at 30 minutes or 1, 2, 3, 6, 24, or 96 hours post injection. The rats fed the choline-adequate diet (ad libitum) followed the same protocol. Thirty minutes after the intraperitoneal injection, [^{3}H] appeared in the serum, with radioactivity disappearing shortly after its initial uptake. Radioactivity in the serum was consistently lower in the rats fed the choline-deficient diet, with

Table 3. Current Cosmetic Product Uses and Concentrations for Aminomethyl Propanol (AMP) and Aminomethyl Propanediol (AMPD)[11,12]

Product Category (Total No of Products in Each Category)	Ingredient Uses in Each Product Category	Use Concentrations, %
Aminomethyl propanol		
Bath products		
Soaps and detergents (594)	7	0.03-0.2
Other (276)	—	2
Eye makeup		
Eyebrow pencils (124)	—	0.7
Eyeliners (639)	—	0.7
Eye shadow (1061)	—	0.7
Eye lotions (32)	1	0.5-0.7
Eye makeup remover (114)	2	0.7
Mascara (308)	6	0.3-1
Other (229)	1	0.7
Fragrance products		
Colognes and toilet waters (948)	2	0.03-0.3
Perfumes (326)	—	0.2-0.3
Powders (324)	—	0.3
Sachets (28)	—	0.3
Other (187)	4	0.04-0.3
Noncoloring hair care products		
Conditioners (715)	24	0.02-2
Sprays/aerosol fixatives (294)	219	0.3-3
Straighteners (61)	1	0.2
Permanent waves (169)	—	0.2
Rinses (46)	—	0.2
Shampoos (1022)	9	0.0001-1
Tonics, dressings, etc. (623)	124	0.5-3
Wave sets (59)	6	1
Other (464)	144	0.3-1
Hair coloring products		
Dyes and colors (1600)	245	0.5-7[a]
Tints (56)	1	0.5
Rinses (15)	5	0.5
Shampoos (27)	7	—
Color sprays (4)	—	0.5
Lighteners with color (14)	—	0.5
Bleaches (103)	—	0.5
Other (73)	—	0.5
Makeup		
Blushers (459)	—	0.5
Face powders (447)	—	0.5
Foundations (530)	—	0.5-0.6
Makeup bases (273)	—	0.5
Makeup fixatives (37)	—	0.5
Other (304)	1	0.3-0.5
Nail care products		
Basecoats and undercoats (43)	—	0.1
Cuticle softeners (20)	—	0.1
Creams and lotions (13)	—	0.0009-0.1
Nail extenders (1)	—	0.1
Nail polishes and enamels (398)	—	0.1
Nail polish and enamel removers (39)	—	0.1
Other (58)	—	0.1
Personal hygiene products		
Underarm deodorants (281)	10	0.0009-0.4
Douches (8)	—	0.2
Feminine deodorants (7)	—	0.2
Other (390)	1	0.08-2
Shaving products		
Aftershave lotions (260)	6	0.5-0.8
Shaving creams (135)	—	0.2-2
Shaving soaps (2)	—	0.2

(continued)

Table 3 (continued)

Product Category (Total No of Products in Each Category)	Ingredient Uses in Each Product Category	Use Concentrations, %
Other (64)	—	0.2
Skin care products		
Skin cleansing creams, lotions, liquids, and pads (1009)	4	0.1-1
Depilatories (49)	—	0.5
Face and neck creams, lotions, powders, and sprays (546)	2	0.07-0.5[b]
Body and hand creams, lotions, powders, and sprays (992)	5	0.05-1[c]
Foot powders and sprays (43)	1	0.03-0.5[d]
Moisturizers (1200)	6	0.5[e]
Night creams, lotions, powders, and sprays (229)	—	0.5[f]
Paste masks/mud packs (312)	2	0.5
Skin fresheners (212)	—	0.1-0.5
Other (915)	7	0.09-2[g]
Suntan products		
Suntan gels, creams, liquids, and sprays (138)	—	0.4
Total uses/ranges for aminomethyl propanol	853	0.0001-7
Aminomethyl propanediol		
Eye makeup		
Eyebrow pencils (124)	—	1
Eyeliners (639)	—	0.1
Eye makeup remover (114)	—	0.5
Mascara (308)	37	0.2-2
Other (229)	1	—
Noncoloring hair care products		
Sprays/aerosol fixatives (294)	1	—
Shampoos (1022)	1	—
Other (464)	3	—
Makeup		
Blushers (459)	—	1
Face powders (447)	—	1
Foundations (530)	—	1
Makeup bases (273)	—	0.8-1
Makeup fixatives (37)	—	1
Other (304)	1	1
Skin care products		
Skin cleansing creams, lotions, liquids, and pads (1009)	1	1
Depilatories (49)	—	1
Face and neck creams, lotions, powders, and sprays (546)	1	1[h]
Body and hand creams, lotions, powders, and sprays (992)	—	1[i]
Foot powders and sprays (43)	—	1
Moisturizers (1200)	1	1[j]
Night creams, lotions, powders, and sprays (229)	—	1[k]
Paste masks/mud packs (312)	—	1
Skin fresheners (212)	—	1
Other (915)	—	0.1-1[l]
Suntan products		
Suntan gels, creams, liquids, and sprays (138)	—	1
Total uses/ranges for aminomethyl propanediol	47	0.1-2

[a] 7% before dilution.
[b] 0.5% in face and neck sprays; 0.07%-0.5% in face and neck creams, lotions, and powders.
[c] 0.1%-0.5% in body and hand sprays; 0.05%-1% in body and hand creams, lotions, and powders.
[d] 0.4% in foot cream; 0.03%-0.5% in foot powders and sprays.
[e] 0.5% in moisturizing sprays; 0.5% in moisturizing creams, lotions, and powders.
[f] 0.5% in night sprays; 0.5% in night creams, lotions, and powders.
[g] 0.09% in an antibacterial hand soap; 0.2% in a hand sanitizer; 2% in pore strips; 2% in a body polish.
[h] 1% in face and neck sprays; 1% in face and neck creams, lotions, and powders.
[i] 1% in body and hand sprays; 1% in body and hand creams, lotions, and powders.
[j] 1% in moisturizing sprays; 1% in moisturizing creams, lotions, and powders.
[k] 1% in night sprays; 1% in night creams, lotions, and powders.
[l] 0.1% in exfoliating scrubs (hand and body; foot).

the exception of the 6-hour value, at which time the activity was approximately equal for both dietary groups.

Radioactivity in the urine followed the same pattern as that in the serum, with the rats on the choline-adequate diet excreting a greater amount of radioactivity in their urine than the rats on the choline-deficient diet. Paper chromatography results suggested that the radioactivity in the urine was the [^{3}H]AMP, which had been excreted unchanged, as indicated by samples of [^{3}H]AMP that were chromatographed concurrently.

From 0.5 to 6 hours, the uptake of radioactivity by the brain, skeletal muscle, heart muscle, intestine, and spleen was greater in the rats fed the choline-adequate diet. At 6 hours, this trend was completely reversed and remained so until the end of the study. In the liver, uptake of radioactivity was greater in the choline-deficient group throughout the study. By 96 hours, the radioactivity in the liver of both groups had decreased considerably, but that in the liver of the choline-deficient group remained higher than that in the choline-adequate diet group.

The distribution of the radioactive AMP in the phospholipids of the liver was also examined. At 0.5 hours, the amount of free radioactive AMP in hepatic mitochondria from rats fed the choline-adequate diet was approximately 72%, the remaining 28% being incorporated into phospholipids. In the choline-deficient rats, about 29% of the AMP was free; the remaining 71% was present in the phospholipids. This same trend was seen in hepatic microsomes, with the exception that a greater amount of the AMP (81%) in rats fed the choline-deficient diet was incorporated into phospholipids. There was no indication that the AMP had been phosphorylated.

At all times, the livers of the choline-deficient rats had a higher amount of [^{3}H]AMP in all subcellular fractions. The cytosol of both the liver and kidneys cells contained the most [^{3}H]AMP. In the rats receiving a choline-adequate diet, the radioactivity in the kidneys and liver decreased with time, whereas the opposite was true for rats fed the choline-deficient diet; radioactivity increased in the hepatic subcellular fractions and remained constant in the renal subcellular fractions.

In the choline-deficient rats, this change was most pronounced in the liver microsomal fraction. The authors also noted that the radioactivity found in the cytosol was not free AMP because no free AMP was identified after 30 minutes, and that the radioactive AMP was redistributed among several phospholipid fractions. This latter observation indicated that incorporation of AMP into phospholipids may occur with other derivative forms of AMP other than the phosphatidyl derivative.[27]

No distribution or excretion data on AMPD were found.

Animal Toxicology

Acute Oral

Aminomethyl propanol. A review by Power[28] described an acute oral toxicity study in young adult male fasted rats (strain not specified; 5 groups of 10 rats each) that received a single oral dose of AMP diluted in an equal volume of saline. Doses were 2200, 2400, 2800, 3600, or 4000 mg/kg. Animals were observed closely for 4 hours immediately following dosing and then daily for 14 days after treatment. Rats receiving 3600 or 4000 mg/kg experienced rapid absorption into the circulatory system that resulted in gross damage to the liver, kidney, spleen, and respiratory system that was followed by respiratory collapse. Irritation to the stomach and duodenum was observed in rats receiving 2800 mg/kg or more. The LD_0, LD_{50}, and LD_{100} were 2200, 2900 $\pm$ 140, and 4000 mg/kg, respectively.

An acute toxicity study of a hair spray containing 0.25% AMP was performed using 10 albino rats, 5 of each sex.[29] After fasting overnight, the animals received a dose by gavage of 5.0 mL/kg of the undiluted hair spray (sprayed into glass beakers to collect test material) and then were observed for 14 days thereafter, during which they were allowed feed and water ad libitum. One female rat died during the second week of observation. Most of the rats had either slightly decreased activity or decreased activity up to 3 hours after administration of the test material, and all appeared normal from the 6-hour point until the end of the study. All of the survivors gained weight during the study. At necropsy, no abnormalities were observed in the survivors or in the rat that died during the study.

The same protocol was also performed with a hair spray (specific gravity 0.81) containing 0.58% AMP.[30] All rats survived the 14-day oral observation period. The rats had severely decreased activity an hour after administration of the test material. Their activity remained decreased through the 6-hour observation point and then returned to normal for the remainder of the study. All animals gained weight during the study, and no gross abnormalities were noted at necropsy.

Using the same protocol as the previous study, a hair spray (specific gravity 0.74) containing 0.59% AMP was tested for oral toxicity in albino rats.[31] All rats had some degree of decreased activity for the first 24 hours. All of the rats died before the end of the study, and 7 of the 10 rats died on or before day 2. The 3 rats that survived through day 2 appeared normal on the second day but had recurring slightly decreased activity on day 3. These 3 rats all died within the first week. The following observations were noted at necropsy: the 3 rats that died within 1 hour had severely reddened pyloric mucosae, the 2 rats that died at 24 hours had moderately reddened pyloric and duodenal mucosae, the 2 rats that died on day 2 had severely reddened pyloric and duodenal mucosae, the rat that died on day 5 had necrosis of the pyloric mucosa, the rat that died on day 6 had consolidation of the superior and inferior lobes of the right lung, and the rat that died at 1 week had moderately reddened pyloric and duodenal mucosa and gas-filled stomach and intestines.

A fourth test following the same protocol was performed with 3 cosmetic formulations containing either 0.58% or 0.59% AMP (0.59% AMP at specific gravity of 0.80, 0.58% AMP at specific gravity of 0.79, and 0.58% AMP at specific gravity of 0.85).[32] No animals in the 3 test groups died during the study, and all rats gained weight. All of the test animals had varying degrees of decreased activity; in no case did the decreased activity last beyond 24 hours. No gross abnormalities were observed at necropsy, and the 3 formulations containing 0.58% or 0.59% AMP were not toxic to rats by the oral route

under the conditions of the study. Predicated on the different specific gravity values for the tested materials, these materials are different formulations. Because no control formulations without AMP were tested, it is not possible to conclude that any adverse reactions are related to AMP.

The oral LD_{50} values in Cox strain albino mice for both AMP and AMP-95 (95% AMP solution) were estimated at 2.15 $\pm$ 0.2 g/kg and 2.4 $\pm$ 0.089 g/kg, respectively, but no experimental details were provided.[13]

Aminomethyl propanediol. Bio-Test Laboratories tested an aerosol spray containing 0.40% AMPD for acute oral toxicity using Charles River albino rats.[33] The rats were divided into groups of 2 males and 2 females for each of 4 dosage groups (no control group was described). The rats received the test material undiluted at the following doses: 10.2, 15.4, 23.1, and 34.6 g/kg. The pH of the test material was 8.7. The animals were observed for 14 days following administration of the test material, at which time all surviving animals were killed and necropsied. Animals that died during the study were also necropsied. One rat in the 15.4 g/kg group died during the study, and none of the rats of the low-dose group died. All rats in the 2 high-dose groups died, with those in the 23.1 g/kg group dying within the first week and those in the high-dose group dying 45 minutes to 3 hours after administration of the test material. The 7- and 14-day LD_{50} doses were both 17.0 $\pm$ 1.7 g/kg. Schafer and Bowles[34] reported an approximate oral LD_{50} for AMPD in the deer mouse of 0.140 g/kg, and CTFA[35] stated that albino mice all survived an oral dose of 5.0 g/kg.

Inhalation

Aminomethyl propanol. A group of 10 Wistar rats, equally divided by sex, were exposed for 1 hour to an atmosphere containing 200 mg/L of a hair spray (particle size not available) containing AMP at a concentration of 0.59%.[36] The test animals were observed for 2 weeks following the exposure. All but 1 rat survived the duration of the study. All survivors gained weight during the study, and all, including the rat that died, appeared normal during the observation period. At necropsy, the left lung of the rat that died (day 3) was adhered to the dorsolateral body wall; none of the other rats had any abnormalities.

In a second study following the above protocol, 3 cosmetic formulations containing 0.58%, 0.59%, and 0.58% AMP (groups 1, 2, and 3, respectively) were tested.[37] All rats survived the 2-week observation period, and all but 1 gained weight (1 rat maintained a steady weight). The rats of groups 1 and 2 appeared normal throughout the observation period, whereas those of group 3 had slightly decreased activity at hour 1 and were normal thereafter. The only abnormality noted upon necropsy was in 1 rat of group 2; all lobes of the right lung were consolidated and had adhered to the ventral body wall. The formulations containing 0.58%, 0.59%, and 0.58% AMP were not toxic by inhalation to rats under the conditions of the study.

A group of 20 Sprague-Dawley rats, 10 of each sex, were exposed for 1 hour to an atmosphere containing 168.2 mg/L of a spray (particle size not available) containing AMP at a concentration of 0.26%.[38] Except for the hour during which they were exposed to the test material, the rats were allowed feed and water ad libitum. After exposure to the test material, the rats were rinsed, dried, and placed in clean cages. The rats were observed during the exposure, and half of the rats of each sex were killed 24 hours later. The remainder of the test animals were observed for 14 days.

During exposure to the test material, all rats had decreased activity and exhibited labored respiration, squinting, and ataxia. The decreased activity, labored/slow respiration, and squinting continued after the exposure; in addition, the rats had depressed righting and placement reflexes. One female rat had tremors and prostration upon removal from the test chamber; another female had intermittent tremors. All rats, with the exception of 1 male rat with a slight nasal discharge, appeared normal at 24 hours. One male rat was wheezing on days 2, 3, and 14. All of the remaining rats appeared normal through the remainder of the observation period. One female rat in the control group was wheezing on days 13 and 14; all other control rats appeared normal.

There were no differences in weights and weight gains between the control and test animals. The kidney weights and ratios of kidney to body weight were significantly higher for the treated rats. No treatment-related lesions were observed at necropsy. One hour of exposure to an atmosphere containing 168.2 mg/L of a spray containing 0.26% AMP caused no significant histopathological changes in rats.[38]

No deaths occurred when rats were exposed for 1 hour to atmospheres containing 200 mg/L of an aerosol (particle size not available) containing AMP at concentrations of 0.25% or 2.5% in alcohol and propellant.[13]

In a study of the effects of metal working fluid components, Detwiler-Okabayashi and Schaper[39] exposed a group of 4 male Swiss-Webster mice to aerosolized AMP (concentration range of 185-1160 mg/m^3; mass median aerodynamic diameter range of 1-2 μm) for 3 hours. The exposure period was followed by a 20-minute recovery period. The mice were observed for sensory irritation and pulmonary irritation during the exposure period and for recovery response and mortality for a week following the exposure. Sensory irritation and pulmonary irritation (measured by evaluating the individual breathing patterns of mice) occurred during the exposure period and recovery response was poor. No deaths occurred in the test group.

Aminomethyl propanediol. An acute inhalation toxicity study of a hair spray containing 0.50% AMPD was performed using 10 male Sprague-Dawley rats.[40] The rats were exposed for 1 hour to an aerosol atmosphere containing approximately 200 mg/L of the hair spray formulation (particle size not available). The animals were observed during exposure and for 14 days thereafter. The rats were weighed before the study and on days 7 and 14. At the end of the study, the rats were necropsied, and tissues were examined microscopically. All rats survived the duration of the study, and body weights and weight gains were normal. The animals had no pharmacotoxic signs during or after exposure to the test material. There was no evidence of toxicity with respect

to organ weights and gross lesions. The results of the microscopic evaluations were unavailable.

Dermal

Aminomethyl propanol. Parekh[41] performed an acute dermal toxicity study of 99.19% AMP using rabbits (strain unknown). Twelve rabbits (3.0 ± 0.5 kg) were divided into 3 groups, with each group consisting of 2 males and 2 females. The abdomens were shaved and 1 of each sex in each group was abraded on the shaved site. Rats received 1000, 1500, or 2000 mg/kg body weight applied to the shaved abdomen and covered with gauze and rubberized cloth. After 24 hours, the patches were removed and the exposure area was cleaned and observed for skin irritation. The animals were further observed for 2 weeks for toxicity. The test was repeated with 8 more rabbits (2.5 ± 0.2 kg, 4 of each sex). After the abdomens were shaved, the skin was abraded in all rabbits and each was topically treated with 2000 mg/kg body weight for 24 hours. After the exposure period, the rabbits were observed for an additional 2 weeks. Upon completion of the observation period, all rabbits from both tests were weighed, killed, and necropsied. After the 24-hour exposure, all intact and abraded skin sites were severely irritated and black in color. The sites became necrotic in 2 to 3 days and remained necrotic for the rest of the observation period. Severe eschar formation was observed by day 14. Rabbits in all treatment groups experienced a loss in body weight over the 2 weeks. No systemic toxicity was observed and the organs at necropsy appeared normal. AMP was determined to be systemically nontoxic but a severe skin irritant. The LD_{50} was greater than 2000 mg/kg.

Short-Term Toxicity

Oral

Aminomethyl propanol. The International Research and Development Corporation (IRDC) conducted a study in which Charles River CD-1 mice were fed AMP in the diet for 8 weeks.[42] Concentrations were 0, 200, 400, 800, 1600, or 3200 ppm; 10 mice of each sex were in each diet group. The mice were observed daily, and weights and feed consumption were recorded weekly. At the end of the study, all mice appeared normal. Livers and gross lesions found in test animals were examined (all of the 3200 ppm mice and 4 mice from each of the other dosage groups). No compound-related gross lesions and no microscopic lesions in the liver were observed.

A similar study was undertaken with Charles River CD rats.[43] The test protocol was the same as in the mouse study except that the dietary concentrations were 0, 1000, 2000, 4000, 8000, or 16 000 ppm.[42] At study termination, the rats of the 16 000 ppm group were emaciated and had rough hair coats, small skin lesions, and loss of hair. Two female rats in the highest dose group died before the end of the study. Alopecia and focal skin erosions were observed in the rats of the 16 000 ppm group, and these were considered compound induced. Microscopically, hepatocyte vacuolation was noted in all rats of 16 000 and 8000 ppm groups and 4 from each of the remaining dose group. This change was considered compound-induced.

Eight beagle dogs were used in a study of the toxic effects of AMP over a 28-day period.[44] AMP was administered in the diet at concentrations of 600, 1800, 5400, or 16 200 ppm to 2 dogs, 1 of each sex for each dose. Feed consumption was recorded daily, and the dogs were weighed once weekly. Hematologic evaluations and urinalyses were performed once before the administration of AMP and at week 4 during the study. Both of the dogs in the 1800 and 16 200 ppm groups, as well as the female dog in the 5400 ppm group, had frequent soft stools or diarrhea. High-dose dogs had marked weight loss and anorexia, and at week 2, both had dry noses and mouths. The male dog of the 5400 ppm group had similar but less severe reactions. All dogs survived the duration of the study. Urinalyses were normal throughout the study. The hematologic changes at 4 weeks included elevated hemoglobin, packed cell volume, and erythrocyte count for the female high-dose dog. The male dogs of the 5400 and 16 200 ppm groups had slight neutropenia. For all dogs, except those of the 600 ppm group, serum glutamate pyruvate transaminase (SGPT) and alkaline phosphatase activities were moderately to markedly increased; for the dogs of the 5400 and 16 200 ppm groups, serum glutamic oxaloacetic transaminase (SGOT) activity was slightly to moderately increased.

No gross lesions attributable to the AMP treatment were found at necropsy. Microscopic lesions in the liver included hepatocytic vacuolation, necrosis of hepatocytes, pigment deposits, centrilobular inflammatory infiltrate, and fibrosis and atrophy of centrilobular parenchymal tissue; these were observed in all dogs except the male exposed to 600 ppm. The damage to the liver, as well as a decrease in liver weight, was dose dependent.

Inhalation

Aminomethyl propanol. An inhalation study was performed with a hair spray containing AMP at a concentration of 0.58%.[45] A group of 16 Wistar rats, 8 of each sex, was exposed to an atmosphere containing 200 mg/L of the hair spray for 1 hour per day, 5 days per week for 2 weeks. Four rats were killed at the end of the first week, another 4 at the end of the second week, and the remainder after a 1-week recovery period. All rats were examined for gross lesions, and the respiratory tissues were preserved for possible microscopic examination. None of the rats died as a result of exposure to the test material. All rats had slightly decreased activity 1 hour after exposure that returned to normal by 3 hours and once again had slightly decreased activity at 24 hours. The rats in the 1-week recovery group appeared normal by day 14 of the study. No gross changes were noted at necropsy, and weight gains were comparable between the test animals and the control group.

Aminomethyl propanediol. No short-term inhalation studies on AMPD were found.

Subchronic Toxicity

Oral

Aminomethyl propanol. In a 90-day study, AMP solutions (pH 7 and 11+) were administered to rats by gavage.[46] At each pH, the AMP solution was administered at doses of 0.5, 0.75, 1.1, or 1.7 g/kg/d. The dosage groups consisted of 20 rats, divided equally by sex. The rats were observed daily, and body weights and feed consumption were recorded weekly. All rats that died during the study were necropsied, and those that survived to the end of the study were killed and necropsied after samples were taken for hematologic, urologic, and clinical chemistry measurements. Because the pH 11+ AMP solution is so different from cosmetic preparations, these results are not discussed here.

The noted behavior changes were hyperventilation and hyperirritability. All surviving rats gained weight and consumed feed in a normal manner, although the test rats did appear to drink more water.

In the pH 7 group, some occurrences of increased SGPT and OCT activities were noted, and the males of the 1.7 g/kg group had significant decreases in packed cell volume and hemoglobin. Urinalyses were performed only on the rats from the pH 11+ group; some samples contained protein. No gross lesions were found at necropsy.[46]

In a 3-month study by Parekh,[47] AMP-hydrochloride (pH 7) was administered to groups of 20 male and 20 female rats (strain unknown) in their diet at concentrations of 0, 2.5, 15, 25, or 250 mg/kg (0, 25, 150, 250, or 2500 ppm). Prior to administration and at 1 and 3 months of the treatment period, urinalyses, hematology, and plasma chemistries were monitored. A complete histopathological examination was performed at study completion (no further details were available). No physical or ophthalmoscopic changes due to treatment with AMP-hydrochloride were observed. Body weight gains, food consumption, hematology, and urinalysis were comparable to the control animals. Rats in both the control and treatment groups became infected with a virus at weeks 5 and 6 of the study, but it was determined that the infection had no effect on the outcomes of the study. At the 1-month observation, animals in the 250 mg/kg dose group had increases in total serum proteins, immunoglobulins, and alkaline phosphatase activity. At the 3-month observation, this same treatment group had increases in SGOT, SGPT, and LDH activities. Livers of 3 males and 4 females in the 250 mg/kg dose group exhibited patchy hepatocellular vacuolization.

For 3 months, groups of 4 male and 4 female beagle dogs were fed diets containing 0.63, 15.0, or 62.5 mg AMP/kg.[48] The AMP was used as AMP-hydrochloride, pH 7.0. The physical conditions and feed consumptions of the dogs were monitored, and urinalyses, hematology, and clinical chemistries were obtained at the start of the study and at 1 and 3 months. At the end of the study, some tissues were examined microscopically (further details not provided in this study). Except for the high-dose group, body weight gains were normal during the study. The high-dose group also had increased activities of SGOT, SGPT, and alkaline phosphatase at 1 and 3 months. The liver weights and ratio of liver to body weight were slightly increased in the dogs of the high-dose and mid-dose groups at necropsy. In addition, 2 females and 1 male of the high-dose group had tan and mottled livers. At microscopic examination, vacuolization, lipid deposits, and bile duct hyperplasia were found in the livers of all of the high-dose dogs and in 1 of the mid-dose dogs. The author stated that no other organs appeared to be affected. No other comments were made about the effects in dogs of 90 days of dietary consumption of AMP.

Inhalation

Aminomethyl propanol. A 90-day inhalation toxicity study of 2 pump sprays (mass median diameters ranging from 4.82 to 7.45 μm), each containing 0.40% AMP, was performed using cynomolgus monkeys.[49] The test animals were divided into groups consisting of 3 males and 6 females each. One group (group 2) was exposed to the test material under static conditions by automatic dispensation of 1 pump sprayer every 7.5 seconds per 10-minute period per day, for a total of 800 sprays per day. The monkeys of group 3 were exposed to the test material following the same spraying regimen but under dynamic conditions (in an air flow of 622 L/min) for the first 25 days, followed by static exposure for the remaining 64 days. The other 2 groups of monkeys were the control group and a group exposed to a different test material. The monkeys were fed after the daily exposure, and water was available ad libitum. All monkeys tested negative for tuberculosis and had clear chest X-rays prior to the start of the study.

The monkeys were weighed prior to the start of the study and weekly thereafter. They were observed daily during and after exposure for signs of behavioral abnormalities or toxicity. Prior to the start of the study and after 89 days, the following respiratory function parameters were assessed: distribution of ventilation, diffusion capacity, mechanics of respiration, mid-maximum expiratory flow, and spontaneous anesthetized tidal volume and respiratory rate. These tests were accomplished by anesthetizing the monkeys and placing them on a whole-body respirator. Hematology and clinical chemistry values were performed on blood samples from each monkey prior to the start of the study and at 30 and 89 days. After 89 days of exposure, the monkeys were killed and necropsied; organ weights were obtained and various organs were preserved for microscopic examination. The monkeys in group 2 were exposed to a mean gravimetric concentration of 6.63 $\pm$ 1.50 μg/L, and the monkeys in group 3 were exposed to a mean gravimetric concentration of 6.06 $\pm$ 1.99 μg/L during the study.

All animals survived the study, and no exposure-related clinical signs were noted. Only the monkeys in group 3 failed to gain weight during the study (body weights were slightly but significantly lower for weeks 3-12). Monkeys in group 3 required a significantly greater number of breaths and cumulative tidal volume to wash out to 2% nitrogen and had a significantly higher pulmonary flow resistance. No significant

hematological differences were noted. The test animals had decreased blood urea nitrogen values and increased SGPT activities compared with the controls at weeks 4 and 13. These differences were not considered significant, because the values were still within the normal range for the species and because there was no microscopic evidence of damage to the affected organs. An increase in serum CO_2 was noted for all test groups, but because there was no evidence of hyperventilation, the authors stated that the cause was believed to be ingestion of the acidic resin, causing a nonrespiratory acidosis.

Group 3 monkeys had increased ratios of liver to body weight that resulted from increased mean liver weights coupled with decreased average body weights. No compound-related alterations were found upon histopathological evaluation of the tissues in the monkeys of groups 2 and 3. No other compound-related adverse effects were reported after 89 days of exposure to atmospheres containing either 6.06 or 6.63 μg/L of hair spray containing 0.40% AMP.

In another 90-day study, groups of 8 cynomolgus monkeys, divided equally by sex, were exposed for 1 hour per day to a hair spray formulation (particle size not available) containing 0.21% AMP.[50] Groups were exposed to high and low concentrations of the hair spray, as well as to the vehicle control. There was a room air control group. The 90-day high and low mean values for the hair spray concentrations were 27.0 ± 3.1 μg/L and 2.73 ± 0.56 μg/L, respectively. No treatment-related effects were noted in body weights, weight gains, organ weights, ratios of organ to body weight, ratios of organ to brain weight, hematology, clinical chemistry, neurologic and ophthalmic parameters, or at necropsy. Histopathologic examination of the pulmonary tissues indicated increased numbers of free macrophages and macrophage aggregates in the alveolar spaces as well as foci of interstitially located particle-laden alveolar macrophages. Inflammation or interstitial fibrosis was not evident. Pulmonary alveolitis was noted in the high-dose hair spray group, and a slight to moderate increase in hepatocellular lipid was noted in all test animals.

In a 13-week inhalation study, CD-Crl:CS(SD)BR Charles River albino rats, 11 of each sex, were exposed to an aerosolized form of a pump hair spray (particle size not available) containing 0.44% AMP for 4 hours per day, 5 days per week for a total of 67 exposures.[51] The control group was a chamber control. The exposure concentration was 0.23 mg/m^3 (calculated to be a 100-fold increase over normal human exposure). The animals were observed daily and weighed weekly, and blood and urine samples were obtained on weeks 7 and 13. The animals were killed after the 67th exposure, gross observations were made, and various tissues and organs were removed for weighing and microscopic study. All animals survived the duration of the study. There were decreases in body weight gains for female rats during weeks 1 to 3 compared with controls, but they were considered within normal limits for the species in this laboratory.

Statistically significant hematologic changes included increased packed cell volume and erythrocyte counts for males at weeks 7 and 13, increased hemoglobin values for males at week 7, and increased packed cell volume for females at week 7. Although these differences were significant with respect to the controls, they were still within the normal range established by the laboratory for the strain of rat used. Male rats had a statistically significant increase in serum glucose concentration at week 7, and females had a significant decrease in blood urea nitrogen at week 13. The authors stated that these differences were not considered toxicologically significant when included with the other study results. No abnormalities were noted in urinalyses, and no lesions were found at necropsy. Female rats had a significant decrease in uterine and lung weights; there were also significant increases in heart- and liver-to-body weight ratios for the females.

No treatment-related microscopic changes were found in the heart or liver; frequency and severity of noted changes were equivalent for both the treated and control rats. The authors stated that microscopic changes observed in the lungs and upper respiratory tract of both the treated and control rats were consistent with chronic murine pneumonia in rats and were unrelated to treatment. The authors concluded that the pump hair spray formulation containing 0.44% AMP was safe under the exaggerated inhalation conditions of the test.

Aminomethyl propanediol. A 13-week inhalation toxicity study in female Chr/CD Charles River albino rats and female outbred Syrian golden hamsters was performed with 2 hair spray formulations (particle size not available) containing 0.135% AMPD.[52] One hair spray formulation also contained 3.00% ethylene maleic anhydride copolymer, 50%; this formulation was referred to as the hair spray, whereas the second formulation, without the ethylene maleic anhydride copolymer, was labeled the hair spray vehicle. Dosage groups consisted of 16 animals of each species. All animals were allowed feed and water ad libitum. The following concentrations were used: 10 mg/m^3 hair spray, 100 mg/m^3 hair spray, 100 mg/m^3 vehicle, and controls. Animals were exposed to the formulations 4 hours per day, 5 days per week for 13 weeks. The aerosol concentrations in the inhalation chambers were monitored hourly and adjusted as necessary; the temperature, pressure, and humidity were also closely monitored. After 32 exposure days, 5 animals of each species from each group were killed. The remaining test animals were killed starting 3 days after the last day of exposure. Blood analyses were performed on all of the test animals. Gross and microscopic examinations were also performed.

During the study, 5 animals either died or were killed when moribund (1 rat and 1 hamster of the low-dose hair spray group, 1 hamster of the high-dose hair spray group, and 1 animal of each species of the vehicle group). The authors stated that none of the deaths were the result of the aerosol treatment. The low- and high-dose hair spray group hamsters had a decreased body weight gain; these values were statistically significant for the hamsters of the high-dose group. The high-dose hair spray hamsters also had lower body weights at the end of the study, but this result was not statistically significant. There were no significant body weight changes in the rats.

In both species, there were scattered incidences of statistically significant differences in various hematology and clinical chemistry parameters, but no dose- or exposure-dependent trends were noted, and so these differences were not considered toxicologically significant. The same was true for the gross observations made at necropsy. The organ weights and histopathological findings did not include any comments on the animals exposed to the hair spray vehicle. The authors concluded that exposure of female Chr/CD Charles River rats and Syrian golden hamsters to atmospheres containing 144 mg/m^3 of a hair spray vehicle containing AMPD at a concentration of 0.135% was not harmful.

Chronic Toxicity

Aminomethyl propanol. A chronic oral toxicity study of AMP in beagle dogs was reported by Griffin.[53] Male and female dogs (number not specified) received 0, 1.1, 11.0, or 110.0 ppm AMP in their diets for a period of 1 year. The dogs were observed daily for general pharmacologic or toxicologic effects. Ophthalmology, hematology, and urinalysis evaluations were performed and serum chemistry was measured before dosing began and after 3, 6, 9, and 12 months of treatment with AMP. Two dogs of each sex and treatment group were killed after 6 months and the remaining animals were killed after the year-long treatment. All dogs were necropsied. No effects in appearance, behavior, food consumption, body weights, vision, blood chemistry, or urine attributable to AMP were observed in the dogs at any dose level. No gross or microscopic effects were observed. An amendment to the study in 1993 reported the details of seizures that occurred in 2 female dogs during the study. The authors stated that because the breed of dogs used is prone to primary epilepsy, AMP likely was not the cause of the seizures. The no observable effect level (NOEL) was reported to be 110.0 ppm or greater.

Aminomethyl propandiol. No chronic toxicity studies on AMPD were found.

Dermal Irritation and Sensitization Irritation

Aminomethyl propanol. A group of 6 rabbits was tested for primary skin irritation to AMP at a concentration of 0.25% in ethanol.[54] This single-insult, occlusive patch test was modified to include abraded and nonabraded skin. The test sites were graded for erythema and edema 24 and 72 hours after patch removal. Neither the abraded nor the nonabraded skin of any of the rabbits had a reaction during the study. The 0.25% AMP in ethanol was not irritating to rabbit skin.

The primary skin irritation potential of 2 formulations containing 0.26% AMP was determined in albino rabbits.[55] The test formulations (0.5 mL) were applied under an occlusive patch to the intact and abraded skin (2 rabbits of each sex per test formulation; 1 rabbit of each sex in each group was abraded). The patch was removed 24 hours later and the sites were graded at 25 hours (1 hour after patch removal) and 72 hours.

With the first formulation, all of the rabbits had erythema at both sites at both time points, with slight desquamation at the 72-hour time point. One rabbit also had edema at the abraded site; this reaction had subsided by 72 hours. The reactions of the rabbits tested with the second formulation were essentially the same. All rabbits had erythema at both time points, with slight desquamation at 72 hours. One rabbit had edema at the abraded site at 25 hours but was negative at 72 hours.

The primary irritation indices (PIIs) for the 2 formulations containing 0.26% AMP were 1.13 and 1.31 (maximum possible score = 8), respectively. The reactions to the second formulation were slightly more severe than those to the first formulation, accounting for the differences in the PIIs. The formulations containing 0.26% AMP were considered mildly irritating to intact and abraded rabbit skin.

Dermal irritation studies were performed on 3 cosmetic formulations containing AMP.[56-58] In each test, 0.5 mL of the formulation was applied under an occlusive patch to the abraded and nonabraded skin of 6 rabbits (3 per sex). After 24 hours, the patch was removed. The test sites were graded upon patch removal and at 72 hours.

A hair spray containing 0.25% AMP caused no irritation to either the intact or abraded skin of rabbits; the PII was 0.0.[56]

The PII of a hair spray containing 0.58% AMP was 0.38.[57] At the 24-hour grading, 3 of the rabbits had erythema at both the intact and abraded sites, whereas the other 3 rabbits had erythema at the abraded sites only. All of the reactions had cleared by 72 hours.

The PII for a hair spray containing 0.59% AMP was 0.35.[58] Four of the 6 rabbits had erythema at both the intact and abraded skin sites at the 24-hour grading. All reactions at 72 hours were negative. The authors concluded that the hair spray containing 0.59% AMP was not a primary dermal irritant.

A study was conducted using the same procedure as described above, in which 3 products containing 0.58, 0.59, and 0.58% AMP had PIIs of 0.75, 1.40, and 0.35, respectively.[59] With the first formulation (0.58% AMP), 5 of the 6 rabbits had erythema at both the abraded and intact sites at 24 hours and the irritation persisted through the 72-hour grading period, with 1 rabbit having edema in addition to the erythema at both sites. With the second formulation (0.59% AMP), 4 rabbits had both erythema and edema at 24 hours. A fifth rabbit had erythema alone, which had subsided by 72 hours. Of the other 4 rabbits with reactions, 1 had no reaction at 72 hours, 1 had erythema only, 1 had increased erythema and continued edema, and the last had increased erythema and edema. All of the reactions noted occurred at both the intact and abraded sites. With the third formulation (0.58% AMP), 1 rabbit had erythema at the abraded site, and 2 rabbits had erythema and edema at both sites at the 24-hour grading. All of the reactions had subsided by 72 hours. None of the formulations were considered primary dermal irritants under the conditions of the test.

In a limited summary, CTFA stated that AMP at concentrations of 0.25% and 2.5% in aqueous and alcoholic vehicles caused no irritation in single insult occluded patch tests in rabbits, but no details were available.[13]

In another study, an unspecified cosmetic formulation containing AMP-95 at a concentration of 0.22% was tested for primary skin irritation potential in a group of 9 rabbits using a single-insult, occlusive patch test procedure.[60] The skin reactions were graded 2 and 24 hours after patch removal. Three rabbits had erythema 2 hours after patch removal; of these 3, 1 had undiminished erythema 24 hours after patch removal. A fourth rabbit had erythema at the 24-hour grading. The group PII for the formulation containing AMP-95 at a concentration of 0.22% was 0.56 (maximum 8.00), leading the authors to conclude that the formulation was minimally irritating.

Aminomethyl propanediol. A hair care product containing 0.715% AMPD was tested in 4 New Zealand albino rabbits for primary dermal irritation.[61] The undiluted test material, 0.5 mL, was applied under an occlusive patch to the intact and abraded skin of each rabbit, where it remained for 24 hours. The sites were graded 1 hour after patch removal and at 72 hours. No adverse reactions were noted. The authors stated that the hair care product containing 0.715% AMPD was nonirritating when applied to intact and abraded rabbit skin.

A hair spray formulation containing 0.50% AMPD was tested for irritation following the protocol outlined in the previous paragraph.[62] Two rabbits had erythema and edema at the intact skin site; the reactions had cleared by 72 hours. One rabbit had erythema persisting through 72 hours at the intact site. The fourth rabbit had no reaction at the intact skin site. At the abraded skin sites, 3 rabbits had erythema and edema; the erythema persisted through 72 hours whereas the edema subsided in all but 1 of the rabbits. The fourth had continuing erythema and no edema. The PII for the hair spray was 1.38.

Sensitization

Aminomethyl propanol. The intradermal sensitization potential of AMP was studied in guinea pigs.[63] Three groups of 10 male guinea pigs each were used in the study: negative control (saline), positive control (0.3% dinitrochlorobenzene, or DNCB), and test group receiving 0.1% AMP. The backs and flanks of the guinea pigs were shaved, and 0.05 mL of the appropriate solution was injected intradermally. The injection sites were graded 24 hours later. At 48 hours, 0.1 mL of the appropriate solution was injected, and the injections were repeated 2 to 3 times a week for a total of 10 injections. Two weeks after the last injection, the animals received challenge injections at a previously untreated site. The challenge injections for the test and control groups consisted of 0.1 mL each of 0.01% and 0.05% solutions of AMP. The challenge sites were chemically depilated 24 hours after the injection; grading of the sites was performed 3 hours later and again at 48 hours. During the first 2 injections of the induction phase, 1% and 0.5% AMP solutions, respectively, caused necrotic lesions, and so the remainder of the induction injections were made with a 0.1% AMP solution. One guinea pig of the test group had a slight reaction at the 24-hour grading of the 0.05% AMP challenge site. This reaction had cleared by 48 hours. No reactions were noted in the test group at the second challenge. At the second challenge with AMP solutions, 4 guinea pigs of the saline control group had reactions to 0.05% AMP and 1 had a reaction to 0.01% AMP. All of these reactions had cleared by 48 hours. The positive control animals had the expected reactions. The authors concluded that AMP was not a sensitizer in guinea pigs.

Another sensitization potential study of AMP in guinea pigs was performed by the International Minerals & Chemicals Corporation.[64] The test was conducted in 3 groups of 10 male guinea pigs (250-300 g).[65] The test group was topically treated on shaved backs and flanks with 0.5 mL of 10% AMP solution applied under an occlusive patch. The positive control group received 0.5 mL of 0.3% DNCB and the negative control received 0.5 mL of saline in the same manner. The patches were removed from all animals 24 hours after the application, the skin was cleaned, and the patch sites were scored for reaction at 24 and 48 hours post application. The procedure was repeated every 48 hours, 2 to 3 times a week, with each group of animals for a total of 10 applications. Following a 2-week rest period, the guinea pigs received challenge patches on virgin sites. The test group and the negative control group were patched with 0.5 mL of 2.5% and 5.0% AMP solution. The positive control group and the negative control group were patched with 0.3% and 0.03% DNCB. After 24 hours, the patches were removed and the sites were cleaned and depilated. Three hours after depilation and 48 hours later, the challenge sites were scored for skin reactions. During the first 2 patches of the induction phase, the 10% AMP solution was found to be mildly irritating to all the animals in the test group. Because of this, the concentration of AMP was lowered to 5% for the remaining 8 topical application. In the positive control group, DNCB caused mild to strong skin reactions at all 10 applications. One animal in the positive control group died on day 6 from a lung infection. During the challenge phase, the test group and the negative control did not have any observable skin reactions from the AMP solution at 24 hours; however, at the 48-hour scoring, 2 animals in the negative control group had mild skin reactions. In the positive control group that was patched with 0.3% DNCB, 9 animals at the 24-hour scoring and 7 animals at the 48-hour scoring had skin reactions. The negative control group had 4 animals at the 24-hour scoring period with skin reactions and none at the 48-hour scoring. It was concluded that AMP was nonsensitizing under these conditions.

Ocular Irritation

Aminomethyl propanol. An ocular irritation study of AMP-Regular and AMP-95 was conducted using Draize techniques.[66] The eyes of 6 rabbits (strain not specified) were instilled with an unspecified amount and concentration of the test materials and were not rinsed. The eyes were scored at 110, the highest score possible, in all rabbits at both the 3-hour and 24-hour evaluation. Vision was destroyed in all the rabbits. Another test group of 6 rabbits received 0.1 mL of materials for either a 15-second or 30-second exposure period followed by a 30-second wash. Scores for AMP-Regular were

69.3 and 89.3 for the 15-second and 30-second exposures, respectively. Scores for AMP-95 were 69.6 and 82.6 for the 15-second and 30-second exposures, respectively. It was concluded that flushing had little beneficial effect following exposure to these materials and that AMP-Regular and AMP-95 were severe ocular irritants.

Twelve New Zealand White rabbits received a single 1-second spray, from a distance of 4 inches, of a hair spray containing 0.25% AMP.[67] The eyes of 6 of the rabbits were rinsed 30 seconds after the spraying. The animals were observed for 3 days. Two of the 6 rabbits of the no-rinse group had signs of irritation. One had slight iritis and conjunctivitis on day 1, with the conjunctivitis continuing through day 2 and clearing by day 3. The second rabbit had slight corneal opacity, iritis, and conjunctivitis; the corneal opacity had cleared by day 2 and the remainder of the irritation had cleared by day 3. Three rabbits of the rinsed group had slight conjunctivitis on day 1, which was cleared by day 2.

A second test following the protocol described in the previous paragraph was performed with a hair spray containing 0.58% AMP.[68] Of the rabbits that did not have their eyes rinsed, 3 had slight conjunctivitis on day 1; the conjunctivitis had cleared by day 2 in 2 of the rabbits and by day 3 in the third. The remaining 3 rabbits of the group had no reactions. Of the rabbits receiving a rinse, 1 had slight corneal opacity and conjunctivitis on day 1. The opacity had cleared by day 2, and the conjunctivitis cleared by day 3. None of the other rabbits of the rinsed group had adverse reactions.

Five New Zealand White rabbits received a single spray of a formulation (pH 8.3) containing 0.26% AMP.[69] The spray was directed from a distance of 6 inches from the left eye; the right eye was untreated and served as a control. Observations of the eyes were made at 1 and 24 hours and at 3, 4, and 7 days post exposure. At 1 hour, 2 rabbits had slight conjunctivitis and dull corneas that cleared by 24 hours. A third rabbit had slight conjunctivitis at 1 hour that also cleared by 24 hours. The fourth rabbit had slight conjunctivitis that persisted through 24 hours and was cleared by day 3. The fifth rabbit had no reaction. All rabbits had negative fluorescein stains 7 days after exposure to the test material. Details pertaining to the control eyes were not available. The spray containing 0.26% AMP was minimally irritating when not rinsed from sprayed rabbit eyes.

A hair spray containing 0.59% AMP was instilled into the eyes of 12 New Zealand White rabbits.[70] The volume of the material tested was 0.1 mL. Six of the rabbits received no eye rinse, whereas the eyes of the other 6 were rinsed 30 seconds after instillation of the test material. The rabbits were observed for 3 days after the instillation. Of the rabbits that did not receive eye rinses, 1 had scattered areas of opacity over most of the cornea as well as slight redness and chemosis on day 1. On day 2, this rabbit had obvious translucent areas over a small part of the cornea, and by day 3 the eye appeared normal. The remaining rabbits in the test group had no ocular reaction. Of the rabbits that received eye rinses, 1 rabbit had scattered areas of opacity over a small portion of the cornea and moderate chemosis, both of which had cleared by day 2. None of the other rabbits had adverse reactions. The hair spray containing 0.59% AMP was considered a mild ocular irritant to rabbits under the conditions of the test. Rinsing reduced the extent of the irritation.

In a limited summary, CTFA stated that AMP at a concentration of 0.25% in an aqueous vehicle caused slight transient irritation when instilled in the eyes of rabbits with and without rinsing.[13] The irritation had cleared by days 2 and 4, respectively.

A cosmetic formulation containing 0.22% AMP-95 was tested in 6 rabbits for eye irritation potential.[71] The test material was not rinsed from the eyes of the rabbits, and the reactions were graded on days 1 to 4 and on day 7 after instillation. Three different rabbits had conjunctivitis, 1 each on days 1 to 3. No reactions were observed on days 4 and 7. The formulation containing 0.22% AMP-95 had a mild eye irritation potential according to the Draize classification system.

A bovine corneal opacity and permeability test was performed using a waving gel containing 6.3% AMP.[72] Five bovine corneas were treated with 0.75 mL of test product. Opacity measurements and sodium fluorescein permeability were determined. A corrected mean opacity score was calculated to be 0.5. The corrected mean optical density (permeability measurement) was 0.008. The in vitro score was 0.62 (mild ocular irritant) for the test material. No further details are available.

Because these studies lacked a vehicle control, the irritation cannot be conclusively attributed to AMP. In the absence of other data, however, these results need to be considered.

Aminomethyl propanediol. A hair spray containing 0.40% AMPD was sprayed into the left eye of each of 5 New Zealand White rabbits for a duration of 1 second.[73] The right eyes served as controls. The eyes were observed for signs of irritation at 1 and 24 hours and on days 2, 3, 4, and 7. All of the rabbits had severe iritis and slight conjunctivitis at 1 hour. In 4 of the rabbits this was reduced at 24 hours and cleared by day 2. In the fifth rabbit, the severe iritis persisted, along with the slight conjunctivitis, through day 2 and was cleared by day 3.

A hair care product (0.1 mL) containing 0.715% AMPD was instilled into the left eye of each of 10 New Zealand White rabbits.[74] Half of the rabbits had their eyes rinsed 4 seconds after instillation of the test material. Ocular reactions were graded at 1 and 24 hours and on days 2, 3, 4, and 7. Sodium fluorescein examinations were performed on day 7 as well as at other times during the study as necessary. One rabbit of the nonrinsed group had moderate conjunctivitis at 1 hour, clearing by 24 hours. Two rabbits had moderate conjunctivitis that diminished steadily and was cleared by day 3. One rabbit had moderate iritis and conjunctivitis at 1 hour; the iritis had cleared by 24 hours and the conjunctivitis by day 2. The fifth rabbit had moderate iritis at 1 hour, clearing by 24 hours. In addition, this rabbit had moderate conjunctivitis at 1 hour; this reaction gradually diminished through day 4 and was clear by day 7. Two of the rabbits of the rinsed eye group had moderate iritis and conjunctivitis, with the iritis clearing by 24 hours and

the conjunctivitis diminishing at 24 hours and clearing by day 2. Two rabbits had moderate conjunctivitis at 1 hour, clearing by 24 hours. The fifth rabbit had moderate conjunctivitis, which had diminished at 24 hours and cleared by day 2. The rabbits of both test groups had negative fluorescein dye examinations on day 7.

Reproductive and Developmental Toxicity

Aminomethyl Propanol

A reproductive and developmental toxicity study of AMP hydrochloride salt was performed on groups of 12 male and 12 female CD rats, 8 weeks of age.[75] AMP was administered to rats via diet at doses of 0, 100, 300, or 1000 mg/kg of body weight per day. The males were dosed 2 weeks prior to breeding, during breeding, and after until necropsy on day 38. The females were dosed 2 weeks before breeding and during breeding and gestation until day 4 postpartum. They were killed and necropsied on day 5 postpartum. General toxicity and reproductive effects were evaluated (cage side observations twice daily, clinical examinations weekly), with body weights and food consumption monitored throughout the study. Organ weights were measured, and a histopathological examination of tissues was conducted at necropsy in the adult rats. Litters were measured for size, pup survival, weight, and physical abnormalities after delivery.

No mortalities were observed in any groups. The male rats that received 1000 mg/kg/d in their diet had increases in absolute and relative liver weights. Very slight microvacuolation of periportal hepatocytes was also observed, with and without vacuolization of hepatocytes consistent with fatty change. The male rats in this dose group also had increased absolute and relative kidney weights, but there were no histopathological effects. The female rats in all test groups had increased incidences of microvacuolization of the hepatocytes when compared with the controls, but there were no significant changes in the liver weights. No effects on mating or conception were observed. However, a dose-related increase in embryo resorption was noted. 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 the effects on the liver. Complete litter resorption was seen in all females in the 1000 mg/kg/d dose group. In the 300 mg/kg/d dose group, resorption was 70%. In the control group, resorption was 10%. The 300 mg/kg/d dose group also had decreased litter size, increased pup body weight, and decreased gestation body weight and body weight gain. No treatment-related fetal effects were observed in the 100 mg/kg/d dose group. Litters had no visible morphologic alterations.

Carney and Thorsrud[76] performed a dermal developmental toxicity study of 94.85% AMP in CRL:CD(SD) female rats. AMP (pH ~9.5) was administered once daily (dose volume of 1 mL/kg body weight; each exposure was ~6 hours) to 4 groups of 26 time-mated females (10-11 weeks old, 200-250 g) via dermal wrapping at dose levels of 0, 30, 100, or 300 mg/kg/d from gestation days 6 to 20. The rats were observed twice daily, and body weights and food consumption were monitored. After the wrapping material was removed each exposure period, test sites were graded for erythema, edema, scaling, and fissuring. On the last day of dosing (3 hours after exposure), blood samples were collected from 4 rats in each dose group in order to determine the amount of dermal absorption of AMP. All rats were killed on gestation day 21 and necropsied. The reproductive organs were studied in detail, and the number and position of implantations, viable fetuses, dead fetuses, and resorptions were recorded. Fetuses were examined and measured for variations or malformations, and the sex and fetal body weights were recorded. No signs of systemic toxicity were noted during the treatment period. There also were no significant differences in body weight and feed consumption in the dose groups compared with the control group. Blood sampling indicated that dermal absorption of AMP occurred in a dose-responsive manner. A disproportional increase in mean blood concentration at 300 mg/kg/d was thought to be due to a compromised skin barrier. Localized dermal effects were most pronounced in the 300 mg/kg/d dose group: 1 female had very slight edema from gestation days 6 to 13, and almost all of the rats (92%) in this dose group had slight scaling, with 36% of the rats having moderate to severe scaling during the last half of the treatment period. One, 2, and 7 rats in the 0, 30, and 100 mg/kg/d dose groups, respectively, had slight scaling that was not considered adverse. Scabbing was mainly observed in the 300 mg/kg/d dose group, with 77% of the rats in this group having scabs on up to 25% of the test site. Rats in the lower dose groups did not have scabbing that was considered to be toxicologically significant. At necropsy, there were no significant gross findings. One rat in the 300 mg/kg/d dose group had a hemorrhagic placenta in the right uterine horn that correlated with the occurrence of red vulvar discharge on gestation day 19. No other adverse effects were observed in this rat, which produced a normal litter, so the finding was considered not related to treatment. There were no significant treatment effects on reproductive parameters and fetal development. The small number of malformations observed in the fetuses did not follow a pattern consistent with treatment. The maternal no observable adverse effect level (NOAEL) was 100 mg/kg/d and the NOEL for fetuses was 300 mg/kg/d.

Genotoxicity

Microbial Assays

Aminomethyl propanol. A plate assay mutagenicity test, with and without metabolic activation, was performed using AMP and *Saccharomyces cerevisiae* strain D4 and *Salmonella typhimurium* strains TA1535, TA1537, TA1538, TA98, and TA100.[77] Positive activation and nonactivation controls were used; the controls were positive for either frameshift or base-pair substitution mutations. AMP was tested over a range of concentrations from 0.01 to 5 μL. The high dose produced some toxic effects, and the low dose was below a toxic level.

The results indicated that AMP was not mutagenic, with and without metabolic activation, under the conditions of the test.

Wagner and Bonvillain[78] tested the mutagenicity of AMP (98.81% pure) in a bacterial reverse mutation assay using *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537 and *Escherichia coli* strain WP2 uvrA. The assays were performed with and without the presence of Aroclor-induced rat liver S9.[78] The study was performed in 2 phases: a preliminary toxicity assay, which established the dose ranges used in the second phase, and a mutagenicity assay. Both phases used the plate incorporation method. In both phases, water served as the solvent at 100 mg/mL. In the first phase, the maximum dose of AMP was 5000 μg per plate. No precipitates or significant toxicity was observed in this phase of the study, with and without the metabolic activation. In the second phase, 5000 μg AMP per plate was again the maximum dose. The assay consisted of both an initial and a confirmatory stage. Neither precipitates nor significant toxicity was observed in either stage of this phase, with and without metabolic activation. It was concluded that AMP was negative for mutagenic activity.

Aminomethyl propanediol. RCC NOTOX tested AMPD for mutagenic potential using *S typhimurium* strains TA1535, TA1537, TA98, and TA100 to detect frameshift and base-pair substitution-type mutations.[79] AMPD was tested at concentrations of 100, 333, 1000, 3330, or 5000 μg per plate, with and without metabolic activation. The test was performed twice. There were no dose-related increases in the number of revertants in either study over the concentration range tested, and AMPD was not considered mutagenic under the conditions of the study.

Mammalian Cell Assays

Aminomethyl propanol. The mutagenic potential of AMP was studied by San and Clarke[80] in an L5178Y/TK$^{+/-}$ mouse lymphoma assay with and without metabolic activation. The study was performed in 2 phases, with the first phase, a preliminary toxicity assay, establishing the dose range for the mutagenesis assay of the second phase, which was made up of an initial mutagenesis assay and an independent repeat assay. The maximum concentration of AMP in the first phase was 5000 μg/mL, where substantial toxicity (growth was ≤50% of the solvent control) was observed, with and without metabolic activation. The dose range in the second phase was 500 to 5000 μg/mL. In the initial and the independent repeat mutagenesis assay, no treated cultures had mutant frequencies that were at least 55 mutants per 10^6 clonable cells over the control. There was no dose-response trend in either assay. Cloned cultures exhibited toxicity at doses 1500 μg/mL or greater without activation and at 3500 μg/mL with activation in the initial assay. Toxicity in the independent repeat assay was observed at 2500 μg/mL or greater without activation and at 3500 μg/mL with activation. AMP was concluded to be negative in this mutagenesis assay.

Animal Assays

Aminomethyl propanol. Gudi[81] studied the mutagenic effects of AMP (>95% pure) in a mouse micronucleus assay.[81] The assay was performed in 2 phases: phase I consisted of a pilot assay and toxicity study to set the doses for phase II, the micronucleus assay. In both phases of the study, test and control material were administered at a volume of 20 mL/kg body weight by a single intraperitoneal injection. In the pilot assay, male mice (species and number not specified) were dosed with 1, 10, 100, or 1000 mg of AMP per kilogram of body weight, and both male and female mice were dosed with 2000 mg/kg. Mortality occurred in 2 of 2 male mice in the 1000 mg/kg dose group and in all mice (5/5 of each sex) in the 2000 mg/kg dose group.

In the toxicity assay, male and female mice were dosed with 200, 400, 600, or 800 mg of AMP per kilogram of body weight. Mortality occurred in all mice (5/5 of each sex) in the 400, 600, and 800 mg/kg dose groups and in 3 of 5 males and 5 of 5 females in the 200 mg/kg dose group. Clinical signs following dosing were lethargy in both sexes at all dose levels and piloerection in males and females and crusty eyes in males in the 200 mg/kg dose group. From this toxicity assay, the high dose for the micronucleus test was set at 60 mg/kg (80% of $LD_{50/3}$). Male and female mice were dosed with 16, 30, or 60 mg of AMP per kilogram of body weight in the micronucleus assay. No mortality was observed. Lethargy was observed in both sexes at 60 mg/kg. Bone marrow cells were collected at 24 and 48 hours post treatment and were examined microscopically for micronucleated polychromatic erythrocytes (PCE). Reductions of up to 10% in the ratio of PCE to total erythrocytes were observed in some AMP-treated groups compared with the controls. No significant increases in micronucleated PCE in any AMP treatment groups of either sex were observed ($P > .05$). It was concluded that AMP was not a mutagen in a mouse micronucleus assay.

Clinical Assessment of Safety

Irritation

Aminomethyl propanol. The skin irritation potential of a cosmetic formulation containing 0.22% AMP-95 was examined in a single-insult, occlusive patch test using 15 panelists.[82] One panelist had an equivocal reaction (±), resulting in a group PII of 0.03 (8.0 maximum). The cosmetic formulation containing 0.22% AMP-95 had a negligible primary skin irritation potential.

Aminomethyl propanediol. A hair spray containing 0.40% AMPD was tested for primary irritancy in 15 human subjects.[83] The patches were applied to the arms of the panelists. The test was referred to as a 5-hour, 4-day test with the test beginning on Monday and the readings being made on the mornings of Tuesday, Wednesday, Thursday, and Friday. Four panelists had no reactions. There were scattered instances of questionable responses in 9 panelists, with 7 having 1 questionable response and the remainder having 2 questionable responses. In addition,

1 panelist had slight redness on day 4, and 1 panelist had slight redness on days 2 to 4.

Sensitization

Aminomethyl propanol. A conditioning hair mousse containing 0.22% AMP-95 was tested for allergic contact sensitization potential in 97 panelists.[84] None of the panelists (86 females and 11 males) had skin conditions or medical histories that would interfere with the purpose of the study.

Ten formulations were tested simultaneously; 5 patches were placed on either side of the upper back, next to the midline. Only if there was a severe reaction was a patch removed. Approximately 0.1 mL of the test material was applied to the patch. The patches were applied every Monday, Wednesday, and Friday for 3 weeks. The patches were removed by the panelists 24 hours after application, and the patch sites were graded prior to the application of the new patch. The final induction patch sites were graded prior to the challenge phase of the study, which began week 6 of the study. The challenge sites were graded 24 and 48 hours after patch removal. Thirteen panelists had reactions during the induction phase of the test. Of these 13, 9 had single reactions, 2 had 2 reactions each (patches 4 and 7; 1 and 8), 1 had 3 reactions (patches 1, 6, and 7), and 1 had 4 reactions (insults 2, 3, 8, and 9). All of the reactions that occurred during the induction phase were recorded as barely perceptible. Another panelist had a barely perceptible reaction at the 24-hour grading of the challenge phase; the results of the 72-hour grading were not available. The conditioning hair mousse containing 0.22% AMP-95 did not have allergic contact sensitization potential.

TKL Research, Inc[85] performed a repeated insult patch test (RIPT) in 50 volunteer subjects using a hairstyling gel containing 3.8% AMP. During the induction phase, 0.02 mL was applied to the infrascapular region of the subjects' backs with an occlusive Finn Chamber patch.[85] The patches were removed after 48 hours, the sites were evaluated, and new patches were reapplied until a total of 9 consecutive applications were recorded. During the induction phase, 1 subject had a minimal to doubtful response for patches 6 to 9 and another subject had a minimal to doubtful response for patch 6. After the last patch application, the subjects were given a 10- to 15-day rest period. The challenge phase began in the sixth week of the study, with patches applied to the induction phase sites as well as to virgin sites on the subjects' backs. The patches were removed after 48 hours and the application sites were scored at 48 and 72 hours post application. There was no evidence of sensitization from the hairstyling gel formulation containing 3.8% AMP.

AMA Laboratories, Inc[86] reported on an RIPT evaluating a hair dye base containing 3.5% AMP in 108 subjects. The test material was diluted 50% in distilled water to a final concentration of 1.75%. During the induction phase, semiocclusive patches with 0.2 mL of the test material were applied to the infrascapular region of the back of the subjects for 24 hours. The applications were made every Monday, Wednesday, and Friday until a total of 9 applications were made during 3 consecutive weeks. Following a 10- to 14-day rest period, a challenge patch was applied to a virgin site on the subjects' backs for 24 hours. Reactions were scored 24 and 48 hours after the application for signs of sensitization. No reactions were observed, and it was concluded that the test material was a nonprimary irritant and a nonprimary sensitizer.

Harrison Research Laboratories, Inc[87] performed an RIPT evaluating a hair dye containing 1.5% AMP. Of 120 initial subjects, 108 completed the investigation. In the induction phase of the study, a Webril patch with 0.2 g of the test material was affixed occlusively to the left back of the subjects for 24 hours. Another patch was affixed after a 24-hour rest period (48 hours on weekends) for 9 applications over 3 consecutive weeks. A 2-week resting phase preceded the challenge phase patch that was adhered to the right (virgin) side of the subjects' backs for 24 hours. The site was scored for irritation reactions at patch removal and again at 48, 72, and 96 hours post patching. During the induction phase, 1 subject had a 1+ edema reaction in 2 different patch sites. The remaining patch applications for the induction phase were suspended for this subject. In the challenge phase, this subject exhibited erythema and edema at 72 hours and erythema and dryness at 96 hours. The subject was then given an open patch test with the test material and breakdown products. Dermal sensitization was not sustained during this procedure. Other subjects exhibited a low-level, transient ($\pm$/1) reaction during the induction phase. Another subject had faint, minimal erythema at the 48- through 96-hour observation periods of the challenge phase.

Another RIPT conducted on a body polish (rinse-off) containing 1.625% AMP was reported by Consumer Product Testing Co.[88] Of 114 initial subjects, 105 completed the study. The test material was prepared as a 1% dilution using distilled water. During the induction phase, approximately 0.2 mL of the prepared material was applied to the interscapular region of the subjects with an occlusive patch for 24 hours. The patches were applied 3 times a week for a total of 9 applications. After the final induction patch, subjects were given a 2-week rest period before a challenge patch was applied to a virgin site adjacent to the induction site. The patch was removed after 24 hours, and the site was scored for reaction at 24 and 72 hours post application. It was concluded that the test material containing AMP did not indicate potential for dermal irritation or allergic contact sensitization.

Harrison Research Laboratories, Inc[89] performed an open RIPT with the dyeless base of a hair dye product containing 7% AMP. Of 121 initial subjects, 99 completed the investigation. In the induction phase, approximately 0.2 g of the test material was applied to a test site on the left side of the subjects' backs and allowed to air dry. The subjects were instructed to keep the test area dry and untouched for 24 hours. Another application of test material was made 48 hours after the previous application (72 hours if the application was performed on a Friday) until a total of 9 applications were made. The test sites were observed following each application, and any reactions were scored and recorded. There was a 2-week rest period between the induction and challenge phases of the

study. In the challenge phase, approximately 0.2 g of test material was applied to a virgin site on the right side of the subjects' backs and allowed to air dry. Again, the subjects were instructed to keep the site dry and untouched. The challenge sites were observed 24, 48, 72, and 96 hours post application, and any reactions were scored and recorded. One subject had faint, minimal erythema (±) at the ninth induction reading. This subject did not have any reaction in the challenge phase. No other reactions were observed in any of the other subjects during the induction and challenge phases. It was concluded that the test material containing 7% AMP was not a dermal sensitizer in humans.

The Consumer Product Testing Company[90] performed an RIPT with a body polish (rinse-off) containing 1.65% AMP on 113 subjects (108 subjects completed the study). The test material was prepared as a 1% dilution using distilled water. Approximately 0.2 mL of the prepared material was applied to the interscapular region of the subjects with a semiocclusive patch for 24 hours. The patches were applied 3 times a week for a total of 9 applications during the induction phase of the study. Following a 2-week rest period after the final induction patch, a challenge patch was applied to a virgin site adjacent to the induction patch site. The patch was removed after 24 hours, and the site was scored for reaction at 24 and 72 hours post application. The study did not indicate a potential for dermal irritation or allergic contact sensitization in a test material containing 1.65% AMP.

Aminomethyl propanediol. A cosmetic formulation containing 0.073% AMPD was tested for sensitization potential in a group of 30 human test subjects using an open RIPT.[91] The test material was applied to the arm daily, 4 days per week for 2 weeks, alternating arms daily. In addition, an occlusive patch was applied on the first day of the test. After the 2-week application period, there was a 2-week nontreatment period. After this 2-week period, the test subjects were challenged with a reapplication of the formulation to the test site along with an occlusive patch at an adjacent site. The original patch, challenge patch, and open challenge test sites were read at 24, 48, and 96 hours. No reactions were observed in any of the test subjects. The formulation containing 0.073% AMPD was neither a primary irritant nor a sensitizer, and the formulation was safe under the conditions of the study.

A modified RIPT of a cosmetic formulation containing 0.50% AMPD was performed on a panel of 39 women and 20 men.[92] The test material (0.5 mL) was applied to a semiopen patch on the arm of each panelist every Monday, Tuesday, Wednesday, and Thursday for 2 weeks. The patch sites were graded approximately 24 hours after application. In addition, a closed patch was applied to each panelist on the first day of the study and on the day of challenge. No patches were applied for 2 weeks after the induction phase. On the Monday following the nontreatment period, challenge patches were applied to the original test site and an adjacent site; the second closed patch was also applied at this time. The challenge sites were graded 1, 2, and 4 days after application. Slight erythema was noted at 1 adjacent application site at each of the grading times, but it was not clear whether these reactions occurred in the same panelist. The formulation containing 0.50% AMPD was not a sensitizer under the conditions of the test.[92]

A RIPT study by Consumer Product Testing Company[93] tested for the irritation and sensitization potential of a mascara containing 1.92% AMPD using 113 subjects (107 subjects completed the study). A semioccluded patch was used to apply 0.2 mL of the test material to the interscapular region of the subjects, and the patch was affixed 24 hours before removal. The induction phase consisted of patch applications 3 times a week for a total of 9 applications. Following a 2-week rest period, a challenge patch was applied to a virgin site adjacent to the induction patch site. The challenge patch was removed after 24 hours, and the site was scored for reaction at 24 and 72 hours post application. No indication of potential dermal irritation or allergic contact sensitization by the test material containing AMPD was observed.[93]

Case Studies

Aminomethyl propanol. Two cases of airborne contact dermatitis were described by Cipolla et al[94] in patients who were exposed to AMP 100 in a cosmetic company during production of a hair dye. The patients had periorbital erythema and itching skin, which improved when they were away from their workplace (weekends and holidays). Patch testing with AMP 100 and other substances in the production line was performed on the 2 patients and on 8 asymptomatic subjects from the same company (2 of the 8 were on the same production line as the 2 patients). The dilutions were 0.1%, 0.5%, 1%, 2%, 5%, 10%, and 20% in distilled water and in ethyl alcohol, respectively. The patch tests proved positive (+/++) in the 10% and 20% dilutions of both distilled water and ethyl alcohol in all the subjects.[94]

Summary

AMP and AMPD are substituted aliphatic alcohols used as cosmetic ingredients. 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 containing 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 AMP 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 containing 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 hour per day to a hair spray containing 0.21% 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 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 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.

Discussion

The CIR Expert Panel considered that acute, short-term, subchronic, and chronic oral, inhalation, and dermal toxicity studies are adequate to support the safety of AMP and AMPD with respect to systemic toxicity end points. These ingredients did not produce significant toxicity to the reproductive systems or development of fetuses in animal studies. AMP and AMPD did not demonstrate genotoxicity in bacterial, mammalian cell, or animal assays.

In past ingredient safety assessments, the CIR Expert Panel has expressed concern over N-nitrosation reactions in ingredients containing amine groups. The 2 ingredients in this report, AMP and AMPD, are primary amines that are not substrates for N-nitrosation. However, these ingredients may contain secondary amines as impurities in finished products that may undergo N-nitrosation. Because of the possible presence of secondary amine contamination, the Expert Panel recommends that these ingredients should not be included in cosmetic formulations containing N-nitrosating agents.

In its earlier safety assessment, the Expert Panel determined that the available skin irritation and sensitization data at the time were able to support the safety of AMP and AMPD in cosmetic products up to a concentration of only 1%. The Expert Panel now has considered safety test data for a hairstyling gel containing 3.8% AMP; hair dye bases with 1.5%, 3.5%, and 7% AMP; and body polishes with 1.625% and 1.650% AMP and determined that these test materials did not cause dermal irritation or allergic contact sensitization in human subjects. In addition, the Expert Panel determined that a mascara with 1.92% AMPD did not cause dermal irritation or allergic contact sensitization. Although the reported use concentration of AMPD is 2% in mascara, the Expert Panel considers the new clinical data adequate to support safety to the higher concentration.

After reviewing inhalation toxicity data on AMP and AMPD, the Expert Panel determined that AMP and AMPD can be used safely in hair sprays because hair spray aerosols are nonrespirable.

Conclusion

The CIR Expert Panel concluded that AMP and AMPD are safe as cosmetic ingredients in the practices of use and concentrations as described in this safety assessment.

Authors' Note

The 2009 Cosmetic Ingredient Review Expert Panel members are Wilma F. Bergfeld, MD, FACP, Chair; Donald V. Belsito, MD; Curtis D. Klaassen, PhD; James G. Marks, Jr., MD, Ronald C. Shank, PhD; Thomas J. Slaga, PhD; and Paul W. Snyder, DVM, PhD. The CIR director is F. Alan Andersen, PhD. This report was prepared by Christina L. Burnett, CIR scientific analyst.

Unpublished sources cited in this report are available from the Director, Cosmetic Ingredient Review, 1101 17th Street, Suite 412, Washington, DC 20036, USA.

Conflicts of Interest

No potential conflict of interest relevant to this article was reported. F. Alan Andersen, PhD, and Christina L. Burnett are employed by the Cosmetic Ingredient Review.

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1803 BUILDING
17 December 2012

Dr. F. Allen Anderson
Director, Cosmetic Ingredient Review
1101 17 St NW Suite 412
Washington DC 20036-4702

Dear Dr. Anderson:

In response to the 60 day comment period, The Dow Chemical Company is submitting additional data for the document entitled, "Safety Assessment of Tromethamine as Used in Cosmetics." TRIS AMINO™ is the registered tradename for TRIS[Hydroxymethyl]Aminomethane, aka Tromethamine, from ANGUS Chemical Company, a subsidiary of The Dow Chemical Company. Enclosed you will find two documents. The first document is the completed Tromethamine IUCLID dataset which will be submitted to the European Chemicals Agency (ECHA) to meet REACH registration requirements in 2013 (see http://echa.europa.eu/ for more information). This REACH dossier in the IUCLID data format [International Uniform Chemical Information Database], provides robust summaries of available data including more recent acute oral and dermal testing, skin and eye irritation, and an OECD Reproductive/Developmental study.

The second document is the read across justification for Tromethamine (2-Amino-2-(Hydroxymethyl)-1,3-Propanediol) using 2-amino-2-methyl-1,3-propanediol and 2-amino-2-ethyl-1,3-propandiol as surrogates for some endpoints. Please be advised that twice we have notified the Mark Townsend, EPA, Chief, HPV Chemicals Branch that 2-Amino-2-methyl-1-propanol (AMP) was not a suitable surrogate for Tromethamine. The appropriate surrogate chemicals for Tromethamine (a tri-hydroxy primary aminoalcohol) are the other 2-amino-1,3-propanediols rather than AMP (a monohydroxy primary aminoalcohol). Therefore, any reference to AMP in the safety assessment is inappropriate and should be omitted.

Should you need to discuss any of the information enclosed, please call me at 989-636-9870.

Sincerely,

Brian J. Hughes, PhD, MPH, DABT
The Dow Chemical Company

cc: Carol Eisenmann, Cosmetic Ingredient Review
Linda Troester, ANGUS Chemical Company, a subsidiary of The Dow Chemical Company

KNOELL CONSULT

REACH registration of TRIS AMINO (CAS No. 77-86-1)

Analogue Approach

1.) Hypothesis for the analogue approach

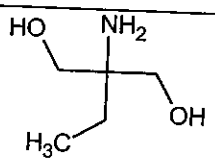
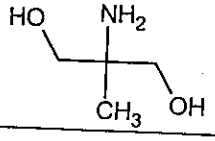
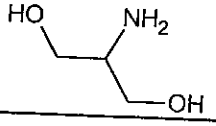
Potential analogues for the substance 2-amino-2-(hydroxymethyl)-1,3-propanediol /TRIS AMINO, CAS No. 77-86-1) are other 2-amino-1,3-propanediols, i.e. substances that share with the target substance a common propane backbone with an amine group at 2-carbon position and primary alcohols at 1 and 3 positions. Candidate source substances are thus members of the aminopropanediol category: 2-amino-2-ethyl-1,3-propanediol (AEPD, CAS No. 115-70-8), 2-amino-2-methyl-1,3-propanediol (AMPD, CAS No. 115-69-5) and 2-amino-1,3-propanediol (APD, CAS No. 534-03-2). The only structural difference between TRIS AMINO and AEPD is a replacement of a hydroxyl group with a methyl group. Further analogues differ in the length of the alkyl side-chain at position 2 so that the following sequence is obtained: from 0 carbon atoms (APD) through 1 (AMPD) to 2 (AEPD). There are no other functional groups present in these molecules.

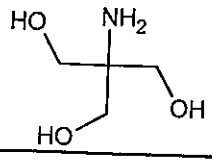
It is expected that the target substance and the source substances share similar physico-chemical properties, as well as properties in regard to environmental fate, environmental toxicology, and mammalian toxicology.
Their class with respect to the mode of action in the environment can be characterised as narcotic aliphatic amines. If TRIS AMINO is absorbed in-vivo, it is not metabolised and rapidly excreted via urine. The available data support this hypothesis and underpin the read-across between target and source substances to fill data gaps while minimising additional animal testing where possible.

The list of the endpoints for which analogue approach is applied:

Environmental fate (biodegradability, photo-transformation in the air, adsorption)
Short term aquatic toxicity to fish
Long term aquatic toxicity to daphnia
Skin sensitisation
Repeated dose toxicity
Genetic toxicity
Toxicity to reproduction
Developmental toxicity

2.) Source and target chemical(s)

Substance	CAS No.	Structural formula
Source chemical No.1		
2-amino-2-ethyl-1,3-propanediol; AEPD	115-70-8	
Source chemical No.2		
2-amino-2-methyl-1,3-propanediol; AMPD	115-69-5	
Source chemical No.3		
2-amino-1,3-propanediol; APD	534-03-2	

Target chemical		
2-amino-2-(hydroxymethyl)-1,3-propanediol, TRIS AMINO	77-86-1	

3.) **Purity / Impurities**

Target substance and source substance are monoconstituents. All known components of the target substance are specified in Sec. 1.2 of the technical dossier and in Sec. 1.1 of the CSR. There are no impurities that could influence the read-across.

4.) **Analogue approach justification**

PHYSICO-CHEMICAL PROPERTIES

Target substance TRIS AMINO as well as source substances AMPD and APD are solids at room temperature. AEPD (melting point: -3 °C) is liquid. Measured boiling point values under ambient pressure are similar (from 259 °C, APD, to 288 °C, TRIS AMINO) for the target substance and source substances except AMPD, where only data at reduced pressure is available probably due to decomposition occurring. Decomposition is reported for all four substances. Relative densities depend on the aggregate state: the lowest value of 1.08 at 20 °C is reported for AEPD which is liquid; the densities of solid TRIS AMINO and APD are about 1.3 and there is no data for AMPD available.
Vapour pressures are low, from ca. 0.0003 Pa (TRIS AMINO, at slightly lower temperature of 20 °C, while the others were measured at 25 °C) to ca. 0.3 Pa (AEPD). Partition coefficients in octanol-water are also low, taking negative values for all substances. As expected, low partition coefficients are accompanied by very high water solubilities: target substance and source substances are miscible with water. All four substances are alkaline, with pKa values between 8.2 (TRIS AMINO) and 9 (AEPD).

ENVIRONMENTAL FATE AND TOXICITY

For environmental fate and toxicity, only AEPD is regarded as source substance.
TRIS AMINO and AEPD are both soluble in water, non-volatile and have low potential for adsorption, thus their main target environmental compartment is water.
In the air, the substances undergo indirect photolysis. Half-lives estimated by QSAR are 11.5 h and 12.6 h for TRIS AMINO and AEPD, respectively (AOPWIN v1.92, 0.5E+6 OH radicals/cm^3, 24h-day).
Sorption to soil or sediment is low. As the substances are primary amines and will be charged to a great extent in the environmentally relevant pH range, the calculation model by Franco and Trapp (2008) taking into account both charged and uncharged species can be applied. The estimated log K_{oc} values are 1.87 and 2.96 for TRIS AMINO and AEPD, respectively.
Hydrolysis is not relevant for neither of the substances, because they do not contain any hydrolysable groups. Amines are known to be hydrolytically stable under environmental conditions.
Both substances are rapidly biodegradable. TRIS AMINO was shown to be readily biodegradable, and with AEPD rapid biodegradation was demonstrated in an inherent biodegradation test (> 90% in 7 days).
Because of very low log K_{ow} values (TRIS AMINO -1.02; AEPD -2.31), significant bioaccumulation in organisms is not to be expected.
As a conclusion, biodegradation by microorganisms in water is the main environmental pathway for both substances.

OECD QSAR Toolbox (v2.3) profiling in regard to aquatic toxicity is presented in Table I.

Profiling	Target chemical, TRIS AMINO CAS No. 77-86-1	Source chemical, AEPD CAS No. 115-70-8
Acute aquatic toxicity MOA by OASIS	Narcotic amine	Narcotic amine
Aquatic toxicity classification by ECOSAR	Aliphatic amine	Aliphatic amine
Organic functional groups (nested)	Alcohol, aliphatic amine (primary)	Alcohol, aliphatic amine (primary)

Table I OECD QSAR Toolbox characteristics of environmental toxicity profiles of target and source substances

Both OASIS and ECOSAR classification models indicate the amine group and narcosis associated with it as the mode of action for all category members, however it is known that for amines the toxicity can be enhanced with respect to baseline and the mode of action can be classified as "amine narcosis". Nevertheless, as in the general case of narcosis (i.e. with absence of specific and reactive effects – what is confirmed by the presence of only amine and alcohol functional groups), log K_{ow} is the main toxicity trigger. This descriptor takes very low values for both substances as discussed before and this fact is reflected in high experimental acute toxicity values for fish, Daphnia and algae (see Data Matrix, Sec. 5).

Short-term studies are available for both TRIS AMINO and AEPD for aquatic invertebrates, algae and microorganisms. With Daphnia magna, EC50 values of > 980 mg/L and 668 mg/L were obtained for TRIS AMINO and AEPD, respectively, indicating that both substances are of low toxicity with TRIS AMINO being even less toxic than AEPD. The EC50 values obtained with algae, 548 mg/L and 397 mg/L, are considered to be in the same range of low toxicity. The NOEC value is however lower for AEPD (31.3 mg/L) than for TRIS AMINO (100 mg/L). Toxicity to microorganisms is low for both substances as well, with EC50 values of > 1000 mg/L and 640 mg/L for TRIS AMINO and AEPD, respectively. In conclusion, although the definite values differ at some endpoints, it is clear that both substances show low toxicity to aquatic organisms, TRIS AMINO being even somewhat less toxic than AEPD. A read-across from AEPD to TRIS AMINO is therefore justified, considering aquatic toxicity.

In the ready biodegradation test (301 F), TRIS AMINO reached 100% degradation in less than 10 days. AEPD is not readily biodegradable, but was rapidly degraded in the inherent biodegradation test (> 90% within 7 days). Since TRIS AMINO is better degradable than AEPD, AEPD .represents the worst case for long-term effects.

The read-across from AEPD to TRIS AMINO for the two endpoints short-term toxicity to fish and long-term toxicity to aquatic invertebrates is justified by the data comparison discussed above as well as by structural and physico-chemical similarity of the two substances.

The NOEC value of 3.99 mg/L for Daphnia determined for AEPD indicates low toxicity concern. Taking into account this fact, as well as all short-term data, rapid biodegradability and low bioaccumulation potential, further testing of long-term toxicity to fish is scientifically unjustified and should not be performed also for reasons of animal welfare.

Terrestrial studies are not available for TRIS AMINO or for potential analogue substances. However, the substances are expected to partition mainly into the aquatic compartment and to be removed quickly due to rapid biodegradation. Additionally, significant bioaccumulation is not likely due to the low K_{ow}, and the data available for aquatic organisms indicates low toxicity concern. Therefore, no terrestrial testing is proposed.

MAMMALIAN TOXICITY

OECD QSAR Toolbox (v2.3) profiling in regard to mammalian toxicity is presented in Table II.

Profiling	Target chemical, TRIS AMINO CAS No. 77-86-1	Source chemical No.1, AEPD CAS No. 115-70-8	Source chemical No.2, AMPD CAS No. 115-69-5	Source chemical No.3, APD CAS No. 534-03-2
Cramer rules (original or with extension)	low (class I)	low (class I)	low (class I)	low (class I)
Protein binding by OASIS	no alert found	no alert found	no alert found	no alert found
Protein binding by OECD	no alert found	no alert found	no alert found	no alert found
DNA binding by OASIS	no alert found	no alert found	no alert found	no alert found
DNA binding by OECD	no alert found	no alert found	no alert found	no alert found
In vitro mutagenicity (Ames test) alerts by ISS	no alerts	no alerts	no alerts	no alerts
In vitro mutagenicity (developmental) alerts by ISS	H-acceptor-path3-H-acceptor	H-acceptor-path3-H-acceptor	H-acceptor-path3-H-acceptor	H-acceptor-path3-H-acceptor
Carcinogenicity (genotox and nongenotox) alerts by ISS	no alerts	structural alert for nongenotoxic carcinogenicity: substituted n-alkyl/carboxylic acid *)	no alerts	no alerts

*) The substance is not a "substituted n-alkyl/carboxylic acid", and thus it can be concluded that the alert implementation in the Toolbox is not precise.

Table II OECD QSAR Toolbox characteristics of mammalian toxicity profiles of target and source substances

Systemic toxicity

In a reproduction/developmental screening test performed according to OECD 421 with TRIS AMINO, the NOAEL for reproduction, fertility and systemic toxicity was set at the highest dose level of 1000 mg/kg bw/day in the absence of systemic effects (Ellis-Hutchings, 2012). Various studies via the oral, dermal, intravenous or intraperitoneal route on TRIS AMINO indicate low acute and repeated dose toxicity. The results of these studies demonstrate that the main cause of toxicity was only the intrinsic alkalinity of TRIS AMINO at the site of contact.

As no 90-day repeated dose toxicity study (according to OECD 408) and no prenatal developmental toxicity study (according to OECD 414) are available for TRIS AMINO, read-across within an analogue approach will be used. Potential analogues for the target substance TRIS AMINO are other 2-amino-1,3-propanediols. Therefore, source substances are members of the aminopropanediol category: 2-amino-2-ethyl-1,3-propanediol (AEPD), 2-amino-2-methyl-1,3-propanediol (AMPD) and 2-amino-1,3-propanediol (APD). TRIS AMINO and the 2-amino-1,3-propanediols are expected to show comparable toxicokinetic characteristics, and it is anticipated that the absorbed amounts of all the aminopropanediols have limited systemic bioavailability and are rapidly eliminated by the kidneys. No relevant metabolism is expected, based on experimental data and on QSAR predictions. The modelling of potential metabolites using the OECD QSAR toolbox v.2.0 (2010) did not predict relevant metabolites of TRIS AMINO or of any of the 2-amino-1,3-propanediols. Therefore, no metabolism by cytochrome P450 enzymes in-vivo is expected.

A combined oral repeated dose toxicity study and reproduction/developmental screening test was performed with AEPD according to OECD 422 (Ishida, 2004). No effects on reproduction or fertility and no systemic toxicity were observed up to and including the highest dose level of 1000 mg/kg bw/day. In a non-guideline developmental toxicity screening study performed in rats, AMPD did not shown any potential for causing developmental toxicity at doses of 1000 mg/kg bw/day (Rasoulpour and Andrus, 2011). Furthermore, in an in-vitro developmental screen (limb bud assay) using AMPD, a lack of developmental toxicity (within the scope of the assay) was indicated (Ellis-Hutchings and Marshall,

2011). Available studies via the oral, dermal or intraperitoneal route on these substances also caused no systemic toxicity. The results of the acute studies, as well as the repeated dose studies, demonstrate that the main cause of toxicity was the intrinsic alkalinity of the respective test substances at the site of contact. Inhalation is of no concern, because the low vapour pressure of the pure substances means that exposure is unlikely to occur. In case of spray applications of technical products containing the neat substance, the concentration is very low (< 1%). The Cramer classification (related mainly to the oral route) also indicates a low toxicological concern for TRIS AMINO and the 2-amino-1,3-propanediols. Thus, both TRIS AMINO and 2-amino-1,3-propanediols are of low concern with regard to systemic toxicity.

Due to the structural similarity and the similar toxicological properties between TRIS AMINO and the 2-amino-1,3-propanediols, read-across using an analogue approach is justified.

In order to meet the standard information requirements according to Regulation (EC) 1907/2006 Annex IX, Column I, 8.6.2, a GLP-compliant subchronic toxicity study according to OECD 408 is proposed with APD. According to Regulation (EC) 1907/2006 Annex IX, Column I, 8.7.2, also a GLP-compliant prenatal development toxicity study (following OECD 414) is proposed with APD. Based on the information that APD represents the basic molecular structure compared with TRIS AMINO, AMPD and AEPD, which contain an additional hydroxyl, methyl or ethyl group, APD has been selected as the most appropriate test candidate. The tests will be performed with APD, and the results will be read-across to TRIS AMINO, based on an analogue approach. This is in accordance with Regulation (EC) 1907/2006, Annex XI, which specifies that read-across of data from a suitable substance may be used to avoid unnecessary animal testing.

Skin/eye irritation/corrosion

TRIS AMINO is not irritating to the skin and the eye. The analogue substances AEPD and APD are not skin irritants, but AEPD and APD are corrosive in contact with the eye. No data are available for eye irritation with AMPD. However, read-across for irritation/corrosion is not applied.

Skin sensitisation

Experimental results in regard to skin sensitisation are available with AEPD and AMPD. These findings (negative, "non-sensitising") can be read-across to TRIS AMINO. Furthermore, there are no structural alerts indicating possible interaction of TRIS AMINO and the 2-amino-1,3-propanediols with skin proteins (see Table II). Therefore, read-across using an analogue approach is justified based on molecular similarity and on absence of substructures indicating a sensitisation potential.

Genetic toxicity

All available studies with TRIS AMINO and the analogue substances are negative. With respect to molecular structures, no mutagenic potency was predicted and no structural alerts were detected. "H-acceptor-path3-H-acceptor" was alerted in a large number of molecules and thus of practically no prediction value. With respect to carcinogenicity (no concern for TRIS AMINO and the read-across substances), the alert detected for AEPD is not useful for prediction purposes. The substance is not a "substituted n-alkyl/carboxylic acid", and thus it can be concluded that the alert prediction is not precise. The available data support read-across between analogue substances to cover data gaps. Therefore, read-across from AEPD to TRIS AMINO using an analogue approach is justified. TRIS AMINO has no functional groups associated with carcinogenicity and did not produce any evidence of neoplasia in the available repeated dose toxicity studies.

5.) Data matrix

PYSICO-CHEMICAL PARAMETERS

Endpoint	Target chemical, TRIS AMINO CAS No. 77-86-1	Source chemical No.1, AEPD CAS No. 115-70-8	Source chemical No.2, AMPD CAS No. 115-69-5	Source chemical No.3, APD CAS No. 534-03-2
Molecular weight, g/mol	121.14	119.16	105.14	91.11
Melting point, °C	169	-3	110	52
Boiling point at 1013 hPa, °C	288 (decomposition)	259-260 (decomposition)	151 (at 10 mmHg)	274 (at 1010 hPa, decomposition)
Relative density at 20 °C	1.32	1.08	no data available	1.28

Vapour pressure at 25 °C, Pa	0.000267 (at 20 °C)	0.29	0.159 (QSAR)	0.01
log Kow at 20 °C	-2.31	-1.02	< -0.8	-1.82
Water solubility at 20 °C, g/L	678-689	> 950	2500	859-898
Dissociation constant at 25 °C	8.22	9.03	8.76	8.51

ENVIRONMENTAL FATE AND TOXICITY

Endpoint	Target chemical, TRIS AMINO CAS No. 77-86-1	Source chemical, AEPD CAS No. 115-70-8
Photo-transformation in air, half-life, hours	11.5 (QSAR)	12.6 (QSAR)
Biodegradation	readily biodegradable	rapidly biodegradable
Adsorption, log Koc	1.87 (QSAR)	2.96 (QSAR)
Short-term toxicity to fish, 96h-LC50, mg/L	RA from AEPD: 460	460
Short-term toxicity to daphnia, 48h-EC50, mg/L	> 980	668
Long-term toxicity to daphnia, 21d-NOEC, mg/L	RA from AEPD: 3.99	3.99
Toxicity to algae, 72h-EC50, mg/L	397	548
Toxicity to algae, 72h-NOEC, mg/L	100	31.3
Toxicity to microorganisms, 16h-EC50, mg/L	> 1000, 3 h	640
Toxicity to microorganisms, 16h-EC10, mg/L	> 1000 (3h-NOEC)	300

MAMMALIAN TOXICITY

Endpoint	Target chemical, TRIS AMINO CAS No. 77-86-1	Source chemical No.1, AEPD CAS No. 115-70-8	Source chemical No.2, AMPD CAS No. 115-69-5	Source chemical No.3, APD CAS No. 534-03-2
Acute toxicity, oral LD50, mg/kg bw	> 5000	5000	3500	> 5000
Acute toxicity, dermal LD50, mg/kg bw	> 5000	> 2000	RA from APD and AEPD: > 2000	> 2000
Skin irritation/corrosion	not irritating	not irritating	WoE incl. RA from AEPD and APD: not irritating	not irritating
Eye irritation/corrosion	not irritating	corrosive	no data available	corrosive
Skin sensitisation	RA from AEPD and AMPD: not sensitising	WoE incl. RA from AMPD: not sensitising	WoE incl. RA from AEPD: not sensitising	RA from AEPD and AMPD: not sensitising
Repeated dose toxicity according to Regulation (EC) 1907/2006 Annex	≥ 1000 (based on OECD 421)	≥ 1000 (based on OECD 422)	- (not to be registered)	RA from AEPD: ≥ 1000

VIII, Column I, 8.6.1 NOAEL, mg/kg bw/day				
Repeated dose toxicity according to Regulation (EC) 1907/2006 Annex IX, Column I, 8.6.2 NOAEL, mg/kg bw/day	RA from APD	RA from APD	- (not to be registered)	Testing proposal: 90-day study (OECD 408)
Genetic toxicity	WoE incl. RA from AEPD: negative	negative	WoE incl. RA from AEPD: negative	WoE incl. RA from AEPD and APD: negative
Screening for reproductive/developmental toxicity according to Regulation (EC) 1907/2006 Annex VIII, Column I, 8.7.1, NOAEL, mg/kg bw/day	≥ 1000 (reproduction, systemic)	≥ 1000 (reproduction, systemic, fertility)	≥ 1000 (reproduction, systemic)	RA from AEPD: ≥ 1000 (reproduction, systemic, fertility)
Toxicity to reproduction according to Regulation (EC) 1907/2006 Annex IX, Column I, 8.6.2, NOAEL, mg/kg bw/day	Waiving	Waiving	Waiving	Waiving
Developmental toxicity according to Regulation (EC) 1907/2006 Annex IX, Column I, 8.7.2, NOAEL, mg/kg bw/day	RA from APD	RA from APD	predicted not embryotoxic, in vitro, Limb bud micromass assay	Testing proposal, OECD 414

6.) **Conclusions per endpoint for C&L, PBT/vPvB and dose descriptor**

Classification and labelling

SUBSTANCE NAME	CAS No.	Classification according to Directive 67/548/EEC	Classification according to Regulation (EC) 1272/2008
2-amino-2-(hydroxymethyl)-1,3-propanediol	77-86-1	not classified	not classified
2-amino-2-ethyl-1,3-propanediol (AEPD)	115-70-8	Self classification: Xi; R41; Risk of serious damage to eyes.	Self classification: Category 1; H318: Causes serious eye damage.
2-amino-2-methyl-1,3-propanediol (AMPD)	115-69-5	data lacking	data lacking
2-amino-1,3-propanediol (APD)	534-03-2	Self classification: Xi; R41; Risk of serious damage to eyes.	Self classification: Category 1; H318: Causes serious eye damage.

Conclusion on classification of TRIS AMINO, AEPD, AMPD and APD:

The available data on 2-amino-2-(hydroxymethyl)-propane-1,3-diol (TRIS AMINO) do not meet the criteria for classification according to Regulation (EC) 1272/2008 and Directive 67/548/EEC. The analogue substances AEPD and APD have the same classification according to Directive 67/548/EEC (Xi; R41) and according to Regulation (EC) 1272/2008 (Category 1; H318). Due to the lack of data, classification is not available for AMPD.

PBT and vPvB assessment of TRIS AMINO, AEPD, AMPD and APD

The relevant PBT (persistent, bioaccumulative, toxic) criteria according to the REACH regulation and the corresponding properties of the aminopropanediols are compiled in the following table:

PBT criteria	vPvB criteria	TRIS AMINO	AEPD	AMPD	APD
P-criteria - T1/2 > 60 d in marine water, or - T1/2 > 40 d in fresh- or estuarien water, or - T1/2 > 180 d in marine sediment, or - T1/2 > 120 d in fresh- or estuarien sediment, or - T1/2 > 120 d in soil	**vP-criteria** - T1/2 > 60 d in marine, fresh- or estuarine water, or - T1/2 > 180 d in marine, fresh- or estuarine sediment, or - T1/2 > 180 d in soil	no	no	no	no
B-criteria BCF > 2000 L/kg	**vB-criteria** BCF > 5000 L/kg	no	no	no	no
T-criteria - NOEC < 0.01 mg/L for marine or freshwater organisms, or	Not applicable	no	no	no	no
- carcinogen (cat. 1 or 2), mutagen (cat. 1 or 2), toxic for reproduction (cat. 1, 2 or 3), or		no	no	no	no
- other evidence of chronic toxicity, as identified by the classifications: T, R48, or Xn, R48 according to Directive 67/548/EEC		no	no	no	no

Persistence (P):
A substance is not considered to be persistent if it can be demonstrated that it has potential to degrade (*via* photolysis, hydrolysis and/or biodegradation).
The ready biodegradability of APD and TRIS AMINO was demonstrated in the OECD ready biodegradation test (301 F). Although AEPD does not meet the criteria for ready biodegradability, the inherent study conducted according to the standardised DIN EN 29888 Part 25 guideline resulted in > 90% biodegradation in 7 days, indicating rapid biodegradation. AMPD was shown to be inherently biodegradable, with 96.7% biodegradation in the inherent biodegradability test OECD 302 C.
Therefore, TRIS AMINO, AEPD, APD and AMPD do not meet the Persistent (P) or very Persistent (vP) criteria.

Bioaccumulation (B):
Since the log Kow values of TRIS AMINO, AEPD, APD and AMPD are below 4.5, the substances are not expected to be bioaccumulative, based on the screening criteria for bioaccumulation, stipulated in the ECHA's Guidance on information requirements and chemical safety assessment - Part C: PBT Assessment (2008).

Toxicity (T):
Environmental Toxicity:
According to Annex XIII of REACH Regulation (EC) No 1907/2006, a substance is considered to be toxic in terms of PBT criteria if long-term NOEC values for aquatic organisms are lower than 0.01 mg/L. The lowest NOEC 3.99 mg/L was obtained in a long-term study with D. magna performed with AEPD. Since no long-term aquatic invertebrate studies are available for TRIS AMINO, APD or AMPD, this study was used for the assessment. Thus, TRIS AMINO, AEPD, APD and AMPD are not considered as toxic substances regarding ecotoxicological endpoints.

Human Health Hazards Assessment:
TRIS AMINO is not classified as carcinogenic, mutagenic or toxic for reproduction, nor is there any evidence of chronic toxicity according to Directive 67/548/EEC and Regulation (EC) No 1272/2008. This can be concluded by using TRIS AMINO data and data within the analogue approach.
AEPD is not classified as carcinogenic, mutagenic or toxic for reproduction, nor is there any evidence of chronic toxicity according to Directive 67/548/EEC and Regulation (EC) 1272/2008. The same applies for AMPD and APD. This is concluded by using screening studies and data within the analogue approach. There is no indication of a mutagenic or carcinogenic potential and neither systemic toxicity nor toxicity to reproduction are evidenced for AMPD and APD. Thus, according to Directive 67/548/EEC and Regulation (EC) 1272/2008, AMPD and APD are not classified as carcinogenic, mutagenic or toxic for reproduction and no evidence of chronic toxicity was found.

Thus, TRIS AMINO, AEPD, AMPD and APD are not considered to be toxic, based on the available data on ecotoxicological and toxicological endpoints, and therefore the substances do not meet the toxicity criterion (T).

Conclusion:
According to Annex XIII of the REACH Regulation (EC) No 1907/2006, a PBT substance has to fulfill all three of the criteria for persistence, bioaccumulation and toxicity, as listed in part 1 of Annex XIII, and a vPvB substance has to fulfill all criteria for persistence and bioaccumulation, as listed in part 2 of Annex XIII.
TRIS AMINO, AEPD, AMPD and APD are not identified as PBT or as a vPvB substance on the basis of the relevant data provided and discussed.

Dose descriptors
In general the dose descriptors of the analogue substances which were taken into consideration for hazard assessment were equivalent or similar to the dose descriptors of the substance to be registered.
Adaptations of the dose descriptors were conducted where appropriate. The dose descriptor of the source substances were converted into the corrected dose descriptor of the target substance in a worst case assumption.

There are no uncertainties in the read-across used that need to be addressed.

Contents

Physical and chemical properties

Property	Results	Value used for CSA / Discussion
Physical state at 20 °C and 1013 hPa	white crystalline solid	**Value used for CSA:** solid Visual inspection in the lab. Handbook data is supporting.
Melting / freezing point	169 ± 0.5 °C, assumed to be measured under ambient pressure.	**Value used for CSA:** 169 °C at 1013 hPa DSC method compatible with EU Method A.1 and OECD Guideline 102. Handbook data (171 -172 °C) is supporting.
Boiling point	boiling with decomposition temperature: 288 ± 0.5 °C at ca. 101 kPa	**Value used for CSA:** 288 °C at 1013 hPa DSC method compatible with EU Method A.2 and OECD Guideline 103. QSAR result (291 °C) and handbook data under reduced pressure of 10 mmHg (220 °C) are supporting.
Relative density	relative density: 1.32 at 20.4 °C	**Value used for CSA:** 1.32 at 20 °C Air comparison pycnometer (OECD 109). Available result from a database (0.932) is disregarded due to inconsistency.
Vapour pressure	0.000267 Pa at 20 °C	**Value used for CSA:** 0.000267 Pa at 20 °C Estimated according to ASTM Method E1719 -97. QSAR result (0.0003 Pa at 25 °C) is supporting.
Surface tension	not surface active	Based on the chemical structure surface activity is not expected.
Water solubility	between 678 and 689 g/L at 20 °C (pH not reported)	**Value used for CSA:** 684 g/L at 20 °C Measured in accordance with the ASTM E1148 method. Other reported data from handbooks (from 550 g/L at

Property	Results	Value used for CSA / Discussion
		25 °C to 800 g/L at 20 °C, pH = 10.4) confirm a very good solubility of the substance in water.
Partition coefficient n-octanol/water (log value)	log Kow = -2.31 at 20 °C	**Value used for CSA:** Log Kow (Pow): -2.31 at 20 °C OECD 107 with acceptable restrictions
Flash point	not applicable	The substance is a solid.
Explosive properties	not explosive	**Value used for CSA:** non explosive There are no chemical groups associated with explosive properties present in the molecule.
Self-ignition temperature	No relative self-ignition below the melting temperature of the test item	Procedure designed to be compatible with EU Method A.16.
Oxidising properties	not oxidising	**Value used for CSA:** Oxidising: no On the basis of the chemical structure the substance is incapable of reacting exothermically with combustible materials.
Granulometry	particle size < 100 µm: 19% particle size < 10.0 µm: 2.46% particle size < 5.5 µm: 1.13 %	The method satisfies the requirements of OECD Guideline 110. Particle size < 100 µm: inhalable fraction (screening test, sieve method); particle size < 10 µm: thoracic fraction and particle size < 5.5 µm: respirable fraction (both: definitive test, cascade impactor method).
Stability in organic solvents and identity of relevant degradation products	not relevant	The stability of the substance is not considered to be critical.
Dissociation	pKa = 8.22 at 25 °C	Titration method, no guideline named. Data from a peer reviewed database is

Property	Results	Value used for CSA / Discussion
constant		supporting (8.3 at 20 °C, 8.03 at 25 °C, 7.8 at 30 °C).
Viscosity	not applicable	The substance is a solid.

Toxicological information

Toxicokinetics, metabolism and distribution

Endpoint summary: Toxicokinetics, metabolism and distribution

Discussion

There are only few experimental studies available in which the toxicokinetic properties of 2-amino-2-(hydroxymethyl)-1,3-propanediol (TRIS AMINO) were investigated. Therefore, whenever possible, toxicokinetic behaviour was assessed taking into account the available information on physicochemical and toxicological characteristics of TRIS AMINO according to the "Guidance on information requirements and chemical safety assessment Chapter R.7c: Endpoint specific guidance (ECHA, 2009)".

In its pure state, TRIS AMINO is a white crystalline solid (121.14 g/mol) and is highly soluble in water (684 g/L), but shows low lipid solubility. It has a very low vapour pressure (0.0003 Pa) and the relatively low partition coefficient (log Kow of -2.31) indicates a low potential to accumulate in biological systems. Moreover, TRIS AMINO is a highly alkaline substance with a pH of 10.4 of a 1% solution.
In general, TRIS AMINO is a biologically inert amino alcohol of low toxicity, which buffers carbon dioxide and acids in-vitro and in-vivo. As weak base with a pKa of 7.8 at 37 °C, TRIS AMINO acts as proton acceptor. In-vivo, it supplements the buffering capacity of the blood bicarbonate system, accepting a proton and generating bicarbonate (NH3+/HCO3-), but without forming CO2.
Due to the fact that TRIS AMINO is used in the therapeutic treatment of severe respiratory or metabolic acidosis and in other clinical indications for example salicylate and barbiturate intoxication, pharmacokinetic studies are available. However, since TRIS AMINO is most effective when administered intravenously, pharmacokinetic studies were performed predominantly via the intravenous route.

Absorption and distribution
Animal data
In the study of Brasch and Iven (1981), the pharmacokinetics of TRIS AMINO were investigated in rabbits after intravenous administration (121 mg/kg; pH 7.4). TRIS AMINO was infused in 2 min and blood samples were collected up to 24 h after the infusion. 2 min after the end of infusion, TRIS AMINO concentration in plasma was 550 µg/mL. Thereafter, TRIS AMINO levels declined steadily and equalled 2.9 µg/mL after 24 h. TRIS AMINO exhibited three-compartment characteristics with a half-life of 12.9 h. In erythrocytes, TRIS AMINO levels increased during the first hour after infusion. After 2 h, TRIS AMINO concentrations in erythrocytes were about 2.5 times higher than those in plasma and remained well above plasma levels during the rest of the observation period. The final volume of distribution (3700 mL/kg) was much larger than the volume of total body water (275 mL/kg), indicating accumulation of TRIS AMINO inside cells, but the equilibration between body compartments was slow.
When 14C labelled TRIS AMINO was intravenously administered to nephrectomised rats, the distribution of TRIS AMINO was determined between the extra- and the

intracellular space as a function of time at constant plasma pH of 7.4 (Rothe and Heisler, 1984). The equilibrium in distribution of TRIS AMINO between the extra- and the intracellular space was not observed before 6-12 hours after administration. This indicates that TRIS AMINO permeates very slowly into the intracellular compartment. TRIS AMINO disappears from the extracellular space in a multi-exponential fashion, indicating that it equilibrates with the different body tissues at largely variable rates.
Human data
The pharmacokinetics of TRIS AMINO were also investigated in healthy volunteers (121 mg/kg bw; pH 7.4) and in patients (109-376 mg/kg; pH 10.9) suffering from metabolic acidosis (Brasch et al., 1982). Venous blood samples were collected during the 30 min infusion and after it had ended (5 min – 24 h). At the end of the infusion, in healthy volunteers TRIS AMINO concentrations in plasma averaged 565 µg/mL. There was a bi-exponential decline of plasma TRIS AMINO levels and after 24 h the level was only 3.8 µg/mL. Two-compartment characteristics were observed with a half-life of 6.6 h. TRIS AMINO concentration in erythrocytes rose more slowly, with a maximum 20 min after the end of infusion. After 2 h TRIS AMINO levels in erythrocytes were about 2.5 times higher than those in plasma, and they remained well above the corresponding plasma levels during the rest of the observation period. The final volume of distribution (894 mL/kg) was somewhat larger than the volume of total body water (580- 630 mL/kg), indicating uptake of TRIS AMINO into tissues, but equilibration between compartments was slow.
In contrast to healthy volunteers, the half-life in patients suffering from metabolic acidosis was much longer (16.3-45.6 h) and the final volumes of distribution were much larger (658-5247 mL/kg).
In conclusion, after intravenous administration, TRIS AMINO is rapidly distributed through the extracellular space and slowly penetrates the intracellular space, except for erythrocytes and hepatocytes. A half-life of 6.6 h was calculated for TRIS AMINO in healthy humans.
After intravenous injection of TRIS AMINO, approximately 70% of the administered dose is available in the ionised form at physiological blood pH; if pH is decreased from pH 7.4, the ionised fraction of the drug is increased (Nahas, 1962, 1963; Nahas et al., 1998). While the ionised fraction reacts only with acids in the extracellular fluids, the fraction of the dose which remains unionised at physiologic pH is thought to be capable of penetrating the cell membrane (Nahas, 1962, 1963). TRIS AMINO is not bound to plasma proteins (Holmdahl and Nahas, 1962; Goldberg et al., 1962) and the low lipid solubility of ionised and unionised TRIS AMINO may be the factor that limits the rate of the intracellular uptake.
After oral intake TRIS AMINO is poorly absorbed from the gastro-intestinal tract and acts as a powerful osmotic laxative in humans. When given orally, TRIS AMINO is rapidly ionised by the acid content of the stomach and is not absorbed (Brinkman et al., 1960).

Absorption and distribution based on acute toxicity studies
Based on the lack of toxicokinetic data using the oral, inhalation or dermal exposure route, additional information on the physico-chemical and toxicological characteristics of TRIS AMINO will be given below.
Acute oral toxicity:
Acute oral toxicity studies have been conducted in rats, mice and rabbits. In a study

performed by Kumar (2011), acute oral toxicity testing was carried out using an up and down procedure in rats, according to OECD 425. There was no mortality during the study period and no clinical signs were observed. Histopathological examinations revealed no substance-related findings. Therefore, the LD50 was determined to be > 5000 mg/kg bw. In another study, the acute oral toxicity of a 20% solution of TRIS AMINO was assessed in rats (Giroux and Beaulaton, 1961). The animals were administered 1000, 3000, 5000, 6000 and 7000 mg/kg bw of the test substance by gavage. The mortality was 0/10, 0/10, 3/10, 6/10 and 7/10, listed by increasing dose. The estimated LD50 was ~ 6000 mg/kg bw. Giroux and Beaulaton (1961) also administered TRIS AMINO to mice by gavage, using a 10% TRIS AMINO solution. For dose levels of 1000, 2000, 3000, 5000, 6000 and 7000 mg/kg bw, the mortality was 0/10, 0/10, 0/10, 0/10, 4/10 and 10/10, respectively. The animals that died had muscle tension and breathing difficulties, while the surviving mice were calm. The calculated LD50 was 6100 mg/kg bw. In the study performed by Rubenkoenig (1955), mice were administered 2000, 3500, 5000, 7000 and 10000 mg/kg bw TRIS AMINO. The mortality was 0, 2, 3, 9 and 10, respectively, by increasing dose. 10 animals per dose were used. The estimated LD50 was 5500 mg/kg bw. The effect of a 25% TRIS AMINO solution in water, either pH-neutralised or unchanged, was assessed in rabbits (Machle, 1940). In the animals that died, damage to the gastrointestinal tract associated with the alkalinity of the test substance was observed. The effect of the alkalinity was also apparent in the LD50 levels, which were 1000-2000 mg/kg bw for the unchanged solution and > 5000 mg/kg bw for the pH-neutralised solution.
The study of Darby and Anderson (1966) confirmed that TRIS AMINO orally administered caused diarrhoea in rats and dogs. In general, the findings of the acute oral toxicity studies evidenced that the main cause of acute toxicity was most probably local irritation due to the highly alkaline properties of TRIS AMINO. With regard to the dose administered and the nature of effects observed, systemic bioavailability of the test substance is considered to be rather low via the oral route. Acute oral toxicity in rats, mice and rabbits showed low toxicity and confirmed the previous pharmacokinetic data of TRIS AMINO via the intravenous route in test animals and humans.
In conclusion, when given orally, TRIS AMINO is rapidly ionised by the acid content of the stomach and is therefore poorly absorbed from the gastrointestinal tract. After oral intake, it acts as a powerful osmotic laxative.

Acute inhalation toxicity:
No data on acute inhalation toxicity are available. However, as a consequence of the very low vapour pressure(0.0003 Pa at 20 °C)of TRIS AMINO, inhalation is not considered to be a significant route of exposure. The particle size distribution shows that the inhalable fraction, particles < 100 µm, constitutes 19% of the total(White and Woolley, 2011).Only 1.13% of the particles are < 5.5 µm. Most of the inhaled particles will therefore remain in the upper airways and/or will be swallowed, with few or no particles reaching the pulmonary alveoli.
Industrial and professional workers may be exposed via spray applications containing < 1% TRIS AMINO, meaning that the potential for acute toxicity via the inhalation route will be negligible. The general population is not exposed to the pure substance, but may be exposed to TRIS AMINO in spray applications. However, the concentration will be < 1% during normal handling and use of TRIS AMINO, and therefore the potential for acute toxicity via the inhalation route is considered to be

negligible.

Acute dermal toxicity:
According to OECD 402, dermal acute toxicity was assessed by exposing rats skin to 5000 mg/kg bw TRIS AMINO under semi-occlusive conditions (Kumar, 2011). There was no mortality, and no signs of toxicity were observed during the study period. The necropsy and gross pathological examination revealed no substance-related findings. The test substance did not cause skin irritation effects on the application site. Therefore, the LD50 value is considered to be > 5000 mg/kg bw.
Also, a quantitative study of percutaneous absorption in-vitro was carried out on human abdominal skin placed in a FRANZ diffusion cell (Noel-Hudson, 1993). After 24 h, the percutaneous absorption of TRIS AMINO through human skin was very low regardless of the concentration of the TRIS AMINO solution (10% and 0.1% TRIS AMINO solutions were tested). Less than 1% of the applied dose was found. For both solutions, the maximum value of flux was reached after 4 h and remained essentially constant during the rest of the experiment (totally 24 h). However, the value of flux was about 150 times higher for the 10% solution (6.922 ± 6.179 µg/cm²/h) than for the 0.1% solution (0.039 ± 0.052 µg/cm²/h). After washing, the retention of TRIS AMINO in the epidermis and dermis was also less than 1% of the applied dose. Therefore, TRIS AMINO did not retain in the horny layer but was almost totally eliminated by washing the skin. The washing waters contained more than 90% of the applied dose.
For neat TRIS AMINO (i.e. a solid substance resulting in a basic solution), a QSAR based modelling published by Potts and Guy (1992), taking into account molecular weight and low Kow, estimated a dermal permeability constant Kp of 7.54E-06 cm/h. Similar to the approach taken by Kroes et al. (2007), the maximum flux Imax (Imax = Kp [cm/h] x water solubility [mg/cm³]) was calculated, resulting in dermal absorption of 5.2µg/cm²/h TRIS AMINO. Usually, this value is considered as indicator for a dermal absorption of 40% (Mostert and Goergens, 2011).However, given the fact that experimental data are available, the information on dermal absorption generated from experiments is more relevant for the risk assessment compared to QSAR based calculations. Taking into account that the washing waters contained more than 90% of the applied dose (Noel-Hudson, 1993), a dermal uptake of 10% has to be regarded as a worst case scenario.

In conclusion, all available data from the pharmacokinetic studies and from acute toxicity studies indicated low toxicity of TRIS AMINO. The effects observed were likely due to alkalinity of the test substance. Moreover, it can be assumed that administration of TRIS AMINO via the oral, inhalation or dermal exposure route yields only small quantities that can be absorbed based on the bio- and physico-chemical properties of the test substance. In addition, systemic bioavailability of TRIS AMINO is considered to be rather low. This is in accordance with the low toxicity observed in the acute and repeated dose toxicity studies.

Metabolism
After intravenous administration of TRIS AMINO to animals or humans, no evidence of a metabolism was found (Nahas, 1962, 1963; Brasch et al., 1982). Several studies confirmed that the protonated form of TRIS AMINO was solely detected in plasma and urine of test animals (rat, rabbits and dogs) and humans (Nahas, 1962; Holmdahl and Nahas, 1962; Christensen and Clifford, 1962; Nahas, 1963).

According to the chemical structure of TRIS AMINO, it is also expected that the test substance will remain unmetabolised. Modelling of potential metabolites via OECD QSAR toolbox v.2.0 (2010) confirms this assumption. No relevant metabolites were generated by the liver metabolism simulator, by the skin metabolism simulator or by the microbial metabolism simulator. Based on this information, it seems to be very unlikely that TRIS AMINO will be metabolised by cytochrome P450 enzymes in-vivo. Moreover, available studies on genetic toxicity in-vitro (Ames test, gene mutation in mammalian cells in-vitro, chromosome aberration in-vitro) were negative, indicating that there is no evidence of reactivity under in-vitro test conditions. With respect to skin sensitisation data, there was no evidence of direct protein reactivity which would cause skin sensitisation. Since no interactions with proteins were determined and no relevant metabolites were generated using QSAR modelling, reactivity of the test substance is considered rather unlikely under in-vitro and in-vivo conditions.

Excretion
TRIS AMINO is a highly water soluble substance and its elimination mainly occurs by glomerular filtration in the kidneys after intravenous injections (Linn and Roberts, 1961; Nahas and Reveillaud, 1961; Brown et al., 1961; Thompson et al., 1965; Bräunlich, 1975). Already 30 min after infusion of TRIS AMINO, 25% of the administered dose was found in human urines and after 24 h, 82% had been eliminated in this way (Brasch et al., 1982). However, this was less than the total elimination of TRIS AMINO, which, from the area under the curve, was calculated to be 97% during 24 h. Only insignificant amounts were found in gastric juice and bile (Brasch et al., 1982). No TRIS AMINO was detected in the expired air of nephrectomised cats and dogs, when intravenously given the test substance (Holmdahl and Nahas, 1962). TRIS AMINO is renally excreted in its ionised form at a rate that slightly exceeds creatinine clearance (Nahas et al., 1963; Brasch et al., 1982). The strong correlation observed between the clearance of creatinine and TRIS AMINO confirms that the kidney is the dominant excretory organ for test substance (Brasch et al., 1982). It may take between 24 to 72 h to achieve 80% elimination of TRIS AMINO in healthy humans after intravenous injection (Nahas, 1998; Brasch et al., 1982).
In conclusion, when given via the oral, inhalation or dermal exposure route, TRIS AMINO will only be absorbed in small amounts based on the fact that predominantly the unionised form of TRIS AMINO can permeate the cell membrane and based on its physico-chemical properties. As TRIS AMINO acts as proton acceptor in-vivo, it is rapidly ionised e.g. by the acid content of the stomach and is therefore not absorbed. The limited amounts of TRIS AMINO that are systemically bioavailable are not bound to plasma proteins and exist in the ionised form. At physiological blood pH, TRIS AMINO supplements the buffering capacity of the blood bicarbonate system, accepting a proton. Bioavailable levels of TRIS AMINO are rapidly eliminated by the kidneys and found not metabolised in the urine. No relevant metabolism is expected, based on experimental data and QSAR modelling. Therefore, the potential to accumulate in biological systems is also expected to be low if TRIS AMINO is ingested using the oral, inhalation or dermal exposure route. This is also confirmed by the low toxicity observed in acute and repeated dose toxicity studies.

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Basic toxicokinetics

Endpoint study record: WoE, Brasch and Iven, 1981, pharmacokinetics, rabbit, RL2

Administrative Data

Purpose flag	weight of evidence
Study result type	experimental result
Reliability	2 (reliable with restrictions)
Rationale for reliability incl. deficiencies	Acceptable, well documented publication which meets basic scientific principles.

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	Brasch, H. and Iven, H.	1981	Pharmacokinetics of TRIS (hydroxymethyl-)aminomethane in the Rabbit.	Arch. int. Pharmacodyn. 254:4-12					

Data access

data published

Materials and methods

Type of method

in vivo

Objective of study

distribution

Principles of method if other than guideline

Pharmocokinetics of TRIS were investigated in rabbits after a short-time infusion of a single low dose.

GLP compliance

no

Identity of test material same as for substance defined in section 1 (if not read-across)

yes

Radiolabelling

no

Test materials

Details on test material

- Analytical purity: analytical grade

Test animals

Species

rabbit

Strain

no data

Sex

male/female

Details on test animals and environmental conditions

TEST ANIMALS- Weight at study initiation: 3600-4400 g

Administration / exposure

Route of administration

intravenous

Vehicle

no data

Duration and frequency of treatment / exposure

24 h

Doses / concentrations

121 mg/kg (1 mmol/kg)

No. of animals per sex per dose

6

Control animals

no

Details on dosing and sampling

PHARMACOKINETIC STUDY- Tissues and body fluids sampled: blood- Time and frequency of sampling: 2, 4, 8, 15 and 30 min, 1, 2, 3, 4, 6, 8, 12 and 24 h- Method type(s) for identification: Hewlett-Packard 5710A GC equipped with a FID together with a Hewlett Packard 3380A integrator

Statistics

The experimental data were fitted by mulitexponential functions, using the method of least squares. The kinetic parameters of two- and three compartment models were calculated according to Gibaldi and Perrier (1975) and Wagner (1975) with a Hewlett-Packard 9820 A desk top calculator.

Any other information on materials and methods incl. tables

The rabbits were anaesthetised with pentobarbital-Na (30 mg/kg) and a catheter was implanted into the right jugular vein. 24 h later, TRIS was infused in 2 min and a 0.3 mol/L solution titrated to pH 7.4 with HCl was used. After the end of infusion, blood samples of approximately 2 mL were collected. The heparinised blood was centrifuged and plasma and erythrocytes were kept frozen separately until analysis. One week later experiments were repeated using a 0.3 mol/L solution of TRIS pH 10.9. In 5 of the 6 experiments in each series the solution in addition to TRIS contained 30 mg/mL inulin for the determination of the extracellular space.

The determination of TRIS concentrations was carried out via a gas chromatographic assay, a slightly modified version of the method described by Hulshoff and Kostenbauder (1979).

Plasma inulin levels were determined with the anthrone-reagent according to Handelsman and Drabkin (1954).

Literature:

Hulshoff and Kostenbauder, 1979, J. Chromatogr. 145: 155 -159

Handelsman and Drabkin, 1954, Proc. Soc. exp. Biol. Med. 86: 356 -360

Results and discussions

Main ADME results

Type	
distribution	A half-life of 12.9 h was calculated. The final volume of distribution (3641 mL/kg) indicated drug accumulation inside cells, but equilibration between compartments was slow compared to drug elimination.

Pharmacokinetic studies

Details on distribution in tissues

TRISTwo min after the end of infusion the TRIS (pH 7.4) concentration in plasma was 550 µg/mL. The drug concentration then declined steadily and was only 2.9 µg/mL after 24 h. The drug exhibited three-compartment characteristics with long terminal half-life (12.9 h). TRIS concentration in erythrocytes increased during the first hour after infusion. After 2 h drug levels in erythrocytes were about 2.5 times those in plasma and remained well above plasma levels during the rest of the observation period. This was found in all animals.To calculate pharmacokinetic parameters a linear three-compartment open model with elimination from the central compartment was used. With these parameters the drug concentrations in the peripheral compartments were simulated. The final volume of distribution (3641 mL/kg) indicated drug accumulation inside cells, but equilibration between the body compartments was slow compared to drug elimination. The rate constants of distribution to the peripheral compartments (0.5 h and 0.3 h) were smaller than the elimination constant (0.9 h). A reasonable correspondence between the expected concentrations in the small fast-exchanging compartment and the drug levels observed in erythrocytes was obtained. The large discrepancies during the first minutes after infusion, when irregular changes of TRIS concentrations were found, may be due to a partial equilibration between plasma and erythrocytes in the time between drawing a blood sample and centrifuging it. To evaluate a possible influence of the pH of the TRIS solution on drug distribution, the experiments with TRIS solution of pH 10.9 were repeated in the same animals. The results were the same as with TRIS solution of pH 7.4 (data of pH 10.9 not shown in the publication).InulinA biexponential decline of inulin plasma levels was found in experiments with neutral alkaline TRIS. A half-life of 42 min and a final volume of distribution of 275 mL/kg was calculated.

Metabolite characterisation studies

Metabolites identified

not measured

Any other information on results incl. tables

The volume of the central compartment (223 mL/kg) was similar to the volume of the inulin space (data not given). Therefore the authors concluded that TRIS initially distributes to the extracellular space. The final volume of distribution of TRIS (3641 mL/kg) however, is much larger than the inulin space (275 mL/kg), which can only be

explained by an accumulation of the drug inside cells.

Intracellular accumulation would be expected from the physicochemical properties of TRIS. Since the drug is a weak base with a pKa-value of 7.92, 70% of the administered amount exists in the cationic form at normal plasma pH. Assuming that only the undissociated from can pass the cell membrane, the normal transmembrane pH-gradient may create a 2 -3 -fold higher intracellular drug concentration due to pH-partitioning. The observed drug levels in erythrocytes and the drug concentrations calculated for the peripheral compartments fit into this concept. In situations of elevated extracellular pH the accumulation of TRIS inside cells should be even greater. But the buffer capacity of plasma probably prevented a long-lasting rise of extracellular pH after infusion of TRIS solution of pH 10.9 and thus the results obtained with both solutions were identical.

Applicant's summary and conclusion

Interpretation of results

bioaccumulation potential cannot be judged based on study results

Dermal absorption

Endpoint study record: Noel-Hudson, M.S., 1993, in-vitro, human skin, RL2

Administrative Data

Purpose flag	weight of evidence
Study result type	experimental result
Reliability	2 (reliable with restrictions)
Rationale for reliability incl. deficiencies	Acceptable, well documented study report which meets basic scientific principles.

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report	Noel-Hudson, M.S.	1993	Percutaneous absorption of tromethamine hydrochloride. Study on human skin in in-vitro diffusion cells.		Laboratoire de pharmacologie, Unité de recherche en dermopharmacologie, 5, rue Jean-Baptiste Clément, 92296 Châtenay-Malabry Cedex, France	DR 00/7-3524-100	Laboratoires Lancôme, 188 rue P. Hochart, 94150 Chevilly-Larue, France		1993-08-09

Data access

data submitter has Letter of Access

Materials and methods

Type of method

in vitro

Principles of method if other than guideline

Percutaneous absorption of tromethamine hydrochloride was studied in 0.1% and 10% solution in-vitro in 4 samples of human abdominal skin, in a FRANZ diffusion cell.

GLP compliance

no data

Test materials

Radiolabelling

yes (carbon-14 labelled tromethamine solution)

Identity of test material same as for substance defined in section 1 (if not read-across)

yes

Details on test material

- Analytical purity: no data

Test animals

Species

other: in-vitro, human abdominal skin in a FRANZ diffusion cell

Strain

other: in-vitro, human abdominal skin in a FRANZ diffusion cell

Sex

no data

Administration / exposure

Type of coverage

other: not applicable

Vehicle

no data

Doses

- Concentration: 0.1% (1 kg/L) and 10% (100 kg/L)- Dose volume: 100 µL

No. of animals per group

not applicable

Details on in vitro test system (if applicable)

SKIN PREPARATION- Source of skin: human skin comes from biopsies obtained after plastic surgery of the abdomen- Type of skin: human abdominal skin- Preparative technique: skin is cut with a dermatome- Thickness of skin (in mm): 0.3- Storage conditions: stored frozenPRINCIPLES OF ASSAY- Diffusion cell: FRANZ diffusion cell- Flow-through system: jacket with double circulation of water surrounds the lower part- Test temperature: 37 °C

Any other information on materials and methods incl. tables

The quantitative study of percutaneous absorption in-vitro is carried out on dermatomised human skin placed in a FRANZ diffusion cell that permit contact of the dermis with a survival liquid in which the absorbed substance is determined (Franz, T.J., 1975, J. Invest. Dermatol. 64: 190 -195). The tests were carried out on 4 skin samples of different origin for each concentration studied: skin 1 and 4: mulatto skin; skin 2 and 3: white skin.

The cutaneous biopsy is maintained horizontally between the two parts of the FRANZ diffusion cell, thus confining the two parts of the sample to two compartments: one for the epidermis, which consists of a glass cylinder with a precisely defined surface (0.635 cm2), deposited on the upper surface of the skin; the other is for the dermis, on the lower surface of the tegument, which involves a reservoir with a fixed volume that has a lateral adjustment. The lower compartment (dermis) is filled with a survival liquid consisting of a sodium chloride solution with bovine serum albumin. The temperature in the lower part was 37 °C.

The upper part was open toward the outside, thus exposing the surface of the epidermis to the ambient air of the laboratory as in the case of an in-vivo application. The temperature on the surface of the biopsy was between 32 °C and 33 °C.

At regular time intervals (2, 4, 6, 8, 10 h), the totality of the liquid contained in the dermal compartment is taken by lateral adjustment and replaced by new liquid.

At t = 24 h, the survival liquid is removed and the surface of the biopsy is washed with 100 µL of different solvents, according to the following protocol: 1st, Cetavlon/doubly-distilled water; 2nd, doubly-distilled water; 3rd, Cetavlon/doubly-distilled water; 4th, doubly-distilled water; 5th, doubly-distilled water.

The epidermis and the dermis are separated mechanically and digested in Soluene TM (Packard) for 24 h at 37 °C. The detection of the carbon-14 labelled tromethamine molecule is done with a liquid scintillation counter, Packard Tri-carb 4530.

The intensity of percutaneous absorption is evaluated by calculating the percentage absorption of the quantity deposited during the time. The mean flux for each time interval is determined and expressed as µg/cm²/h.

The mean results correspond to at least 10 experimental determinations and are assigned a standard deviation.

Results and discussions

Any other information on results incl. tables

After 24 h, the percutaneous absorption of tromethamine hydrochloride through human abdominal skin in-vitro was very low, regardless of the concentration of the solution. The mean percentages of the applied tromethamine hydrochloride dose that had passed into the survival liquid were 0.506 ± 0.765% for the 0.1% solution and 0.797 ± 0.691% for the 10% solution. These differences are not significant.

For both solutions, the maximum value of flux was reached after 4 h and remained essentially constant during the rest of the experiment. However, the value of flux was about 150 times higher for the 10% solution (6.922 ± 6.179 µg/cm2/h) than for the 0.1% solution (0.039 ± 0.052 µg/cm2/h).

After washing, the retention of the test substance in the epidermis and dermis was low: For the 0.1% solution, 0.14 ± 0.19% tromethamine hydrochloride remained in the epidermis and 0.69 ± 1.33% in the dermis. For the 10% solution, 0.13 ± 0.21% tromethamine hydrochloride was detected in the epidermis and 0.22 ± 0.21% in the dermis. Therefore, the test substance is not retained in the horny layer (epidermis).

The test substance was almost totally eliminated by washing the skin after 24 h. For the 0.1% solution, 91.13 ± 4.67% tromethamine hydrochloride was found in the washing waters and for the 10% solution, a quantity of 90.45 ± 4.06%.

However, significant differences in permeability from one skin sample to the other were recorded. (The experiments were carried out on 4 skin samples of different origin: mulatto skin; skin 2 and 3: white skin.) The differences of the individual measurements of the skin samples were lower for the 0.1% solution.

At the end of the 24 h, regardless of the concentration of the solution, the radioactivity found in the epidermis and the dermis was low (less than 1% of the applied dose) and the washing waters contained more than 90% of the applied dose.

Acute Toxicity

Endpoint summary: Acute Toxicity

Key value for chemical safety assessment

Acute toxicity: oral
Effect level LD50 **in mg/kg bw** >5000
Acute toxicity: dermal
Effect level LD50 **in mg/kg bw** >5000

Discussion

Acute toxicity: oral
The acute oral toxicity of 2 -amino-2 -(hydroxymethyl)-1,3 -propanediol (TRIS AMINO) was assessed in a study performed according to the up-and-down procedure (OECD 425) in rats administered 5000 mg/kg bw (Mohan Kumar, 2011). Single animals were administered the limit dose stepwise, up to a total of 3 rats. There was no mortality, no clinical signs were observed during the 14-day observation period and no effects on the body weight were noted. There were no substance-related findings of the histopathological examination. The LD50 is considered to be > 5000 mg/kg bw.

In addition, several pre-guideline studies with rats (Giroux and Beaulaton, 1961), mice (Giroux and Beaulaton, 1961 and Rubenkoenig, 1955) and rabbits (Machle, 1940) are available confirming the low systemic acute oral toxicity of TRIS AMINO.

Acute toxicity: dermal
In a study performed according to OECD 402, 5000 mg/kg bw TRIS AMINO was applied to the shaved skin of rats and held under a semi-occlusive dressing for 24 hours (mohan Kumar, 2011). There was no mortality, and no signs of toxicity or effects on body weight were observed during the study period. No substance-related findings were noted during the gross pathological examination and the test substance did not cause skin irritation effects on the application site. The LD50 is considered to be > 5000 mg/kg bw.
Acute toxicity: other routes
A number of studies were performed to determine the acute LD50 in several species and via several routes.
The LD50 (intravenous) of TRIS AMINO for rats varied from > 500 to 2300 and 3500-3600 mg/kg bw, (Darby and Anderson, 1966; Giroux and Beaulaton, 1961; Thompson, 1965). Severe dose-related damage to the kidneys was observed at the highest value (Giroux and Beaulaton, 1961). In mice, the LD50 (i.v.) ranged from 1044 – 1980 mg/kg bw, decreasing inversely with the pH (Darby and Anderson, 1966; Giroux and Beaulaton, 1961;Thompson, 1965) The LD50 (i.v.) for rabbits was reported to be > 500 mg/kg bw; while for dogs the LD50 > 125 mg/kg bw, following a single dose (Giroux and Beaulaton, 1961), and approximately 500 mg/kg bw with a slow perfusion (Darby and Anderson, 1966). Lower blood glucose levels were observed following i.v. perfusions of non-lethal doses of TRIS AMINO in dogs and in

humans; the latter also reporting transient discomfort. Temporary changes in respiration rate and depth were the only effects observed in dogs perfused with up to 10 mg/kg bw (Giroux and Beaulaton, 1961). The LD50 (intraperitoneal) values of TRIS AMINO reported for mice were 790 and 3350 mg/kg bw, respectively (Giroux and Beaulaton, 1961; Rubenkoenig, 1955). For dogs, the LD50 (i.p.) was > 2160 mg/kg bw (Darby and Anderson, 1966). Subcutaneous injections of 1000 mg/kg bw TRIS AMINO in rats and did not cause mortality (Giroux and Beaulaton, 1961).

The test substance-related findings were comparable for oral and dermal routes of application in the acute toxicity studies, with the high LD50 -values indicating that TRIS AMINO has a very low potential to cause acute toxicity via these routes.

Justification for classification or non-classification

The available data on acute toxicity of the test substance do not meet the criteria for classification according to Regulation (EC) 1272/2008 or Directive 67/548/EEC, and are therefore conclusive but not sufficient for classification.

Acute toxicity: oral

Endpoint study record: Machle et al, 1940, rabbit, RL4

Administrative Data

Purpose flag supporting study

Study result type experimental result

Reliability 4 (not assignable)

Rationale for reliability incl. deficiencies Original report not available and documentation insufficient for assessment

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	Machle, W. et al.	1940	The physiological response of animals to some simple mononitroparaffins and to certain derivatives of these compounds	Journal of Industrial Hygiene and Toxicology, Oct. 1940; Vol. 22(3): 315-332					

Data access

data published

Cross-reference to same study

7.2.3 Machle et al, 1940,rabbit, RL4; 7.3.1 Machle et al, 1940, RL4

Materials and methods

Test type

standard acute method

Limit test

no

Principles of method if other than guideline

To estimate the oral LD50 of the test substance, a 25% solution (pH neutralised or not pH neutralised) was administered to rabbits. The skin effects, clinical signs and body weight were monitored during the study period; and gross pathology was performed at necropsy.

GLP compliance

no (performed prior to GLP)

Test materials

Identity of test material same as for substance defined in section 1 (if not read-across)

yes

Details on test material

- Name of test material (as cited in study report): 2-amino-2-methylol-1,3-propanediol

Test animals

Species

rabbit

Strain

no data

Sex

no data

Details on test animals and environmental conditions

TEST ANIMALS- Housing: Animals were housed individually

Administration / exposure

Route of administration

oral: gavage

Vehicle

water

Details on oral exposure

VEHICLE- Concentration in vehicle: 25% w/v

Doses

Test 1: 25% w/v in waterTest 2: 25% w/v in water and neutralised with hydrochloric acid

Control animals

yes

Details on study design

- Duration of observation period following administration: Surviving animals were observed for 2-3.5 months- Frequency of observations and weighing: Animals were observed outside the cage for 2-3 hours before returning the animals to the cage, thereafter observed at unknown frequencies. The animals were weighed during the observation period, but the frequency is not reported. - Necropsy of survivors performed: Yes. Gross pathology was performed on all animals.- Other examinations performed: Clinical signs

Results and discussions

Effect levels

Sex	Endpoint	Effect level	Based on	95% CL	Remarks
no data	LD50	1000 — 2000 mg/kg bw	test mat.		25% w/v in water
no data	LD50	> 5000 mg/kg bw	test mat.		25% w/v in water, pH-neutralised

Mortality

The test substance caused mortality.

Clinical signs

The animals became weak and depressed following administration of the test substance. The animals that died then collapsed and became comatose.

Body weight

The body weight did not change markedly compared to the control group.

Gross pathology

In animals that died, hemorrhage and ulceration of the gastric mucosa was observed, with considerable associated congestion of the enteric tract. The surviving animals did not exhibit similar lesions. The damage, therefore, was mainly related to the alkalinity of the substance.

Endpoint study record: Giroux and Beaulaton, 1961, mouse, RL2

Administrative Data

Purpose flag	supporting study
Study result type	experimental result
Reliability	2 (reliable with restrictions)
Rationale for reliability incl. deficiencies	Basic data given

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	Giroux, J. and Beaulaton I.-S.	1961	Recherches Pharmacologiques Sur Le THAM.	Societe Pharmacie Montpellier. 21:206-217					

Data access

data published

Cross-reference to same study

7.2.1 Giroux and Beaulaton, 1961, rat, RL2; 7.2.4 Giroux and Beaulaton, 1961, iv, dog, RL2; 7.2.4 Giroux and Beaulaton, 1961, iv, 1 and 4%, mouse, RL2; 7.2.4 Giroux and Beaulaton, 1961, iv, 1%, mouse, RL2; 7.2.4 Giroux and Beaulaton, 1961, iv, rabbit, RL2; 7.2.4 Giroux and Beaulaton, 1961, iv, rat, RL2; 7.2.4 Giroux and Beaulaton, 1961, subcutaneous, rat, RL2; 7.2.4 Giroux and Beaulaton, 1961, subcutaneous, mouse, RL2; 7.5.1 Giroux and Beaulaton, 1961, 35d, rat, RL4

Materials and methods

Test type

standard acute method

Limit test

no

Principles of method if other than guideline

To estimate the oral LD50 of the test substance, a 10% solution was administered to mice. The mortality, clinical signs and urine production were monitored.

GLP compliance

no (performed prior to GLP)

Test materials

Identity of test material same as for substance defined in section 1 (if not read-across)

yes

Details on test material

- Name of test material (as cited in study report): Tris(hydroxymethyl)aminomethane, 2-amino-2-hydroxymethyl-1,3-propanediol, tromethamine, THAM- Physical state: White, crystalline substance provided by Abbott Laboratories

Test animals

Species

mouse

Strain

Swiss

Sex

no data

Administration / exposure

Route of administration

oral: gavage

Vehicle

no data

Details on oral exposure

The doses were determined in a pilot study, where rats were administered 1000 and 3000 mg/kg bw in a 50% solution; and mice were administered 1000, 2000 and 3000 mg/kg bw in a 5% solution. 10 animals per dose level were used. There was no mortality, no clinical signs and the animals had a normal urine production.In the main study, the mice were dosed with a 10% solution.

Doses

1000, 2000, 3000, 5000, 6000 and 7000 mg/kg bw

No. of animals per sex per dose

10 animals per dose

Control animals

no data

Details on study design

- Other examinations performed: Clinical signs, urine volume

Statistics

The LD50 was calculated using the formula of Behrens and Karber.

Results and discussions

Effect levels

Sex	Endpoint	Effect level	Based on	95% CL	Remarks
no data	LD50	6100 mg/kg bw	test mat.		

Mortality

In the 1000, 2000, 3000, 5000, 6000 and 7000 mg/kg bw groups, the mortality was 0/10, 0/10, 0/10, 0/10, 4/10 and 10/10

Clinical signs

The animals were calm, urinated frequently, and the animals that died had reduced muscle tension and breathing difficulties.

Endpoint study record: Rubenkoenig, 1955, mouse, RL2

Administrative Data

Purpose flag	supporting study
Study result type	experimental result
Reliability	2 (reliable with restrictions)
Rationale for reliability incl. deficiencies	Basic data given

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report	Rubenkoenig, H.L.	1955	Acute toxicities of amines	unpublished data	Hill Top Research Institute Inc., Miamiville, Ohio, USA	D-333	ANGUS, a wholly owned subsidiary of The Dow Chemical Company		1955-01-14

Data access

data submitter is data owner

Cross-reference to same study

See chapter 7.2.4 Acute toxicity: other routes

Materials and methods

Test type

other: pre-guideline acute toxicity study

Limit test

no

Principles of method if other than guideline

To estimate the LD50 value, mice were administered the test substance by gavage and kept for observation for 7 days. A solution of the test substance was prepared on a weight/volume basis. The concentrations were varied to allow administration of a constant volume of 0.05 mL/g bw.

GLP compliance

no (Performed prior to GLP)

Test materials

Identity of test material same as for substance defined in section 1 (if not read-across)

yes

Details on test material

- Name of test material (as cited in study report): R-11- Lot/batch No.: 396658R-11

Test animals

Species

mouse

Strain

no data

Sex

no data

Details on test animals and environmental conditions

TEST ANIMALS- Weight at study initiation: 15 g, on average

Administration / exposure

Route of administration

oral: gavage

Vehicle

no data (A solution of the test substance with an unknown vehicle was prepared)

Details on oral exposure

MAXIMUM DOSE VOLUME APPLIED: 0.05 mL/g bwDOSAGE PREPARATION (if unusual): A solution of the test substance was prepared on a weight/volume basis. The concentrations were varied to allow administration of a constant volume of 0.05 mL/g bw.

Doses

2000, 3500, 5000, 7000 and 10000 mg/kg bw

No. of animals per sex per dose

10 animals per dose

Control animals

no data

Details on study design

- Duration of observation period following administration: 7 days

Any other information on materials and methods incl. tables

The approximate LD50 doses were estimated by the method of Miller and Tainter, Proc. Soc. Exper. Biol. and Med., 57:261, 1944.

Results and discussions

Effect levels

Sex	Endpoint	Effect level	Based on	95% CL	Remarks
no data	LD50	5500 mg/kg bw	test mat.		Range: 5500 ± 250 mg/kg bw

Mortality

In the 2000, 3500, 5000, 7000 and 10000 mg/kg bw dose groups, the mortality was 0, 2, 3, 9 and 10, respectively (see table 1).

Any other information on results incl. tables

Table 1: Mortality per dose group administered the test substance

Dose (mg/kg)	Number of animals treated	Mortality
2000	10	0/10
3500	10	2/10
5000	10	3/10
7000	10	9/10
10000	10	10/10

Endpoint study record: key, Mohan Kumar, 2011, rat, RL1

Administrative Data

Purpose flag key study; robust study summary

Study result type experimental result **Study period** 18 May - 06 June 2011

Reliability 1 (reliable without restriction)

Rationale for reliability incl. deficiencies GLP-guideline study

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report	Mohan Kumar, S.B.	2011	TRIS AMINO ULTRA PURE: ACUTE ORAL TOXICITY STUDY [UP-AND-DOWN PROCEDURE] IN WISTAR RATS		Advinus Therapeutics Limited, Department of Safety Assessment, Post Box No. 5813, Plot Nos. 21 & 22, Peenya II Phase, Bangalore 560 058, India	G7852	ANGUS, a wholly owned subsidiary of The Dow Chemical Company	DR-0384-8860-001	2011-09-30

Data access

data submitter is data owner

Materials and methods

Test type

up-and-down procedure

Limit test

yes

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 425 (Acute Oral Toxicity: Up-and-Down Procedure)	no
according to	EPA OPPTS 870.1100 (Acute Oral Toxicity)	no

GLP compliance

yes (incl. certificate) (National GLP Compliance Monitoring Authority, Department of Science & Technology, New Delhi, India; Food and Consumer Product Safety Authority (VWA), The Hague, the Netherlands; Bundesinstitut für Risikobewertung, Berlin, Germany)

Test materials

Identity of test material same as for substance defined in section 1 (if not read-across)

yes

Details on test material

- Name of test material (as cited in study report): tris amino ultra pure- Physical state: white crystals- Analytical purity: 99.9%- Impurities (identity and concentrations): 0.018 weight% water- Purity test date: 9 Mar 2011- Lot/batch No.: XK0731LA1C- Storage condition of test material: ambient temperature, 18-36 °C- Other: pH 10.4 (1% aqueous solution)

Test animals

Species

rat

Strain

Wistar

Sex

female

Details on test animals and environmental conditions

TEST ANIMALS- Source: inhouse bred (Outbred), Toxicology, Department of Safety Assessment, Advinus Therapeutics Limited, Bangalore 560 058, India- Age at study initiation: 10-11 weeks- Weight at study initiation: 177.3-180.3 g (range)-

Fasting period before study: yes, overnight (16-18 hours)- Housing: the rats were housed individually in standard polysulfone cages (approximately L 425 mm x B 266 mm x H 175 mm) with stainless steel top grill, with facilities for pelleted food and water; steam sterilised corn cob bedding was used and changed along with the cage twice weekly- Diet: Ssniff rats/mice pellet food, maintenance meal (Ssniff Spezialdiaten GmbH, Soest, Germany), ad libitum- Water: deep bore-well water passed through activated charcoal filter and exposed to UV-rays in Aquaguard on-line water filter-cum-purifier (Eureka Forbes Ltd., Mumbai, India), ad libitum- Acclimation period: 5-10 daysENVIRONMENTAL CONDITIONS- Temperature (°C): 22-24- Humidity (%): 58-68- Air changes (per hr): 12-15- Photoperiod (hrs dark / hrs light): 12/12IN-LIFE DATES: From: 13 May 2011 To: 1, 3 and 6 June 2011

Administration / exposure

Route of administration

oral: gavage

Vehicle

water

Details on oral exposure

VEHICLE- Amount of vehicle (if gavage): 20 mL/kg bw- Justification for choice of vehicle: the test substance forms a visibly homogenous solution in water MAXIMUM DOSE VOLUME APPLIED: 20 mL/kg bw (3.5-3.6 mL absolute volume)

Doses

5000 mg/kg bw

No. of animals per sex per dose

3, dosed stepwise with a 2-day interval, according to the up-and-down procedure

Control animals

no

Details on study design

- Duration of observation period following administration: 14 days- Frequency of observations and weighing: the animals were observed for mortality and clinical signs 1/2, 1, 2, 3 and 4 hours after administration on Day 1, and daily thereafter throughout the study period; the animals were weighed on Day 1 (prior to administration), 8 and 15- Necropsy of survivors performed: yes, external surfaces of the body, all orifices, tissues and organs of the thoracic and abdominal cavities were examined, and all gross findings were recorded

Results and discussions

Effect levels

Sex	Endpoint	Effect level	Based on	95% CL	Remarks
female	LD50	> 5000 mg/kg bw	test mat.		

Mortality

There was no mortality during the study period.

Clinical signs

No clinical signs were observed during the study period.

Body weight

No effect on body weight was noted.

Gross pathology

Necropsy and histopathological examination revealed no substance-related findings.

Any other information on results incl. tables

Table 1: Mortality and clinical signs during the study period

Dose[mg/kg bw]	Toxicological results*	Duration of clinical signs	Time of death	Mortality (%)
Females				
5000	0/0/3	---	---	0
LD50 > 5000 mg/kg bw				

* first number = number of dead animals
second number = number of animals with clinical signs
third number = number of animals used

Applicant's summary and conclusion

Interpretation of results

not classified

Criteria used for interpretation of results

EU

Conclusions

CLP: not classifiedDSD: not classified

Endpoint study record: Giroux and Beaulaton, 1961, rat, RL2

Administrative Data

Purpose flag	supporting study
Study result type	experimental result
Reliability	2 (reliable with restrictions)
Rationale for reliability incl. deficiencies	Basic data given

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	Giroux, J. and Beaulaton I.-S.	1961	Recherches Pharmacologiques Sur Le THAM.	Societe Pharmacie Montpellier. 21:206-217					

Data access

data published

Cross-reference to same study

7.2.1 Giroux and Beaulaton, 1961, mouse, RL2; 7.2.4 Giroux and Beaulaton, 1961, iv, dog, RL2; 7.2.4 Giroux and Beaulaton, 1961, iv, 1 and 4%, mouse, RL2; 7.2.4 Giroux and Beaulaton, 1961, iv, 1%, mouse, RL2; 7.2.4 Giroux and Beaulaton, 1961, iv, rabbit, RL2; 7.2.4 Giroux and Beaulaton, 1961, iv, rat, RL2; 7.2.4 Giroux and Beaulaton, 1961, subcutaneous, rat, RL2; 7.2.4 Giroux and Beaulaton, 1961, subcutaneous, mouse, RL2; 7.5.1 Giroux and Beaulaton, 1961, 35d, rat, RL4

Materials and methods

Test type

standard acute method

Limit test

no

Principles of method if other than guideline

To estimate the oral LD50 of the test substance, a 20% solution was administered to rats. The mortality, clinical signs and urine production were monitored.

GLP compliance

no (performed prior to GLP)

Test materials

Identity of test material same as for substance defined in section 1 (if not read-across)

yes

Details on test material

- Name of test material (as cited in study report): Tris(hydroxymethyl)aminomethane, 2-amino-2-hydroxymethyl-1,3-propanediol, tromethamine, THAM- Physical state: White, crystalline substance, provided by Abbott Laboratories

Test animals

Species

rat

Strain

Wistar

Sex

no data

Administration / exposure

Route of administration

oral: gavage

Vehicle

no data

Details on oral exposure

The doses were determined in a pilot study, where rats were administered 1000 and 3000 mg/kg bw in a 50% solution; and mice were administered 1000, 2000 and 3000 mg/kg bw in a 5% solution. 10 animals per dose level were used. There was no mortality, no clinical signs and the animals had a normal urine production.In the main study, the rats were dosed with a 20% solution.

Doses

1000, 3000, 5000, 6000 and 7000 mg/kg bw

No. of animals per sex per dose

10 animals per dose

Control animals

no data

Details on study design

- Other examinations performed: Clinical signs, urine volume

Statistics

The LD50 was calculated using the formula of Behrens and Karber.

Results and discussions

Effect levels

Sex	Endpoint	Effect level	Based on	95% CL	Remarks
no data	LD50	ca. 6000 mg/kg bw	test mat.		

Mortality

In the 1000, 3000, 5000, 6000 and 7000 mg/kg bw groups, the mortality was 0/10, 0/10, 3/10, 6/10 and 7/10

Clinical signs

No data

Acute toxicity: inhalation

No data

Acute toxicity: dermal

Endpoint study record: key, Mohan Kumar, 2011, rat, RL1

Administrative Data

Purpose flag key study; robust study summary

Study result type experimental result **Study period** 13 May - 03 Jun 2011

Reliability 1 (reliable without restriction)

Rationale for reliability incl. deficiencies GLP-guideline study

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report	Mohan Kumar, S.B.	2011	TRIS AMINO ULTRA PURE: ACUTE		Advinus Therapeutics Limited, Department	G7853	ANGUS, a wholly owned subsidiary of	DR-0384-8860-002	2011-09-30

			DERMAL TOXICITY STUDY IN WISTAR RATS		of Safety Assessment, Post Box No. 5813, Plot Nos. 21 & 22, Peenya II Phase, Bangalore 560 058, India		The Dow Chemical Company		

Data access

data submitter is data owner

Materials and methods

Test type

standard acute method

Limit test

yes

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 402 (Acute Dermal Toxicity)	no
according to	EPA OPPTS 870.1200 (Acute Dermal Toxicity)	no

GLP compliance

yes (incl. certificate) (National GLP Compliance Monitoring Authority, Department of Science & Technology, New Delhi, India)

Test materials

Identity of test material same as for substance defined in section 1 (if not read-across)

yes

Details on test material

- Name of test material (as cited in study report): Tris Amino Ultra Pure- Physical state: white crystals- Analytical purity: 99.9%- Impurities (identity and concentrations): 0.018% (w/w) water- Lot/batch No.: XK0731LA1C- Storage condition of test material: at ambient temperature, 18-36 °C - Recertification date: 22 Feb 2013- pH: 10.4 (1% aqueous solution)- Other: soluble in water

Test animals

Species

rat

Strain

Wistar

Sex

male/female

Details on test animals and environmental conditions

TEST ANIMALS- Source: in-house bred (Outbred); Toxicology, Department of Safety Assessment, Advinus Therapeutics Limited, Bangalore, India- Age at study initiation: 11-12 weeks- Weight at study initiation: 264.9-285.8 g (males), 203.6-211.5 g (females)- Housing: the rats were housed individually in standard polysulfone cages (approximately L 425 mm x B 266 mm x H 175 mm), with a stainless steel top grill. Steam sterilized corn cob bedding was used and changed with the cage twice per week. The water bottles were changed weekly. - Diet: Ssniff rats / mice pellet food - maintenance meal (Ssniff Spezialdiäten GmbH., Soest, Germany), ad libitum- Water: deep bore-well water passed through activated charcoal filter and exposed to UV rays in Aquaguard on-line water filter-cum-purifier (manufactured by Eureka Forbes Ltd., Mumbai, India), ad libitum- Acclimation period: males were acclimatised for 7 days, females were acclimatised for 5 daysENVIRONMENTAL CONDITIONS- Temperature (°C): 22-24- Humidity (%): 58-68- Air changes (per hr): 12-15- Photoperiod (hrs dark / hrs light): 12/12IN-LIFE DATES: From: 18 May 2011 (females), 20 May 2011 (males) To: 03 Jun 2011

Administration / exposure

Type of coverage

semiocclusive

Vehicle

unchanged (no vehicle) (water was added to the test substance to form a paste)

Details on dermal exposure

TEST SITE- Area of exposure: approximately 8 cm x 10 cm on the dorsolateral (back and side) thoracic part of the rats- % coverage: approximately 10- Type of wrap if used: the test substance was spread onto a 6-ply cotton gauze patch (9 cm x 5 cm for males, 8 cm x 5 cm for females), which was applied to the shaved skin area of the rats. The gauze patch was held in place with non-allergenic surgical tape that was wound around the torso. REMOVAL OF TEST SUBSTANCE- Washing (if done): the application sites were washed with water and wiped clean with a towel to remove any residual test substance- Time after start of exposure: 24 hTEST MATERIAL- Amount(s) applied (volume or weight with unit): 5000 mg/kg bw- Constant volume or concentration used: no, the dose was calculated based on body weight- For solids, paste formed: yes

Duration of exposure

24 h

Doses

5000 mg/kg bw

No. of animals per sex per dose

5

Control animals

not required

Details on study design

- Duration of observation period following administration: at least 15 days- Frequency of observations and weighing: the animals were observed for mortality and clinical signs of toxicity 4 times on day 1 (the day exposure started) and once daily thereafter. The body weights were recorded on day 1 (prior to exposure), 8 and 15. The treated skin sites were assessed for local skin irritation daily after exposure ended.- Necropsy of survivors performed: yes, the external surface of the body, all orifices, tissues and organs of the thoracic and abdominal cavities of all animals were examined- Other examinations performed: clinical signs, body weight, local skin irritation

Results and discussions

Effect levels

Sex	Endpoint	Effect level	Based on	95% CL	Remarks
male/female	LD50	> 5000 mg/kg bw	test mat.		

Mortality

There was no mortality during the study period.

Clinical signs

No signs of toxicity were observed during the study period.

Body weight

The body weight gains were within the normal ranges in males and females during the study period.

Gross pathology

The necropsy and gross pathological examination revealed no substance-related findings.

Other findings

- Other observations: there were no local skin irritation reactions

Applicant's summary and conclusion

Interpretation of results

not classified

Criteria used for interpretation of results

EU

Conclusions

CLP: not classifiedDSD: not classified

Acute toxicity: other routes

Endpoint study record: Giroux and Beaulaton, 1961, subcutaneous, rat, RL2

Administrative Data

Study result type experimental result

Reliability 2 (reliable with restrictions)

Rationale for reliability incl. deficiencies Basic data given

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date

publication	Giroux, J. and Beaulaton I.-S.	1961	Recherches Pharmacologiques Sur Le THAM.	Societe Pharmacie Montpellier. 21:206-217					

Data access

data published

Cross-reference to same study

7.2.1 Giroux and Beaulaton, 1961, mouse, RL2; 7.2.4 Giroux and Beaulaton, 1961, rat, RL2; 7.2.4 Giroux and Beaulaton, 1961, iv, dog, RL2; 7.2.4 Giroux and Beaulaton, 1961, iv, 1 and 4%, mouse, 7.2.4 Giroux and Beaulaton, 1961, iv, 1%, mouse, RL2; 7.2.4 Giroux and Beaulaton, 1961, iv, rabbit, RL2; 7.2.4 Giroux and Beaulaton, 1961, iv, rat, RL2; 7.2.4 Giroux and Beaulaton, 1961, subcutaneous, mouse, RL2; 7.5.1 Giroux and Beaulaton, 1961, 35d, rat, RL4

Materials and methods

Limit test

no

Principles of method if other than guideline

To estimate the subcutaneous LD50 of the test substance, a 5% solution was administered to rats by subcutaneous injection to assess systemic and local effects. The mortality, clinical signs and skin irritation effects were monitored.

GLP compliance

no (performed prior to GLP)

Test materials

Identity of test material same as for substance defined in section 1 (if not read-across)

yes

Details on test material

- Name of test material (as cited in study report): Tris(hydroxymethyl)aminomethane, 2-amino-2-hydroxymethyl-1,3-propanediol, tromethamine, THAM- Physical state: White, crystalline substance from Abbott Laboratories

Test animals

Species

rat

Strain

Wistar

Sex

no data

Administration / exposure

Route of administration

subcutaneous

Vehicle

no data

Details on exposure

In a pilot study, rats were injected subcutaneously with a 5% solution of the test substance to assess systemic and local effects.

Doses

500 and 1000 mg/kg bw

No. of animals per sex per dose

5 per dose

Control animals

no data

Results and discussions

Effect levels

Sex	Endpoint	Effect level	Based on	95% CL	Remarks
no data	LD50	> 1000 mg/kg bw	test mat.		

Mortality

There was no mortality.

Clinical signs

500 mg/kg bw caused irritation at the injection site and 1000 mg/kg bw caused sloughing at the injection site. There were no systemic effects of the exposure.

Endpoint study record: key, Thompson, 1965, iv, rat, RL2

Administrative Data

Purpose flag key study; robust study summary

Study result type experimental result

Reliability 2 (reliable with restrictions)

Rationale for reliability incl. deficiencies Basic data given

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report	Thompson, S. W.	1965	Toxicity studies with tris(hydroxymethyl)aminomethane		U.S. Army Research and Nutrition Laboratory, Fitzsimons General Hospital, Denver, Colorado 80240, USA	DR-0017-3524-100			1965-03-01

Data access

data submitter is data owner

Cross-reference to same study

7.5.4 Thompson, 1965, iv-ip, 20d, rat, RL2; 7.5.4 Thompson, 1965, iv, 20d, rabbit, RL2

Materials and methods

Limit test

no

Principles of method if other than guideline

To estimate the intravenous LD50 of the test substance, a solution was administered to two groups of rats by rapid infusion. The mortality and clinical signs were monitored and gross pathology was performed at necropsy.

GLP compliance

no (performed prior to GLP)

Test materials

Identity of test material same as for substance defined in section 1 (if not read-across)

yes

Details on test material

- Name of test material (as cited in study report): Tris(hydroxymethyl)aminomethane, THAM- Physical state: White, crystalline solid- Composition of test material, percentage of components: 36.3 g tris(hydroxymethyl)aminomethane, 1.75 g NaCl, 0.37 g KCl; dissolved in 1000 mL water for injection

Test animals

Species

rat

Strain

Sprague-Dawley

Sex

male/female

Details on test animals and environmental conditions

TEST ANIMALS- Weight at study initiation: 200-300 g- Acclimation period: 14 days

Administration / exposure

Route of administration

intravenous

Vehicle

water

Details on exposure

Animals were injected via the tail vein. Group 1 and 2 received the same doses, but while group 1 animals were all exposed one the same day, the dosing of group 2 animals was spread over several days. An additional control group was included to assess the effect of rapid infusion of large volumes, where the rats were administered physiological saline (0.9 g NaCl/100 mL). The infusion rates and volumes resembled those of the treatment groups.The rate of administration was established on the basis of the weight of each individual rat, to administer 450 mg/kg bw/minute. Total infusion time was the same for a given dosage level, while infusion volume varied per rat, based on the weight.

Doses

Group 1: 2000, 2500, 3000, 3500, 4000 and 4500 mg/kg bw (all animals dosed in one day)Group 2: 2000, 2500, 3000, 3500, 4000 and 4500 mg/kg bw (dosing per animal spread over several days)

No. of animals per sex per dose

3

Control animals

other: yes, 4 animals per sex per dose

Details on study design

- Duration of observation period following administration: Surviving animals were observed for 2 hours post-infusion- Necropsy of survivors performed: Yes, all animals were necropsied. Specimens of all organs and tissues were fixed in neutral buffered 10% formalin.

Results and discussions

Effect levels

Sex	Endpoint	Effect level	Based on	95% CL	Remarks
no data	other: LD50 group 1	3500 mg/kg bw	test mat.	3280 — 3830	
no data	other: LD50 group 2	3600 mg/kg bw	test mat.	3280 — 4040	

Mortality

Group 1: In the 2000, 2500, 3000, 3500, 4000 and 4500 mg/kg bw dose groups, the mortality was 0/6, 0/6, 1/6, 2/6, 5/6 and 6/6, respectively.Group 2: In the 2000, 2500, 3000, 3500, 4000 and 4500 mg/kg bw dose groups, the mortality was 0/6, 0/6, 1/6, 3/6, 4/6 and 5/6, respectively.Many of the animals died before the infusion was complete, particularly in the higher dose levels. There was no mortality in the control group.

Clinical signs

Some of the surviving animals were obviously ill during the infusion, but recovered during the observation period. Some animals were lethargic during the observation period.

Gross pathology

No gross lesions were observed.

Other findings

- Histopathology: peracute toxic nephrosis was observed in the kidneys in animals surviving up to 2 hours after the infusion. In rats infused with low doses (2000 and 2500 mg/kg bw), the severity was limited to a moderate degree of pyknosis of the nuclei of isolated segments of the renal tubular epithelium, increasing in severity with the dose. In rats administered from 2500 mg/kg bw, the lesion was characterised by severe pyknosis of the nuclei of swollen renal tubular epithelial cells of varied segments of the cortex. In these animals the cytoplasm of affected cells was coagulated, distinctly granular and intensely eosinophilic. The lumens of affected tubules were frequently distended with eosinophilic, amorphous tissue debris and secretions. Affected tubules were observed adjacent to apparently normal tubules. While only a few animals in the 2500 mg/kg bw group had these lesions, the severity and number of affected animals increased with dose, with all rats affected in the highest dose groups. In the highest dose groups little difference was observed between rats that died during infusion and those surviving until sacrifice. Two rats in the 3000 and one in the 3500 mg/kg bw groups had acute toxic hepatitis, characterised by pyknosis of the nucleus of the hepatocytes and cloudy swelling of the cytoplasm. - Potential target organs: kidneys

Any other information on results incl. tables

Table 1: mortality per dose level and group

Doses	Number of rats			
Both groups	Group 1		Group 2	
Mg/kg bw	Mortality total	Mortality per sex	Mortality total	Mortality per sex
2000	0/6	-	0/6	-
2500	0/6	-	0/6	-

3000	1/6	1 female	1/6	1 male
3500	2/6	1 male, 1 female	3/6	1 male, 2 females
4000	5/6	3 males, 2 females	4/6	1 male, 3 females
4500	6/6	3 males, 3 females	5/6	2 males, 3 females

Endpoint study record: Giroux and Beaulaton, 1961, iv, rabbit, RL2

Administrative Data

Study result type experimental result

Reliability 2 (reliable with restrictions)

Rationale for reliability incl. deficiencies Basic data given

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	Giroux, J. and Beaulaton I.-S.	1961	Recherches Pharmacologiques Sur Le THAM.	Societe Pharmacie Montpellier. 21:206-217					

Data access

data published

Cross-reference to same study

7.2.1 Giroux and Beaulaton, 1961, mouse, RL2; 7.2.1 Giroux and Beaulaton, 1961, rat, RL2; 7.2.4 Giroux and Beaulaton, 1961, iv, dog, RL2; 7.2.4 Giroux and Beaulaton, 1961, iv, 1 and 4%, mouse, 7.2.4 Giroux and Beaulaton, 1961, iv, 1%, mouse, RL2; 7.2.4 Giroux and Beaulaton, 1961, iv, rat, RL2; 7.2.4 Giroux and Beaulaton, 1961, subcutaneous, rat, RL2; 7.2.4 Giroux and Beaulaton, 1961, subcutaneous, mouse, RL2; 7.5.1 Giroux and Beaulaton, 1961, 35d, rat, RL4

Materials and methods

Limit test

no

Principles of method if other than guideline

To estimate the intravenous LD50 of the test substance, rabbits were administered the test substance in a 5% solution by intravenous injection. The mortality and clinical signs were monitored

GLP compliance

no (performed prior to GLP)

Test materials

Identity of test material same as for substance defined in section 1 (if not read-across)

yes

Details on test material

- Name of test material (as cited in study report): Tris(hydroxymethyl)aminomethane, 2-amino-2-hydroxymethyl-1,3-propanediol, tromethamine, THAM- Physical state: White, crystalline substance from Abbott Laboratories

Test animals

Species

rabbit

Strain

no data

Sex

no data

Administration / exposure

Route of administration

intravenous

Vehicle

no data

Details on exposure

In a pilot study, rabbits were administered 250 and 500 mg/kg bw in a 5% solution. The rabbits were injected via an ear vein.

Doses

250 and 500 mg/kg bw

No. of animals per sex per dose

5 per dose

Control animals

no data

Details on study design

- Other examinations performed: Clinical signs

Statistics

The LD50 was calculated using the formula of Behrens and Karber.

Results and discussions

Effect levels

Sex	Endpoint	Effect level	Based on	95% CL	Remarks
no data	LD50	> 500 mg/kg bw	test mat.		

Mortality

There was no mortality.

Clinical signs

The rabbits exhibited slight variations in the respiratory rate and depth at both dose levels.

Endpoint study record: Darby and Anderson, 1966, iv, mouse, RL4

Administrative Data

Study result type experimental result

Reliability 4 (not assignable)

Rationale for reliability incl. deficiencies Original report not available and documentation is insufficient for assessment

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	Darby, T. D. and Anderson, S. J.	1966	Tolerance and toxicity of THAM	Ann. Anesth. Franc. VII, 3, Juillet, Aout, Septembre 1966		DR-0017-3524-10			

Data access

data published

Cross-reference to same study

7.2.4 Darby and Anderson, 1966, iv, rat, RL4; 7.2.4 Darby and Anderson, 1966, ip, dog, RL2; 7.5.1 Darby and Anderson, 1966, 31d, rat, RL4; 7.5.1 Darby and Anderson, 1966, 30d, dog, RL4; 7.5.1 Darby and Anderson, 1966, 15d, rat, RL4; 7.5.4 Darby and Anderson, 1966, 3d, dog, RL2

Materials and methods

Limit test

no

Principles of method if other than guideline

To estimate the intravenous LD50 of the test substance, a solution was administered to mice by rapid injection.

GLP compliance

no (performed prior to GLP)

Test materials

Identity of test material same as for substance defined in section 1 (if not read-across)

yes

Details on test material

- Name of test material (as cited in study report): THAM

Test animals

Species

mouse

Strain

no data

Sex

no data

Administration / exposure

Route of administration

intravenous

Vehicle

no data

Details on exposure

The test substance was injected in 10 seconds.

Control animals

no data

Results and discussions

Effect levels

Sex	Endpoint	Effect level	Based on	95% CL	Remarks
no data	LD50	3500 mg/kg bw	test mat.		

Endpoint study record: Giroux and Beaulaton, 1961, iv, rat, RL2

Administrative Data

Study result type experimental result

Reliability 2 (reliable with restrictions)

Rationale for reliability incl. deficiencies Basic data given

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	Giroux, J. and Beaulaton I.-S.	1961	Recherches Pharmacologiques Sur Le THAM.	Societe Pharmacie Montpellier. 21:206-217					

Data access

data published

Cross-reference to same study

7.2.1 Giroux and Beaulaton, 1961, mouse, RL2; 7.2.1 Giroux and Beaulaton, 1961, rat, RL2; 7.2.4 Giroux and Beaulaton, 1961, iv, dog, RL2; 7.2.4 Giroux and Beaulaton, 1961, iv, 1 and 4%, mouse, 7.2.4 Giroux and Beaulaton, 1961, iv, 1%, mouse, RL2; 7.2.4 Giroux and Beaulaton, 1961, iv, rabbit, RL2; 7.2.4 Giroux and Beaulaton, 1961, subcutaneous, rat, RL2; 7.2.4 Giroux and Beaulaton, 1961, subcutaneous, mouse, RL2; 7.5.1 Giroux and Beaulaton, 1961, 35d, rat, RL4

Materials and methods

Limit test

no

Principles of method if other than guideline

To estimate the intravenous LD50 of the test substance, rats were administered a 1% solution by intravenous injection. The mortality and clinical signs were monitored.

GLP compliance

no (performed prior to GLP)

Test materials

Identity of test material same as for substance defined in section 1 (if not read-across)

yes

Details on test material

- Name of test material (as cited in study report): Tris(hydroxymethyl)aminomethane, 2-amino-2-hydroxymethyl-1,3-propanediol, tromethamine, THAM- Physical state: White, crystalline substance from Abbott Laboratories

Test animals

Species

rat

Strain

no data

Sex

no data

Administration / exposure

Route of administration

intravenous

Vehicle

no data

Details on exposure

In a pilot study, rats were administered 100, 200, 400 and 500 mg/kg bw in a 1% solution.

Doses

100, 200, 400 and 500 mg/kg bw

No. of animals per sex per dose

10 per dose

Control animals

no data

Details on study design

- Other examinations performed: Clinical signs

Statistics

The LD50 was calculated using the formula of Behrens and Karber.

Results and discussions

Effect levels

Sex	Endpoint	Effect level	Based on	95% CL	Remarks
no data	LD50	> 500 mg/kg bw	test mat.		

Mortality

There was no mortality.

Clinical signs

There were no clinical signs.

Endpoint study record: Giroux and Beaulaton, 1961, iv, dog, RL2

Administrative Data

Study result type	experimental result
Reliability	2 (reliable with restrictions)
Rationale for reliability incl. deficiencies	Basic data given

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	Giroux, J. and Beaulaton I.-S.	1961	Recherches Pharmacologiques Sur Le THAM.	Societe Pharmacie Montpellier. 21:206-217					

Data access

data published

Cross-reference to same study

7.2.1 Giroux and Beaulaton, 1961, mouse, RL2; 7.2.1 Giroux and Beaulaton, 1961, rat, RL2; 7.2.4 Giroux and Beaulaton, 1961, iv, 1 and 4%, mouse, 7.2.4 Giroux and Beaulaton, 1961, iv, 1%, mouse, RL2; 7.2.4 Giroux and Beaulaton, 1961, iv, rabbit, RL2; 7.2.4 Giroux and Beaulaton, 1961, iv, rat, RL2; 7.2.4 Giroux and Beaulaton, 1961, subcutaneous, rat, RL2; 7.2.4 Giroux and Beaulaton, 1961, subcutaneous, mouse, RL2; 7.5.1 Giroux and Beaulaton, 1961, 35d, rat, RL4

Materials and methods

Limit test

no

Principles of method if other than guideline

To estimate the intravenous LD50 of the test substance, dogs were administered 125 mg/kg bw in a 5% solution by intravenous injection. The mortality and clinical signs were monitored.

GLP compliance

no (performed prior to GLP)

Test materials

Identity of test material same as for substance defined in section 1 (if not read-across)

yes

Details on test material

- Name of test material (as cited in study report): Tris(hydroxymethyl)aminomethane, 2-amino-2-hydroxymethyl-1,3-propanediol, tromethamine, THAM- Physical state: White, crystalline substance from Abbott Laboratories

Test animals

Species

dog

Strain

no data

Sex

no data

Administration / exposure

Route of administration

intravenous

Vehicle

no data

Details on exposure

In a pilot study, dogs were administered 125 mg/kg bw in a 5% solution.

Doses

125 mg/kg bw

No. of animals per sex per dose

5 per dose

Control animals

no data

Details on study design

- Other examinations performed: Clinical signs

Statistics

The LD50 was calculated using the formula of Behrens and Karber.

Results and discussions

Effect levels

Sex	Endpoint	Effect level	Based on	95% CL	Remarks
no data	LD50	> 125 mg/kg bw			

Mortality

There was no mortality.

Clinical signs

Minor changes in the heart rate and respiratory rate were observed.

Endpoint study record: Roberts and Linn, 1961, iv, mouse, RL2

Administrative Data

Study result type experimental result

Reliability 2 (reliable with restrictions)

Rationale for reliability incl. deficiencies Basic data given

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	Roberts, M. and Linn, S.	1961	Acute and subchronic toxicity of 2-amino-2-hydroxymethyl-1,3-propanediol	Annals of the New York Academy of Sciences, Vol. 92, Art. 2: 724-734					

Data access

data published

Cross-reference to same study

7.5.4 Roberts and Linn, 1961, iv, 10d, mouse, RL4; 7.5.4 Roberts and Linn, 1961, iv, 19d, rabbit, RL4

Materials and methods

Limit test

no

Principles of method if other than guideline

The study was performed to examine the relation between toxicity (LD50) of the test substance and pH. A 0.3M solution of the test substance (pH 10.4) and 0.3M solutions adjusted to pH 5.5 or 7.4 with HCl were used for dosing. An amount of the solution equivalent to 5 mL above and 5 mL below the approximate LD50 was injected intravenously into the mice during 30 seconds. Mortality and clinical signs were monitored.

GLP compliance

no (performed prior to GLP)

Test materials

Identity of test material same as for substance defined in section 1 (if not read-across)

yes

Details on test material

- Name of test material (as cited in study report): 2-amino-2-hydroxymethyl-1,3-propanediol

Test animals

Species

mouse

Strain

no data

Sex

no data

Administration / exposure

Route of administration

intravenous

Vehicle

unchanged (no vehicle)

Details on exposure

The study was performed to examine the relation between toxicity (LD50) of the test substance and pH. The pH of a 0.3M solution is 10.4. Solutions with several pH vales were tested. The following solutions were used in increasing doses, to determine LD50: 0.3M solution, 0.3M solution adjusted to pH 7.4, 0.3M solution adjusted to pH 5.5, 0.3M solution in 5% dextrose, 0.3M solution in 0.9% sodium chloride. An amount of the solution equivalent to 5 mL above and 5 mL below the approximate LD50 was injected intravenously into the mice during 30 seconds.

Doses

0.3M, 0.3M with pH 7.4, 0.3M with pH 5.5, 0.3M in 5% dextrose,).3M in 0.9% sodium chloride

No. of animals per sex per dose

10 animals per dose

Control animals

no data

Details on study design

- Duration of observation period following administration: 24 hours

Results and discussions

Effect levels

Sex	Endpoint	Effect level	Based on	95% CL	Remarks
no data	LD50	1980 mg/kg bw	test mat.		0.3M
no data	LD50	1548 mg/kg bw	test mat.		0.3M, pH 7.4
no data	LD50	1044 mg/kg bw	test mat.		0.3M, pH 5.5
no data	LD50	1728 mg/kg bw	test mat.		0.3M in 5% dextrose
no data	LD50	1764 mg/kg bw	test mat.		0.3M in 0.9% sodium chloride

Mortality

The LD50 decreased with pH, see table 1.

Clinical signs

The mice that died had convulsions immediately before death.

Any other information on results incl. tables

Table 1: LD50 values per solution in mg/kg bw, mL/kg and mM/kg

Solution	LD50 mg/kg bw	mL/kg	mM/kg
0.3M	1980	55	16.5
0.3M solution adjusted to pH 7.4	1548	43	12.9
0.3M solution adjusted to pH 5.5	1044	29	8.7
0.3M solution in 5% dextrose	1728	48	14.4
0.3M solution in 0.9% sodium chloride	1764	49	14.7

Endpoint study record: Giroux and Beaulaton, 1961, iv, 1%, mouse, RL2

Administrative Data

Study result type	experimental result
Reliability	2 (reliable with restrictions)
Rationale for reliability incl. deficiencies	Basic data given

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	Giroux, J. and Beaulaton I.-S.	1961	Recherches Pharmacologiques Sur Le THAM.	Societe Pharmacie Montpellier. 21:206-217					

Data access

data published

Cross-reference to same study

7.2.1 Giroux and Beaulaton, 1961, mouse, RL2; 7.2.1 Giroux and Beaulaton, 1961, rat, RL2; 7.2.4 Giroux and Beaulaton, 1961, iv, dog, RL2; 7.2.4 Giroux and Beaulaton, 1961, iv, 1 and 4%, mouse, RL2; 7.2.4 Giroux and Beaulaton, 1961, iv, rabbit, RL2; 7.2.4 Giroux and Beaulaton, 1961, iv, rat, RL2; 7.2.4 Giroux and Beaulaton, 1961, subcutaneous, rat, RL2; 7.2.4 Giroux and Beaulaton, 1961, subcutaneous, mouse, RL2; 7.5.1 Giroux and Beaulaton, 1961, 35d, rat, RL4

Materials and methods

Limit test

no

Principles of method if other than guideline

To estimate the intravenous LD50 of the test substance, a 1% solution was administered to mice by intravenous injection. The mortality and clinical signs were monitored.

GLP compliance

no (performed prior to GLP)

Test materials

Identity of test material same as for substance defined in section 1 (if not read-across)

yes

Details on test material

- Name of test material (as cited in study report): Tris(hydroxymethyl)aminomethane, 2-amino-2-hydroxymethyl-1,3-propanediol, tromethamine, THAM- Physical state: White, crystalline substance from Abbott Laboratories

Test animals

Species

mouse

Strain

Swiss

Sex

no data

Administration / exposure

Route of administration

intravenous

Vehicle

no data

Details on exposure

In a pilot study, mice were administered 100, 200, 400 and 500 mg/kg bw in a 1% solution.

Doses

100, 200, 400 and 500 mg/kg bw

No. of animals per sex per dose

10 per dose

Control animals

no data

Details on study design

- Other examinations performed: Clinical signs

Statistics

The LD50 was calculated using the formula of Behrens and Karber.

Results and discussions

Effect levels

Sex	Endpoint	Effect level	Based on	95% CL	Remarks
no data	LD50	> 500 mg/kg bw	test mat.		

Mortality

There was no mortality.

Clinical signs

No clinical signs were reported.

Endpoint study record: Rubenkoenig, 1955, ip, mouse, RL2

Administrative Data

Study result type experimental result

Reliability 2 (reliable with restrictions)

Rationale for reliability incl. deficiencies Basic data given

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report	Rubenkoenig, H.L.	1955	Acute toxicities of amines	unpublished data	Hill Top Research Institute Inc., Miamiville, Ohio, USA	D-333	ANGUS, a wholly owned subsidiary of The Dow Chemical Company		1955-01-14

Data access

data submitter is data owner

Cross-reference to same study

See chapter 7.2.1 Acute toxicity: oral

Materials and methods

Limit test

no

Principles of method if other than guideline

To estimate the LD50 value, mice were administered the test substance intraperitoneally and kept for observation for 7 days. A solution of the test substance was prepared on a weight/volume basis. The concentrations were varied to allow administration of a constant volume of 0.015 mL/g bw.

GLP compliance

no (performed prior to GLP)

Test materials

Identity of test material same as for substance defined in section 1 (if not read-across)

yes

Details on test material

- Name of test material (as cited in study report): R-11- Lot/batch No.: 396658R-11

Test animals

Species

mouse

Strain

no data

Sex

no data

Details on test animals and environmental conditions

TEST ANIMALS- Weight at study initiation: 15 g, on average

Administration / exposure

Route of administration

intraperitoneal

Vehicle

other: A solution of the test substance with an unknown vehicle was prepared

Details on exposure

A solution of the test substance was prepared on a weight/volume basis. The concentrations were varied to allow administration of a constant volume of 0.015 mL/g bw.

Doses

2000, 2500, 3250, 3600 and 4000 mg/kg bw

No. of animals per sex per dose

10 per dose

Control animals

no data

Details on study design

- Duration of observation period following administration: 7 days

Any other information on materials and methods incl. tables

The approximate LD50 doses were estimated by the method of Miller and Tainter, Proc. Soc. Exper. Biol. and Med., 57:261, 1944.

Results and discussions

Effect levels

Sex	Endpoint	Effect level	Based on	95% CL	Remarks
no data	LD50	3350 mg/kg bw	test mat.		Range: 3350 ± 170 mg/kg bw

Mortality

In the 2000, 2500, 3250, 3600 and 4000 mg/kg bw dose groups, the mortality was 0, 1, 4, 7 and 10, respectively (see table 1).

Any other information on results incl. tables

Table 1: Mortality per dose group administered the test substance

Dose (mg/kg)	Number of animals treated	Mortality
2000	10	0/10
2500	10	1/10
3250	10	4/10
3600	10	7/10
4000	10	10/10

Endpoint study record: Giroux and Beaulaton, 1961, subcutaneous, mouse, RL2

Administrative Data

Study result type experimental result

Reliability 2 (reliable with restrictions)

Rationale for reliability incl. deficiencies Basic data given

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date

publication	Giroux, J. and Beaulaton l.-S.	1961	Recherches Pharmacologiques Sur Le THAM.	Societe Pharmacie Montpellier. 21:206-217					

Data access

data published

Cross-reference to same study

7.2.1 Giroux and Beaulaton, 1961, mouse, RL2; 7.2.1 Giroux and Beaulaton, 1961, rat, RL2;7.2.4 Giroux and Beaulaton, 1961, iv, dog, RL2; 7.2.4 Giroux and Beaulaton, 1961, iv, 1 and 4%, mouse, 7.2.4 Giroux and Beaulaton, 1961, iv, 1%, mouse, RL2; 7.2.4 Giroux and Beaulaton, 1961, iv, rabbit, RL2; 7.2.4 Giroux and Beaulaton, 1961, iv, rat, RL2; 7.2.4 Giroux and Beaulaton, 1961, subcutaneous, rat, RL2; 7.5.1 Giroux and Beaulaton, 1961, 35d, rat, RL4

Materials and methods

Limit test

no

Principles of method if other than guideline

To estimate the subcutaneous LD50 of the test substance, a 5% solution was administered to mice by subcutaneous injection to assess systemic and local effects. The mortality, clinical signs and skin irritation effects were monitored.

GLP compliance

no (performed prior to GLP)

Test materials

Identity of test material same as for substance defined in section 1 (if not read-across)

yes

Details on test material

- Name of test material (as cited in study report): Tris(hydroxymethyl)aminomethane, 2-amino-2-hydroxymethyl-1,3-propanediol, tromethamine, THAM- Physical state: White, crystalline substance from Abbott Laboratories

Test animals

Species

mouse

Strain

Swiss

Sex

no data

Administration / exposure

Route of administration

subcutaneous

Vehicle

no data

Details on exposure

In a pilot study, mice were injected subcutaneously with a 5% solution of the test substance to assess systemic and local effects.

Doses

500 and 1000 mg/kg bw

No. of animals per sex per dose

5 per dose

Control animals

no data

Statistics

The LD50 was calculated using the formula of Behrens and Karber.

Results and discussions

Effect levels

Sex	Endpoint	Effect level	Based on	95% CL	Remarks
no data	LD50	> 1000 mg/kg bw	test mat.		

Mortality

There was no mortality.

Clinical signs

500 mg/kg bw caused irritation at the injection site and 1000 mg/kg bw caused sloughing at the injection site. There were no systemic effects of the exposure.

Endpoint study record: Darby and Anderson, 1966, ip, dog, RL2

Administrative Data

Study result type experimental result

Reliability 2 (reliable with restrictions)

Rationale for reliability incl. deficiencies Basic data given

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	Darby, T. D. and Anderson, S. J.	1966	Tolerance and toxicity of THAM	Ann. Anesth. Franc. VII, 3, Juillet, Aout, Septembre 1966		DR-0017-3524-10			

Data access

data published

Cross-reference to same study

7.2.4 Darby and Anderson, 1966, iv, rat, RL4; 7.2.4 Darby and Anderson, 1966, iv, mouse, RL4; 7.5.1 Darby and Anderson, 1966, 31d, rat, RL4; 7.5.1 Darby and Anderson, 1966, 30d, dog, RL4; 7.5.1 Darby and Anderson, 1966, 15d, rat, RL4; 7.5.4 Darby and Anderson, 1966, 3d, dog, RL2

Materials and methods

Limit test

no

Principles of method if other than guideline

Dogs were administered the test substance in Inpersol solution for dialysis intraperitoneally once to assess the acute toxicity. The mortality and clinical signs were recorded. The gross pathological effects were recorded at necropsy.

GLP compliance

no (performed prior to GLP)

Test materials

Identity of test material same as for substance defined in section 1 (if not read-across)

yes

Details on test material

- Name of test material (as cited in study report): THAM

Test animals

Species

dog

Strain

no data

Sex

no data

Details on test animals and environmental conditions

TEST ANIMALS- Weight at study initiation: Females: 7.7 and 8.3 kg; males: 9.6 - 11.8 kg

Administration / exposure

Route of administration

intraperitoneal

Vehicle

other: Inpersol dialysis solution

Details on exposure

Doses of the test substance in Ipersol was administered interperitoneally to dogs in 0.075, 0.15 and 0.3M solutions. One male and one female dog were administered Inpersol as control.

Doses

60 mL/kg bw

No. of animals per sex per dose

1 female (0.075M), 1 male (0.15M), 1 male (0.3M)

Control animals

yes

Details on study design

- Duration of observation period following administration: 24 hours- Necropsy of survivors performed: Yes- Other examinations performed: Histopathology

Results and discussions

Effect levels

Sex	Endpoint	Effect level	Based on	95% CL	Remarks
no data	LD50	> 2160 mg/kg bw			

Mortality

No mortality.

Clinical signs

The animal receiving 0.3M had considerable abdominal distention.

Gross pathology

No abnormal findings were reported.

Other findings

- Histopathology: In the dog receiving 0.3M, local areas of haemorrhaging posterior to the descending colon, on the surface of the stomach and on the urinary bladder were noted. Furthermore, the capsules of the kidneys appeared congested. It is unlikely these effects are treatment-related, considering the administration route is intraperitoneal. No effects were seen in any other animals.

Endpoint study record: Power, 1961, iv, RL4

Administrative Data

Purpose flag	supporting study
Study result type	experimental result
Reliability	4 (not assignable)
Rationale for reliability incl. deficiencies	Original report not available and documentation insufficient for assessment

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
other company data	Power, C.	1961	Analytical report		Research Department, Commercial Solvents Corporation		ANGUS, a wholly owned subsidiary of The Dow Chemical Company		1961-03-03

Data access

data submitter is data owner

Materials and methods

Limit test

no

Principles of method if other than guideline

To estimate the intravenous LD50 of the test substance, an unknown species was administered a solution by rapid injection.

GLP compliance

no data

Test materials

Identity of test material same as for substance defined in section 1 (if not read-across)

yes

Details on test material

- Name of test material (as cited in study report): Tris(hydroxymethyl)aminomethane- Other: Sample numbers 477563RF and 81614

Test animals

Species

other: no data

Strain

no data

Sex

no data

Administration / exposure

Route of administration

intravenous

Vehicle

no data

Results and discussions

Effect levels

Sex	Endpoint	Effect level	Based on	95% CL	Remarks
no data	LD0	1500 mg/kg bw	no data		Sample 477563 RF
no data	LD50	1850 mg/kg bw	no data		Sample 477563 RF
no data	LD100	2500 mg/kg bw	no data		Sample 477563 RF
no data	LD0	1500 mg/kg bw	no data		Sample 81614
no data	LD50	1690 mg/kg bw	no data	1630 — 1750	Sample 81614
no data	LD100	2500 mg/kg bw	no data		Sample 81614

Endpoint study record: Giroux and Beaulaton, 1961, iv, 1 and 4%, mouse, RL2

Administrative Data

Study result type experimental result

Reliability 2 (reliable with restrictions)

Rationale for reliability incl. deficiencies Basic data given

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	Giroux, J. and Beaulaton I.-S.	1961	Recherches Pharmacologiques Sur Le THAM.	Societe Pharmacie Montpellier. 21:206-217					

Data access

data published

Cross-reference to same study

7.2.1 Giroux and Beaulaton, 1961, mouse, RL2; 7.2.1 Giroux and Beaulaton, 1961, mouse, RL2; 7.2.4 Giroux and Beaulaton, 1961, iv, dog, RL2; 7.2.4 Giroux and Beaulaton, 1961, iv, 1%, mouse, RL2; 7.2.4 Giroux and Beaulaton, 1961, iv, rabbit, RL2; 7.2.4 Giroux and Beaulaton, 1961, iv, rat, RL2; 7.2.4 Giroux and Beaulaton, 1961, subcutaneous, rat, RL2; 7.2.4 Giroux and

Beaulaton, 1961, subcutaneous, mouse, RL2; 7.5.1 Giroux and Beaulaton, 1961, 35d, rat, RL4

Materials and methods

Limit test

no

Principles of method if other than guideline

To estimate the intravenous LD50 of the test substance, a 1% and 4% solution was administered to two groups of mice by rapid injection. The mortality and clinical signs were monitored.

GLP compliance

no (performed prior to GLP)

Test materials

Identity of test material same as for substance defined in section 1 (if not read-across)

yes

Details on test material

- Name of test material (as cited in study report): Tris(hydroxymethyl)aminomethane, 2-amino-2-hydroxymethyl-1,3-propanediol, tromethamine, THAM- Physical state: White, crystalline substance from Abbott Laboratories

Test animals

Species

mouse

Strain

Swiss

Sex

no data

Administration / exposure

Route of administration

intravenous

Vehicle

no data

Details on exposure

The doses were determined in a pilot study. Rats were administered 100, 200, 400 and 500 mg/kg bw in a 1% solution; mice were administered 100, 200, 400 and 500 mg/kg bw in a 1% solution; rabbits were administered 250 and 500 mg/kg bw in a 5% solution; and dogs were administered 125 mg/kg bw in a 5% solution. Per dose level, 10 rats, 10 mice, 5 rabbits and 5 dogs were used. The rabbits were injected via an ear vein. There was no mortality in the pilot study in any species. The rats and mice tolerated all doses with no effects. The rabbits exhibited slight variations in the respiratory rate and depth at both dose levels. In the dogs, minor changes in the heart rate and respiratory rate were observed. Based on the results, which show low acute toxicity of the test substance in all the tested species, only mice were exposed in the main study. 200, 400, 500, 600, 700, 1000, 1500 and 2000 mg/kg bw was administered intravenously by rapid injection. For the 200, 400, 500, 600 and 700 mg/kg bw dose groups a 1% solution was used. Due to the large volume of solution injected, the 1000, 1500 and 2000 mg/kg bw dose levels were administered in a 4% solution.

Doses

200, 400, 500, 600, 700, 1000, 1500 and 2000 mg/kg bw

No. of animals per sex per dose

10 per dose level

Control animals

no data

Details on study design

- Other examinations performed: Clinical signs

Statistics

The LD50 was calculated using the formula of Behrens and Karber.

Any other information on materials and methods incl. tables

In the dose table for the main study, 1% solution, 200 mg/kg bw is listed twice. One of these groups has therefore been disregarded.

Results and discussions

Effect levels

Sex	Endpoint	Effect level	Based on	95% CL	Remarks

no data	LD50	1150 mg/kg bw	test mat.		

Mortality

In the 200, 400, 500, 600 and 700 mg/kg bw groups, the mortality was 0/10, 0/10, 0/10, 3/10 and 6/10.In the 1000, 1500 and 2000 mg/kg bw groups, the mortality is given as 10%, 75% and 100%. These groups must have been larger than 10 animals, considering the result in the 1500 mg/kg bw group.

Clinical signs

The mice administered up to 500 mg/kg bw did not exhibit any adverse effects. In the 600 and 700 mg/kg bw groups, convulsions were observed before the injection was completed, followed by breathing difficulties and respiratory failure. In the 1000, 1500 and 2000 mg/kg bw groups, the animals that died showed clinical signs like convulsions and respiratory difficulties.

Endpoint study record: Darby and Anderson, 1966, iv, rat, RL4

Administrative Data

Study result type experimental result

Reliability 4 (not assignable)

Rationale for reliability incl. deficiencies Original report not available and documentation is insufficient for assessment

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	Darby, T. D. and Anderson, S. J.	1966	Tolerance and toxicity of THAM	Ann. Anesth. Franc. VII, 3, Juillet, Aout, Septembre 1966		DR-0017-3524-10			

Data access

data published

Cross-reference to same study

7.2.4 Darby and Anderson, 1966, ip, dog, RL2; 7.2.4 Darby and Anderson, 1966, iv, mouse, RL4; 7.5.1 Darby and Anderson, 1966, 31d, rat, RL4; 7.5.1 Darby and Anderson, 1966, 30d, dog, RL4; 7.5.1 Darby and Anderson, 1966, 15d, rat, RL4; 7.5.4 Darby and Anderson, 1966, 3d, dog, RL2

Materials and methods

Limit test

no

Principles of method if other than guideline

To estimate the intravenous LD50 of the test substance, a solution was administered to rats by rapid injection.

GLP compliance

no (performed prior to GLP)

Test materials

Identity of test material same as for substance defined in section 1 (if not read-across)

yes

Details on test material

- Name of test material (as cited in study report): THAM

Test animals

Species

rat

Strain

no data

Sex

no data

Administration / exposure

Route of administration

intravenous

Vehicle

no data

Details on exposure

The test substance was injected in 10 seconds.

Control animals

no data

Results and discussions

Effect levels

Sex	Endpoint	Effect level	Based on	95% CL	Remarks
no data	LD50	2300 mg/kg bw	test mat.		

Irritation / corrosion

Endpoint summary: Irritation / corrosion

Administrative Data

Key value for chemical safety assessment

Skin irritation / corrosion

not irritating

Eye irritation

not irritating

Discussion

Skin irritation:
Several skin irritation studies with 2 -amino-2 -(hydroxymethyl)-1,3 -propanediol (TRIS AMINO) are available.
In a study performed according to OECD 404 on rabbits, 0.5 g TRIS AMINO (moistened with water) was applied to shaved skin and covered with a semi-occlusive dressing for 4 hours (Prakash, 2011). No erythema or edema was observed in any of the animals 1, 24, 48 and 72 hours after exposure ended. This finding is confirmed by the absence of skin irritation effects in an acute dermal toxicity study (OECD 402) performed on rats using semi-occlusive exposure to 5000 mg/kg bw TRIS AMINO for 24 hours (Mohan Kumar, 2011).
In addition, several non-guideline studies with limited information are available. Baldwin (1961) reported that a saturated solution of the test substance caused very slight skin irritation in rabbits after 4 hours exposure, while the undiluted solid and a 25% solution did not lead to any skin effects. In a study by Machle et al. (1940), 4 hours' open exposure to the test substance on 5 consecutive days did not cause skin irritation effects in rabbits. By applying a principle component analysis (TSAR), a prediction can be made regarding the likely corrosive or non-corrosive classification of a substance (Barratt, 1996). Using the known physical-chemical characteristics logP, molecular volume and melting point, TRIS AMINO was predicted to be non-corrosive under the conditions of the TSAR model.
Taking the available data into account, TRIS AMINO is not considered to be skin-irritating.
Eye irritation:
In a study performed by Prakash (2011), according to OECD 405, 0.1 g of TRIS

AMINO was instilled into one eye of each of 3 rabbits. The eyes remained unwashed and were assessed for irritation 1, 24, 48 and 72 hour after the instillation. Slight to moderate effects on the conjunctiva (score 1-2) and chemosis (score 1-2) were observed from the 1-hour reading time point in 3/3 rabbits. The maximum mean scores for both effects on the conjunctiva and chemosis were 1.33 for 1/3 rabbits, and 0.33 for 2/3 rabbits, respectively. The irritation effects had cleared completely in 2/3 rabbits within 48 hours and in the remaining rabbit with the highest mean scores within 72 hours. No damage to the iris or cornea was observed at any reading time point.
In a brief report, Power (1975) noted that the test substance, production TRIS AMINO concentrate, did not cause eye irritation.
Taking the available data into account, TRIS AMINO is considered not to be eye irritating.

Justification for classification or non-classification

The available data on skin and eye irritation of the test substance do not meet the criteria for classification according to Regulation (EC) 1272/2008 or Directive 67/548/EEC, and are therefore conclusive but not sufficient for classification.

Skin irritation / corrosion

Endpoint study record: Baldwin, 1961, 4h, rabbit, RL4

Administrative Data

Study result type experimental result

Reliability 4 (not assignable)

Rationale for reliability incl. deficiencies Original report not available and documentation insufficient for assessment

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report	Baldwin, R. S.	1961	Tris(hydroxymethyl) aminomethane primary skin irritation studies	unpublished data	Pharmacology Laboratories Research and Development, Commercial Solvents Corporation	K-18227-001	ANGUS, a wholly owned subsidiary of The Dow Chemical Company		1961-05-19

Data access

data submitter is data owner

Materials and methods

Type of method

in vivo

Principles of method if other than guideline

The skin irritation potential of the pure form and 2 solutions of the test substance was tested on rabbits. The animals were exposed for 4 hours.

GLP compliance

no (performed prior to GLP)

Identity of test material same as for substance defined in section 1 (if not read-across)

yes

Test materials

Details on test material

- Name of test material (as cited in study report): Tris(hydroxymethyl)aminomethane- Physical state: Solid, crystals- Lot/batch No.: 5

Test animals

Species

rabbit

Strain

no data

Test system

Type of coverage

no data

Preparation of test site

no data

Vehicle

no data

Amount/concentration applied

TEST MATERIAL3 doses were used: 1) An unknown amount of the pure crystalline test substance2) A saturated solution, the percentage and vehicle is unknown3) A 25% solution in an unknown vehicle

Duration of treatment / exposure

4 hours

Control animals

no data

Details on study design

6 test sites were used per dose; it is not reported whether these sites were on the same rabbit or on different animals.SCORING SYSTEM:according to Draize et al. (J. Pharmacol. Expt. Therap. 1940; 82: 377)

Results and discussions

Irritant/corrosive response data

The undiluted crystalline test substance and 25% solution did not cause skin irritation. A saturated solution caused very mild irritation to the skin of the animals, which was fully reversible within 48 hours (see table 1).

Any other information on results incl. tables

Table 1: irritation score

Test substance dose	Test areas	Primary irritation score, intact skin
Crystalline, undiluted substance	6	0
Saturated solution	6	0.16
25% solution	6	0

Applicant's summary and conclusion

Interpretation of results

not irritating

Criteria used for interpretation of results

EU

Endpoint study record: key, Prakash, 2011, rabbit, RL1

Administrative Data

Purpose flag	key study; robust study summary
Study result type	experimental result **Study period** 13 May - 21 May 2011
Reliability	1 (reliable without restriction)
Rationale for reliability incl. deficiencies	GLP-guideline study

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report	Prakash, P.J.	2011	TRIS AMINO ULTRA PURE: ACUTE DERMAL IRRITATION STUDY IN RABBITS		Advinus Therapeutics Limited, Department of Safety Assessment, Post Box No. 5813, Plot Nos. 21 & 22, Peenya II Phase, Bangalore 560 058, India	G7854	ANGUS, a wholly owned subsidiary of The Dow Chemical Company	DR-0384-8860-003	2011-09-29

Data access

data submitter is data owner

Materials and methods

Type of method

in vivo

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 404 (Acute Dermal Irritation / Corrosion)	no
according to	EPA OPPTS 870.2500 (Acute Dermal Irritation)	no

GLP compliance

yes (incl. certificate) (National GLP Compliance Monitoring Authority, Department of Science & Technology, New Delhi, India)

Identity of test material same as for substance defined in section 1 (if not read-across)

yes

Test materials

Details on test material

- Name of test material (as cited in study report): Tris Amino Ultra Pure- Physical state: white crystals- Analytical purity: 99.9%- Impurities (identity and concentrations): 0.018% (w/w) water- Lot/batch No.: XK0731LA1C- Storage condition of test material: at ambient temperature, 18-36 °C - Recertification date: 22 Feb 2013- pH: 10.4 (1% aqueous solution)- Other: soluble in water

Test animals

Species

rabbit

Strain

New Zealand White

Details on test animals and environmental conditions

TEST ANIMALS- Source: in-house bred (Outbred); Toxicology, Department of Safety Assessment, Advinus Therapeutics Limited, Bangalore, India- Age at study initiation: 7-8 months- Weight at study initiation: 2.84-3.06 kg- Housing: the rabbits were housed individually in rabbit cages (approximately L 65 cm x B 65 cm x H 45 cm) with a Noryl shallow cage body and Noryl waste trays. The litter collection trays were changed daily, except on Sundays. The feed hoppers and water bottles were changed weekly. - Diet: rabbit feed (Pranav agro Industries Ltd., Maharashtra, India), ad libitum - Water: deep bore-well water passed through activated charcoal filter and exposed to UV rays in Aquaguard on-line water filter-cum-purifier (manufactured by Eureka Forbes Ltd., Mumbai, India), ad libitum- Acclimation period: 5 days ENVIRONMENTAL CONDITIONS- Temperature (°C): 21-23- Humidity (%): 59-67- Air changes (per hr): 12-15- Photoperiod (hrs dark / hrs light): 12/12IN-LIFE DATES: From: 13 May 2011 To: 21 May 2011

Test system

Type of coverage

semiocclusive

Preparation of test site

shaved

Vehicle

other: water was added to the test substance to make a paste

Amount/concentration applied

TEST MATERIAL- Amount(s) applied (volume or weight with unit): 0.5 g

Duration of treatment / exposure

4 h

Observation period

72 hReading time points: 1, 24, 48 and 72 h

Number of animals

3 males

Control animals

other: not required, untreated sites of the same animal served as the control

Details on study design

TEST SITE- Area of exposure: approximately 6 cm^2- Type of wrap if used: the test substance was applied to the skin and the area was covered with a semi-occlusive patch. The patch was held in place with non-irritating semi-occlusive adhesive tape that was wrapped around the trunk of the animals. The rabbits were also fitted with an Elizabethan collar for 24 hours after application.REMOVAL OF TEST SUBSTANCE- Washing (if done): the application sites were washed with water and wiped clean with a towel to remove any residual test substance- Time after start of exposure: 4 hTEST MATERIAL- Amount(s) applied (volume or weight with unit): 0.5 g- For solids, paste formed: yesSCORING SYSTEM: Draize scoring system

Any other information on materials and methods incl. tables

The animals were observed for mortality and clinical signs of toxicity 4 times on day 1 (the day exposure started) and once on day 2 and 3. The body weights were recorded when the acclimatisation started, on day 1 of the study (prior to exposure), and day 3.

Results and discussions

Irritation / corrosion results

Irritation parameter	Basis	Time point	Score	Max. score	Reversibility	Remarks
erythema score	mean (out of all 3 animals)	mean over 24, 48 and 72 h	0	4	other: reversibility not applicable	
edema score	mean (out of all 3 animals)	mean over 24, 48 and 72 h	0	4	other: reversibility not applicable	

Irritant/corrosive response data

No skin irritation effects were observed; all scores were 0 at all reading time points for 3/3 animals.

Other effects

There was no mortality and no clinical signs of toxicity were observed during the study period. The body weight gains were within the normal ranges during the study period.

Applicant's summary and conclusion

Interpretation of results

not irritating

Criteria used for interpretation of results

EU

Conclusions

CLP: not classifiedDSD: not classified

Endpoint study record: Machle et al, 1940, 5d, rabbit, RL4

Administrative Data

Study result type experimental result

Reliability 4 (not assignable)

Rationale for reliability incl. deficiencies Original report not available and documentation insufficient for assessment

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	Machle, W. et al.	1940	The physiological response of animals to	Journal of Industrial Hygiene					

			some simple mononitroparaffins and to certain derivatives of these compounds	and Toxicology, Oct. 1940; Vol. 22(3): 315-332					

Data access

data published

Cross-reference to same study

7.2.1 Machle et al, 1940, rabbit, RL4; 7.5.3 Machle et al, 1940, 5d, rabbit, RL4

Materials and methods

Type of method

in vivo

Principles of method if other than guideline

To estimate the local dermal and systemic effects of the test substance, an unknown amount was applied to the shaved skin of rabbits and left to dry without covering. The test site was washed after 4 hours. The animals were exposed daily for 5 consecutive days. The skin effects, clinical signs and body weight were monitored during the study period.

GLP compliance

no (performed prior to GLP)

Identity of test material same as for substance defined in section 1 (if not read-across)

yes

Test materials

Details on test material

- Name of test material (as cited in study report): 2-amino-2-methylol-1,3-propanediol

Test animals

Species

rabbit

Strain

no data

Details on test animals and environmental conditions

TEST ANIMALS- Housing: Animals were housed individually

Test system

Type of coverage

open

Preparation of test site

shaved

Vehicle

no data

Duration of treatment / exposure

4 hours daily, for 5 consecutive days

Control animals

yes

Details on study design

TEST SITEThe fur of the animals was clipped closely to the skin of the anterior abdominal wall. The animals were secured and test substance was pipetted onto the clipped skin area. The test substance was left to dry, exposed to the air. REMOVAL OF TEST SUBSTANCE- Washing (if done): the test site was washed with soap and water- Time after start of exposure: 4 hours

Any other information on materials and methods incl. tables

The animals were observed for clinical signs and weighed.

Results and discussions

Irritant/corrosive response data

The test substance did not cause skin irritation.

Other effects

No systemic effects or changes in body weight were observed.

Applicant's summary and conclusion

Interpretation of results

not irritating

Criteria used for interpretation of results

EU

Endpoint study record: Barratt, 1996, QSAR, RL2

Administrative Data

Purpose flag supporting study

Study result type (Q)SAR

Reliability 2 (reliable with restrictions)

Rationale for reliability incl. deficiencies Acceptable, well documented publication that meets basic scientific principles

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	Barratt, M. D.	1996	Quantitative structure-activity relationships (QSARs) for skin corrosivity of organic acids, bases and phenols: principal components and neural network analysis of extended datasets	Toxicology in Vitro; 10: 85-94					

Data access

data published

Materials and methods

Type of method

other: QSAR modelling

Principles of method if other than guideline

The known values of log(octanol/water partition coefficient), molecular volume, melting point and pKa were used to predict the corrosive properties of a substance. Datasets were analysed by principle components analysis (TSAR) (Clementine; Integral Solutions Ltd., Basingstoke, Hants UK). The programme used a dataset with known values and corrosivity as test set data.

Identity of test material same as for substance defined in section 1 (if not read-across)

yes

Test materials

Details on test material

- Name of test material (as cited in study report): Tris- Other: logP -3.818; molecular volume 109.1 Å3; pKa 8.30; melting point 172 °C

Test system

Any other information on materials and methods incl. tables

A principle component analysis was used to predict a characteristic that is classification-related; non-corrosive or corrosive. Variables used to model skin permeability were log(octanol/water partition coefficient) (logP), molecular volume and melting point. pKa values for organic acids, bases and phenols were used as a measure of cytotoxicity (previously described in Barratt, M. D. Quantitative structure-activity relationships for skin permeability. Toxicology in vitro. 1995; 9:27-37).

Chemical structures were constructed using Sybyl 6.1 software (Tripos Associates, Bracknell, UK). After energy minimization and calculation of logP values (clogP) using the CHEMICALC system (Suzuki and Kudo, 1990) linked to Sybyl, the chemical structures were imported into the principal components analysis (TSAR) spreadsheet (Oxford Molecular Ltd., Sandford-on-Thames, UK) where molecular

volumes were calculated.

pKa values were obtained from the Handbook of Chemistry and Physics (1970) and the Handbook of Biochemistry and Molecular Biology (1976). Melting points were obtained from chemical catalogues. All chemicals with melting points of 37°C or lower were assigned a default value of 37 °C on the basis that they would be in the liquid state when applied to the rabbit skin (Barratt M. D. Quantitative structure-activity relationships for skin corrosivity of organic acids, bases and phenols. Toxicology Letters. 1995; 75: 169 -176). Dataset were analysed by principal components analysis (TSAR) and by neural network analysis (Clementine; Integral Solutions Ltd., Basingstoke, Hants UK). The TSAR principal components module uses a Jacobi transform routine to evaluate the eigenvectors and eigenvalues of the covariance matrix; data are standardized by mean and standard deviation.

In principal components analysis, the original variables are transformed into a new orthogonal set of linear combinants called principal components. The variance form the original descriptors is greatest in the first principal component, less in the second component and so on, allowing multicomponent datasets to be reduced to 2- or 3 - dimensional plots without significant loss of information.

For the neural network analysis, back propagation networks based on the principles described by Rumelhart et al. (Learning internal representations by error propagation. In Parallell distributed processing in the microstructures of cognition. Ed. D. E. Rumelhart, J. L. McLelland and the PDP Research Group. pp 318 -362. MIT Press, Cambridge, USA. 1986) were used. Only equivocal test substance results (borderline between non-corrosive and corrosive) were subject to a neural network analysis.

Results and discussions

Any other information on results incl. tables

Tris was predicted to be non-corrosive.

Applicant's summary and conclusion

Interpretation of results

other: not corrosive

Criteria used for interpretation of results

EU

Eye irritation

Endpoint study record: key, Prakash, 2011, rabbit, RL1

Administrative Data

Purpose flag	key study; robust study summary
Study result type	experimental result **Study period** 02 - 11 Jun 2011
Reliability	1 (reliable without restriction)
Rationale for reliability incl. deficiencies	GLP-guideline study

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report	Prakash, P.J.	2011	TRIS AMINO ULTRA PURE: ACUTE EYE IRRITATION STUDY IN RABBITS		Advinus Therapeutics Limited, Department of Safety Assessment, Post Box No. 5813, Plot Nos. 21 & 22, Peenya II Phase, Bangalore 560 058, India	G7855	ANGUS, a wholly owned subsidiary of The Dow Chemical Company	DR-0384-8860-004	2011-09-29

Data access

data submitter is data owner

Materials and methods

Type of method

in vivo

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 405 (Acute Eye Irritation / Corrosion)	no
according to	EPA OPPTS 870.2400 (Acute Eye Irritation)	no

GLP compliance

yes (incl. certificate) (National GLP Compliance Monitoring Authority, Department of Science & Technology, New Delhi, India)

Identity of test material same as for substance defined in section 1 (if not read-across)

yes

Test materials

Details on test material

- Name of test material (as cited in study report): Tris Amino Ultra Pure- Physical state: white crystals- Analytical purity: 99.9%- Impurities (identity and concentrations): 0.018% (w/w) water- Lot/batch No.: XK0731LA1C- Storage condition of test material: at ambient temperature, 18-36 °C - Recertification date: 22 Feb 2013- pH: 10.4 (1% aqueous solution)- Other: soluble in water

Test animals

Species

rabbit

Strain

New Zealand White

Details on test animals and environmental conditions

TEST ANIMALS- Source: in-house bred (Outbred); Toxicology, Department of Safety Assessment, Advinus Therapeutics Limited, Bangalore, India- Age at study initiation: 7-8 months- Weight at study initiation: 2.81-2.95 kg- Housing: the rabbits were housed individually in rabbit cages (approximately L 65 cm x B 65 cm x H 45 cm) with a Noryl shallow cage body and Noryl waste trays. The litter collection trays were changed daily, except on Sundays. The feed hoppers and water bottles were changed weekly. - Diet: rabbit feed (Pranav agro Industries Ltd., Maharashtra, India), ad libitum - Water: deep bore-well water passed through activated charcoal filter and exposed to UV rays in Aquaguard on-line water filter-cum-purifier (manufactured by Eureka Forbes Ltd., Mumbai, India), ad libitum- Acclimation period: 5-6 daysENVIRONMENTAL CONDITIONS- Temperature (°C): 21-23- Humidity (%): 60-67- Air changes (per hr): 12-15- Photoperiod (hrs dark / hrs light): 12/12IN-LIFE DATES: From: 02 Jun 2011 To: 11 Jun 2011

Test system

Vehicle

unchanged (no vehicle)

Amount/concentration applied

TEST MATERIAL- Amount(s) applied (volume or weight with unit): 0.1 g

Duration of treatment / exposure

Single application without washing

Observation period

72 hReading time points: 1, 24, 48 and 72 h

Number of animals

3

Control animals

other: the untreated eye served as the control

Details on study design

REMOVAL OF TEST SUBSTANCE- Washing (if done): the treated eye remained unwashed SCORING SYSTEM: Draize scoring system
TOOL USED TO ASSESS SCORE: a hand-slit lamp was used to evaluate the severity of damage at all reading time points; fluorescein was used at the 24-hour reading time point to assess the extent of corneal damage

Any other information on materials and methods incl. tables

Additional parameters examined:

The animals were observed for mortality and clinical signs of toxicity 4 times on day 1 (the day exposure started) and once on day 2 and 3. The body weights were recorded when the acclimatisation started, on day 1 of the study (prior to exposure), and day 3 (at study termination).

Treatment procedure:

A single rabbit was initially exposed to the test substance. As the test substance was not corrosive, the test was completed using two additional animals. The test substance was finely ground prior to instillation.

Results and discussions

Overall irritation / corrosion results

Irritation parameter	Basis	Time point	Score	Max. score	Reversibility	Remarks
cornea score	mean (of all 3 animals)	mean of 24, 48 and 72 h	0	4	other: reversibility not applicable	
iris score	mean (of all 3 animals)	mean of 24, 48 and 72 h	0	2	other: reversibility not applicable	
conjunctivae score	animal #1	mean of 24, 48 and 72 h	1.33	3	fully reversible within: 72 h	
conjunctivae score	animal #2	mean of 24, 48 and 72 h	0.33	3	fully reversible within: 48 h	
conjunctivae score	animal #3	mean of 24, 48 and 72 h	0.33	3	fully reversible within: 48 h	
chemosis score	animal #1	mean of 24, 48 and 72 h	1.33	4	fully reversible within: 72 h	
chemosis score	animal #2	mean of 24, 48 and 72 h	0.33	4	fully reversible within: 48 h	
chemosis score	animal #3	mean of 24, 48 and 72 h	0.33	4	fully reversible within: 48 h	

Irritant/corrosive response data

Slight to moderate redness (score 1-2) and chemosis (score 1-2) was observed from the 1-hour reading time point in 3/3 rabbits (see Table 1). The irritation effects had cleared completely in 2/3 rabbits within 48 hours and in the remaining rabbit within 72 hours. No damage to the iris or cornea was observed at any reading time point.

Other effects

There was no mortality and no signs of toxicity were observed during the study period. The body weight gains were within the normal ranges during the study period.

Any other information on results incl. tables

Table 1. Individual scores for eye irritation parameters

Rabbit #	Time [h]	conjunctivae		iris	cornea
		redness	swelling		
1	1	1	2	0	0
	24	2	2	0	0

	48	2	2	0	0
	72	0	0	0	0
	average	**1.33**	**1.33**	**0.0**	**0.0**
2	1	1	1	0	0
	24	1	1	0	0
	48	0	0	0	0
	72	0	0	0	0
	average	**0.33**	**0.33**	**0.0**	**0.0**
3	1	1	2	0	0
	24	1	1	0	0
	48	0	0	0	0
	72	0	0	0	0
	average	**0.33**	**0.33**	**0.0**	**0.0**

Applicant's summary and conclusion

Interpretation of results

not irritating

Criteria used for interpretation of results

EU

Conclusions

CLP: not classifiedDSD: not classified

Endpoint study record: Power, 1975, RL4

Administrative Data

EU: REACH

Purpose flag	supporting study
Study result type	experimental result
Reliability	4 (not assignable)
Rationale for reliability incl. deficiencies	Original reference not yet available

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
other company data	Power, C.	1975	Bioassay report		Pharmacology Laboratories Research and Development, Commercial Solvents Corporation	26775	ANGUS, a wholly owned subsidiary of The Dow Chemical Company		1975-05-21

Data access

data submitter is data owner

Materials and methods

Type of method

no data

Principles of method if other than guideline

Rabbits were exposed to the test substance to assess the eye irritation potential.

GLP compliance

no data

Identity of test material same as for substance defined in section 1 (if not read-across)

yes

Test materials

Details on test material

- Name of test material (as cited in study report): Production TRIS AMINO concentrate- Lot/batch No.: SP7450

Test animals

Species

rabbit

Strain

no data

Test system

Vehicle

no data

Results and discussions

Irritant/corrosive response data

Combined average score: 0. Classification: not irritating.

Applicant's summary and conclusion

Interpretation of results

not irritating

Criteria used for interpretation of results

EU

Sensitisation

Endpoint summary: Sensitisation

Skin sensitisation

Key value for chemical safety assessment

Skin sensitisation

not sensitising

Discussion

There are no animal data available on skin sensitisation of 2 -amin-2 -(hydroxymethyl)-1,3 -propanediol (TRIS AMINO). However, there are reliable data for other substances considered suitable for read-across using the analogue approach. Potential analogues for TRIS AMINO are other 2-amino-1,3-propanediols, i.e. substances that share with the target substance a common propane backbone with amine group at 2-carbon position and primary alcohols at 1 and 3 positions: 2-amino-2-ethyl-1,3-propanediol (AEPD), 2-amino-2-methyl-1,3-propanediol (AMPD) and 2-amino-1,3-propanediol (APD). The only structural difference between TRIS AMINO and AEPD is a replacement of a hydroxyl group with a methyl group and two further analogues differ in the length of the alkyl side-chain at position 2: from 0 carbon atoms (APD) through 1 (AMPD) to 2 (AEPD). The target substance and the source substances share similar physico-chemical properties.

TRIS AMINO and the 2-amino-1,3-propanediols are expected to show comparable toxicokinetic characteristics, and it is anticipated that the absorbed amounts of all the aminopropanediols have limited systemic bioavailability and are rapidly eliminated by the kidneys. No relevant metabolism is expected, based on experimental data and on QSAR predictions. The modelling of potential metabolites using the OECD QSAR toolbox v.2.0 (2010) did not predict relevant metabolites of TRIS AMINO or of any of the 2-amino-1,3-propanediols. Therefore, no metabolism by cytochrome P450 enzymes in-vivo is expected.

A combined oral repeated dose toxicity study and reproduction/developmental screening test was performed with AEPD according to OECD 422 (Ishida, 2004). No effects on reproduction or fertility

and no systemic toxicity were observed up to and including the highest dose level of 1000 mg/kg bw/day. In a non-guideline developmental toxicity screening study performed in rats, AMPD did not shown any potential for causing developmental toxicity at doses of 1000 mg/kg bw/day (Rasoulpour and Andrus, 2011). Furthermore, in an in-vitro developmental screen (limb bud assay) using AMPD, a lack of developmental toxicity (within the scope of the assay) was indicated (Ellis-Hutchings and Marshall, 2011). Available studies via the oral, dermal or intraperitoneal route on these substances also caused no systemic toxicity. The results of the acute studies, as well as the repeated dose studies, demonstrate that the main cause of toxicity was the intrinsic alkalinity of the respective test substances at the site of contact. Inhalation is of no concern, because the low vapour pressure of the pure substances means that exposure is unlikely to occur. In case of spray applications of technical products containing the neat substance, the concentration is very low (< 1%). The Cramer classification (related mainly to the oral route) also indicates a low toxicological concern for TRIS AMINO and the 2-amino-1,3-propanediols. Thus, both TRIS AMINO and 2-amino-1,3-propanediols are of low concern with regard to systemic toxicity.

By modelling interactions of TRIS AMINO and of 2-amino-1,3-propanediols with skin proteins, no structural alerts were found. Therefore, no interactions of TRIS AMINO with skin proteins are expected and the read-across between the analogue substances based on molecular similarity and absence of substructures indicating a sensitising potential is justified for the endpoint skin sensitisation.

The available data support read-across between TRIS AMINO and 2-amino-1,3-propanediols based on an analogue approach to cover data gaps and to minimise additional animal testing, where possible.

There are two skin sensitisation studies available using the analogue substance AEPD.

The first skin sensitisation study was conducted similar to OECD 406 (Parekh, 1982). The Buehler's procedure was used to test the sensitisation potential of AEPD via the topical route. During the induction period some of the guinea pigs in the test group showed mild erythema when treated with 0.5% solution of AEPD (first 5 applications), so the last 5 applications were made with 0.05% solution. The animals in the positive control group showed mild skin reactions during the entire induction period.

When challenged with 0.05% AEPD, one animal in the test group showed skin reactions after 48 h and 6 animals in the negative control group at 24 h as well as at 48 h after challenge. A challenge concentration of 1% APED led to skin reactions in more animals of the negative control group (8 of 10) than of the test group (4 of 10) at the 24 h scoring. Also at the second reading, more animals of the negative control group (6/10) than of the test group (1/10) showed skin reactions. At challenge with 0.3% dinitrochlorobenzene (DNCB) solution, the positive control gave a valid response (8/10). Because more animals in the negative control group than in the test group showed skin reactions at challenge, it is not possible to judge the skin sensitisation potential of AEPD based on this study alone. In the skin sensitisation study of Parekh (1982) no data on the pH value of the test substance is given but from other studies it is known that the pH value in a 1% solution of AEPD was 11.18 and that undiluted AEPD had pH 11.78. Therefore, due to the alkaline pH value, it is likely that the skin reactions observed in the present study were caused by irritation rather than by sensitisation.

In the second study, the skin sensitisation potential of AEPD was tested via the intradermal route (Parekh, 1982). During the induction phase the guinea pigs in the test group showed slight skin reactions caused by AEPD. The first 5 injections were made with a 1% solution and the last 5 injections were made with a 0.05% solution. None of the negative control animals showed any skin reactions. The positive control animals showed mild to necrotic skin reactions during the entire induction period.

Following the challenge with 0.05% AEPD, all animals in the test group (10/10) and in the negative control group (10/10) showed skin reactions, but none of the animals in either group showed skin reactions with a 0.01% solution. The DNCB (0.03%) caused positive skin reactions in the positive control group (10/10) and in some animals in the negative control group (2/10).

In conclusion, it is not possible to distinguish between a skin reaction caused by irritation and a skin

reaction due to sensitisation. Therefore, the skin sensitisation potential of AEPD cannot be judged based on this study alone.

To assess the skin sensitisation potential of AMPD, an in-vitro assay method established by Natsch and Gfeller (2008) and modified by Jeong (2011) was used to determine the direct peptide reactivity, which is a key pathway leading to skin sensitisation. In this assay, a standard peptide consisting of one cysteine, two lysines and 4 other amino acids was incubated with the test substance in neutral pH condition to test for peptide depletion by chemical reactions (Jeong, 2011). After 24 h incubation, the test substance showed no peptide depletion comparable to the value of the negative control. In contrast to the positive control which depleted most of free peptides and showed a positive result. Under the in-vitro test conditions, there was no evidence that the test substance exhibits direct protein reactivity which would cause skin sensitisation.

Human data

Human data on skin sensitisation are available for AEPD. In a human skin sensitisation study, dermatitis patients with present or past occupational exposure to water-based metalworking fluids (MWF) were patch tested with 13 – so far untested – MWF components including AEPD.

Only 1 of 160 patients reacted positively to AEPD. This patient did not react to other MWF components, in particular not to monoethanolamine and diethanolamine. Hence, no clinical relevance of a positive test reaction to AEPD could be found. However, not all the MWFs previously used by this patient could be identified. Therefore, previous occupational exposure and relevance could be regarded as possible. The lack of positive test reactions to AEPD may be due to its low sensitising potential or to a relatively low patch test concentration (1% aq.).

In order to assess skin sensitisation a weight of evidence approach using all available data is applied. Two skin sensitisation studies are available in guinea pigs using the analogue substance AEPD. However, due to the outcome of both studies, it was not possible to distinguish between a skin reaction caused by irritation and a skin reaction based on skin sensitisation. Also human data on skin sensitisation are available for AEPD. But also the human data showed no clinical relevance of a positive test reaction to AEPD. In addition, the skin sensitisation potential of AMPD was investigated in an in-vitro assay, determining the direct peptide reactivity, which is a key pathway leading to skin sensitisation. This assay performed with AMPD resulted in a negative result and was found to be valid. By modelling interactions of TRIS AMINO and of 2-amino-1,3-propanediols with skin proteins, no structural alerts were detected. Taking into account all available data on skin sensitisation, the analogue substances as well as TRIS AMINO are considered to have no skin sensitisation potential.

Respiratory sensitisation

Short description of key information

This information is not available.

Discussion

This information is not available.

Justification for classification or non-classification

Based on the weight of evidence of all available data within an analogue approach, the available data on skin sensitisation do not meet the criteria for classification to Regulation (EC) 1272/2008 or Directive 67/548/EEC, and therefore it is expected that TRIS AMINO has no skin sensitisation potential.

Skin sensitisation

Endpoint study record: WoE, RA-A, 115-70-8, Parekh, 1982, Buehler test, RL3

Administrative Data

Purpose flag	weight of evidence; robust study summary		
Study result type	read-across from supporting substance (structural analogue or surrogate)	**Study period**	30 Mar - 10 Sep 1981
Reliability	3 (not reliable)		
Rationale for reliability incl. deficiencies	Relevant methodological deficiencies: inconclusive data (positive results in the negative control); only 10 animals were used in the treatment group		

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report	Parekh, C.	1982	Topical Sensitization of AEPD (P-1050)		International Minerals & Chemical Corporation, Toxicology Laboratory, Animal Science Research, Terre Haute, Indiana, USA	PLR-258/AMR-056	ANGUS, a wholly owned subsidiary of The Dow Chemical Company	DR-0366-3498-007	1982-06-18

Data access

data submitter is data owner

Materials and methods

Type of method

in vivo

Type of study

Buehler test

Test guideline

Qualifier	Guideline	Deviations
equivalent or similar to	OECD Guideline 406 (Skin Sensitisation)	yes (-limited documentation)

GLP compliance

no

Test materials

Identity of test material same as for substance defined in section 1 (if not read-across)

no

Test material identity

Identifier	Identity
EC name	2-amino-2-ethyl-1,3-propanediol

Details on test material

- Name of test material (as cited in study report): 2-amino-2-ethyl-1,3-propanediol, AEPD (P-1050)- Physical state: viscous yellow liquid- Analytical purity: no data

Test animals

Species

guinea pig

Strain

Hartley

Sex

male

Details on test animals and environmental conditions

TEST ANIMALS- Source: Murphy Breeding Laboratories, Inc., Plainfield, Illinois, USA- Age at study initiation: at least 4 weeks-

Weight at study initiation: at least 200 g- Housing: 5 per cage- Diet (e.g. ad libitum): Purina Guinea Pig Chow #5026, ad libitum- Water (e.g. ad libitum): tap water, ad libitum- Acclimation period: at least one week

Test system

Traditional sensitisation test

Route of induction exposure

epicutaneous, occlusive

Route of challenge exposure

epicutaneous, occlusive

Vehicle

other: water and saline

Concentration

induction:group I (treatment group): 0.5 mL of a 0.5% aqueous solution of AEPD (first 5 applications); 0.5 mL of a 0.05% aqueous solution of AEPD (last 5 applications)group IV (positive control group): 0.5 mL of 0.3% dinitrochlorobenzene solution (DNCB, solubilised in a minimum volume of alcohol and made to volume with saline)group V, VII, VIII (negative control groups): 0.5 mL of saline challenge:group I (treatment group): 0.5 mL of a 0.05% and a 1% aqueous solution of AEPD group V (negative control group for AEPD): 0.5 mL of a 0.05% and a 1% aqueous solution of AEPD group IV (positive control): 0.5 mL of 0.3% dinitrochlorobenzene solution (DNCB, solubilised in acetone instead of alcohol)group VIII (negative control group for DNCB): 0.5 mL of 0.3% dinitrochlorobenzene solution (DNCB, solubilised in acetone instead of alcohol)group VII (negative control): 0.5 mL of saline

No. of animals per dose

50 (10 per group)

Details on study design (Traditional tests)

RANGE FINDING TESTS: The test material was topically applied, at different concentrations on the skin of a guinea pig according to the occlusive patch technique for 24 h. After 24 h, the patches were removed and the skin sites were examined for irritation. The concentration of the test material which produces minimal irritation will be used for the test.In the initial test, all animals in the test and the negative controls developed skin rashes and the skin sensitisation reactions could not to be evaluated. Therefore the test was repeated with a new batch of animals.MAIN STUDYA. INDUCTION EXPOSURE- No. of exposures: 10- Exposure period: ca. 3 weeks- Test groups: epicutaneous; 0.5 mL aqueous solution of AEPD - Control group: 0.5 mL saline- Site: the animals´ backs and flanks- Frequency of applications: 2 to 3 times a week- Duration: 24 h- Concentrations: 0.5% (the first 5 applications) and 0.05% (the last 5 applications) solution of AEPDB. CHALLENGE EXPOSURE- No. of exposures: 1- Day(s) of challenge: 1 day (24 h)- Exposure period: 1 day- Test groups: 0.5 mL of aqueous solution of AEPD- Control group: 0.5 mL of aqueous solution of AEPD- Site: the animals´ backs and flanks- Concentrations: 0.05 and 1% solution of AEPD- Evaluation (hr after challenge): 24 and 48 hOTHER:Induction period:After 24 h exposure the patches were removed, the treated skin sites cleaned and scored at 24 and 48 h for erythema and edema according to Draize.At 48 h the topical application procedure was repeated with each group of animals two to three times a week until a total of 10 applications have been made. After the last application, the animals were allowed to rest for two weeks. Challenge:After 24 h exposure, the patches were removed and the skin sites cleaned. The challenge sites were depilated with Nair (a hair removal product with the active ingredients: calcium hydroxide and sodium hydroxide). 3 h after removal of the hair, the challenge sites were scored for inflammatory skin reactions (erythema and edema) according to Draize. These sites were scored again at 48 h.

Challenge controls

Negative controls:group VII: true negative control groupgroup V: negative control group for AEPD group VIII: negative control group for DNCB

Positive control substance(s)

yes (induction: 0.3% solution of dinitrochlorobenzene (DNCB) in alcohol:saline (20:80); challenge: 0.3% solution of dinitrochlorobenzene (DNCB) in acetone instead of alcohol)

LLNA

Any other information on materials and methods incl. tables

The test was conducted according to Buehler´s procedure (Buehler, E.V. (1965) Delayed Contact Hypersensitivity in the Guinea Pig. Arch. Dermat. 91:171 -175.

Results and discussion

Positive control results

Positive control responded as expected with a clear sensitising response at 24 h (8 of 10 animals) and 48 hours (8 of 10 animals).

Traditional sensitisation test

Results of test (except LLNA)

Reading	Hours after challenge	Group	Dose level	No. with + reactions	Total no. in group	Clinical observations
1st reading	24	test group	0.05%	0	10	

2nd reading	48	test group	0.05%	1	10	
1st reading	24	test group	1%	4	10	
2nd reading	48	test group	1%	1	10	
1st reading	24	positive control	0.3%	8	10	
2nd reading	48	positive control	0.3%	8	10	
1st reading	24	other: negative control for AEPD	0.05%	6	10	
2nd reading	48	other: negative control for AEPD	0.05%	6	10	
1st reading	24	other: negative control for AEPD	1%	8	10	
2nd reading	48	other: negative control for AEPD	1%	6	10	
1st reading	24	other: negative control for DNCB	0.3%	2	10	
2nd reading	48	other: negative control for DNCB	0.3%	5	10	
1st reading	24	negative control	saline	0	10	
2nd reading	48	negative control	saline	0	10	

LLNA

Any other information on results incl. tables

During the induction period (initial ten applications) some of the animals in the test group showed mild erythema when treated with 0.5% solution of P-1050, so the last 5 applications were made with 0.05% solution. The animals in the positive control group showed mild skin reactions during the entire induction period.

Applicant's summary and conclusion

Interpretation of results

ambiguous

Endpoint study record: WoE, RA-A, 115-70-8, Parekh, 1982, intradermal, RL3

Administrative Data

Purpose flag weight of evidence; robust study summary

Study result type read-across from supporting substance (structural analogue or surrogate)

Study period 10 Mar - 16 Apr 1980

Reliability 3 (not reliable)

Rationale for reliability incl. deficiencies Relevant methodological deficiencies: inconclusive data (positive results in the negative control); only 10 animals were used in the treatment group

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report	Parekh, C.	1982	Intradermal Sensitization Potential of AEPD (P-		International Minerals & Chemical Corporation,	PLR-254/AMR-059	ANGUS, a wholly owned subsidiary of	DR-0366-3498-006	1982-06-18

			1050)		Toxicology Laboratory, Animal Science Research, Terre Haute, Indiana, USA		The Dow Chemical Company		

Data access

data submitter is data owner

Materials and methods

Type of method

in vivo

Type of study

Intracutaneus test

Principles of method if other than guideline

Guinea pigs were injected into dermis with test substance or positive/negative control substance every 48 h for totally 10 applications. The animals were challenged at 2 weeks after final injection with injection of test material at a virgin site. Scores for erythema and edema were evaluated at 24 and 48 h post challenge to assess sensitising response.

GLP compliance

no

Test materials

Identity of test material same as for substance defined in section 1 (if not read-across)

no

Test material identity

Identifier	Identity
EC name	2-amino-2-ethyl-1,3-propanediol

Details on test material

- Name of test material (as cited in study report): 2-amino-2-ethyl-1,3-propanediol, AEPD (P-1050)- Analytical purity: 85.34%

Test animals

Species

guinea pig

Strain

Hartley

Sex

male

Details on test animals and environmental conditions

TEST ANIMALS- Weight at study initiation: 250 - 300 g

Test system

Traditional sensitisation test

Route of induction exposure

intradermal

Route of challenge exposure

intradermal

Vehicle

physiol. saline

Concentration

induction:group XIV (treatment group): 0.05 mL of a 1% solution of AEPD in saline (first 5 injections); 0.05 mL of a 0.05% solution of AEPD in saline (last 5 injections)group V (positive control group): 0.05 mL of 0.3% dinitrochlorobenzene solution (DNCB, solubilised in a minimum volume of alcohol and made to volume with saline)group XVI, XVII (negative control groups): 0.05 mL of saline challenge:group XIV (treatment group): 0.1 mL of a 0.05% and 0.01% solution of AEPD in saline group XVII (negative control for AEPD): 0.1 mL of a 0.05% and 0.01% solution of AEPD in saline group V (positive control): 0.1 mL of a 0.03% dinitrochlorobenzene solution (DNCB, solubilised in a minimum volume of acetone instead of alcohol and made to volume with saline)group XVI: (negative control DNBC): 0.1 mL of a 0.03% dinitrochlorobenzene solution (DNCB, solubilised in a minimum volume of acetone instead of alcohol and made to volume with saline)

No. of animals per dose

40 (10 per group)

Details on study design (Traditional tests)

MAIN STUDYA. INDUCTION EXPOSURE- No. of exposures: 10- Exposure period: ca. 3 weeks- Test groups: 10 injections with AEPD- Control group: 10 injections with saline- Site: the animals´ backs and flanks- Frequency of applications: 2 to 3 times a week- Concentrations: 1% (the first 5 injections) and 0.05% (the last 5 injections) solution of AEPDB. CHALLENGE EXPOSURE- No. of exposures: 1- Day(s) of challenge: 1 day (24 h)- Exposure period: 1 day- Test groups: injection with AEPD- Control group: injection with AEPD- Site: the animals´ backs and flanks- Concentrations: 0.05 and 0.01% solution of AEPD- Evaluation (hr after challenge): 24 and 48 hOTHER:Induction period:After 24 and 48 h, the injected sites were scored for erythema and edema according to Draize. At 48 h, the intradermal injection procedure was repeated for each group with 0.1 mL of each solution 2 or 3 times a week until a total of 10 injections have been made. Thereafter the animals were allowed to rest for two weeks. Challenge:24 h after the challenge injection, the injected sites were depilated with Nair (a hair removal product with the active ingredients: calcium hydroxide and sodium hydroxide). 3 h after removal of the hair, the injected sites were scored for inflammatory skin reactions (erythema and edema) according to Draize. These sites were scored again at 48 h.

Challenge controls

Negative controls: group XVI: negative control group for DNCB group XVII: negative control group for APED

Positive control substance(s)

yes induction: dinitrochlorobenzene (DNCB) 0.3% solution (solubilised in minimum volume of alcohol and made to volume with saline); challenge: 0.03% DNCB (solubilised in minimum volume of acetone instead of alcohol and made to volume with saline)

LLNA

Any other information on materials and methods incl. tables

The test was conducted according to Landsteiner and Jacobs procedure (Landsteiner, K. and Jacobs, J. (1983)). Studies of Sensitisation of Animals with Simple Chemical Compounds. J. Exp. Med. 61:643-656

Results and discussion

Positive control results

DNCB gave an expected positive response scored at 24 h (10 of 10 animals) and at 48 h (9 of 10 animals).

Traditional sensitisation test

Results of test (except LLNA)

Reading	Hours after challenge	Group	Dose level	No. with + reactions	Total no. in group	Clinical observations
1st reading	24	test group	0.05%	10	10	
2nd reading	48	test group	0.05%	10	10	
1st reading	48	test group	0.01%	0	10	
2nd reading	48	test group	0.01%	0	10	
1st reading	24	positive control	0.3%	10	10	
2nd reading	48	positive control	0.3%	9	10	
1st reading	24	other: negative control for AEPD	0.05%	10	10	
2nd reading	48	other: negative control for AEPD	0.05%	10	10	
1st reading	24	other: negative control for AEPD	0.01%	0	10	
2nd reading	48	other: negative control for AEPD	0.01%	0	10	
1st reading	24	other: negative control for DNCB	0.03%	2	10	
2nd reading	48	other: negative control for DNCB	0.03%	2	10	

LLNA

Any other information on results incl. tables

During the induction phase the guinea pigs in the test group showed some skin reactions with AEPD (P-1050). The first five injections

were made with 1% solution and the last five injections were made with 0.05% solution.

None of the negative control animals showed any skin reactions. The positive control animals showed mild to necrotic skin reactions during the entire induction period.

Applicant's summary and conclusion

Interpretation of results

ambiguous

Endpoint study record: WoE, RA-A, 115-69-5, Jeong, 2011, peptide binding assay, RL2

Administrative Data

Purpose flag	weight of evidence; robust study summary
Study result type	read-across from supporting substance (structural analogue or surrogate)
Reliability	2 (reliable with restrictions)
Rationale for reliability incl. deficiencies	GLP-guideline study, tested with the source substance 2-amino-2-methyl-1,3-propanediol (CAS 115-69-5). In accordance to the ECHA guidance document "Practical guide 6: How to report read-across and categories (March 2010)", the reliability was changed from RL1 to RL2 to reflect the fact that this study was conducted on a read-across substance.

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report	Jeong, Y.C.	2011	EVALUATION OF XU-12398.00 FOR PROTEIN REACTIVITY USING A DIRECT PEPTIDE BINDING ASSAY		Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, Michigan 48674, USA	100259	ANGUS, a wholly owned subsidiary of The Dow Chemical Company	DR-0436-6670-002	2011-09-21

Data access

data submitter is data owner

Materials and methods

Type of method

in vitro

Type of study

other: peptide binding assay

Principles of method if other than guideline

To test direct peptide reactivity which is a key pathway leading to skin sensitisation, the test substance was investigated for peptide depletion by chemical reaction. The assay method established by Natsch and Gfeller (2008) was validated and improved in the testing facility and utilised in this study.

GLP compliance

yes

Test materials

Identity of test material same as for substance defined in section 1 (if not read-across)

no

Test material identity

Identifier	Identity
EC name	2-amino-2-methyl-1,3-propanediol

Details on test material

- Name of test material (as cited in study report): XU-12398.00- Molecular formula (if other than submission substance): C4H11NO2- Molecular weight (if other than submission substance): 105.1- Analytical purity: 99.9%

Test animals

Species

other: not applicable

Strain

other: not applicable

Sex

no data

Test system

Traditional sensitisation test

Route of induction exposure

other: not applicable

Route of challenge exposure

other: not applicable

Vehicle

other: acetone

Concentration

The test substance was dissolved to a final concentration of 4 mM in acetonitrile.

No. of animals per dose

not applicable

LLNA

Any other information on materials and methods incl. tables

Test system: The test system consists of a model peptide selected from an endogenous human protein expressed in skin tissue. The peptide consists of 7 amino acids and has an acetylated N-terminus. Sequence: Ac-NKKCDLF; Purity: 95.9% (HPLC-UV)

Experimental procedure:

The test substance and the positive control were dissolved to a final concentration of 4 mM in acetonitrile.

Peptide stock solution: 10 mM in 5 mM potassium phosphate buffer (pH 4.5); stored at -80 °C as small aliquots

The peptide stock solution was freshly mixed with 100 mM phosphate buffer (pH 7.5) to a final concentration of 0.133 mM. An aliquot of the peptide solution (750 µL) was mixed with 250 µL of the test substance (final concentrations: 1mM of the test substance and 0.1 mM of peptide in 25% acetonitrile in 75 mM phosphate buffer; ratio: peptide:test substance= 1:10).

The samples were mixed thoroughly using a vortex mixer and incubated for 24 h in 37 °C environmental shaker with mild agitation (100 rpm) 5 replicates were tested for the test substance and triplicates for positive control, negative control and test blank. Peptide solutions for the standard calibration were prepared in duplicates at six different concentrations and a blank.

After 24 h incubation, samples were spun down and the supernatants were subjected to HPLC-UV-MS analyses for quantifications of free standard peptide, as

well as for characterisations of covalent adducts.

Assay controls

Negative control (Vehicle): Acetonitrile was used as vehicle for the test substance.

Positive control: Diethyl maleate (DEM), CAS# 141-05-9, purchased from Sigma-Aldrich (Saint Louis, MO)

Test substance blank: Phosphate buffer (750 mL, 100 mM, pH 7.5) without peptide was mixed with 1/3 volume of the test substance stock solution (4 mM). The test substance controls were processed and analysed in the same manner to determine any interference by the test substance or by its degraded products on UV or MS response, which may cause inaccurate interpretation on peptide reactivity of the test substance.

Analysis conditions:

Pump: Agilent 1100 Series; G1312A

Autosampler: Agilent 1100 Series;G1313A

Column Heater: Agilent 1100 Series; G1316A

UV Spectrometer: Agilent 1100 Series; G1314A

Mass Spectrometer: Agilent 1100 Series LC/MSD; G1946D

Analytical Column: Agilent Eclipse XDB-C18, 2.1 x 30 mm, 1.8 µm

HPLC Eluent - A = Milli Q water + 10 mM Ammonium Acetate; B = Acetonitrile

Injection Volume: 20 µL

Column temperature: 35 °C

Data evaluation and prediction

Upon chemical reaction to test substance, the amount of free peptide in the reaction mixture is decreased relative to peptide reactivity of the chemical. Peptide depletions were determined by measuring remaining parent peptide after the 24 h incubation with 10-fold molar excess test material. The standard calibration curve prepared from UV response was used to calculate the amount of free peptide in the sample solution.

The peptide depletion is calculated relative to 100 µM peptide standard in percentile.

Peptide depletion (%) = 100(1- free peptide concentration in final sample/(100

µM))

The average peptide depletion is calculated from 5 replicates for the test substance or from three replicates for controls.

With the evaluation of 80 chemicals, Natsch and Gfeller (2008) proposed that a chemical covalently bound and depleted free peptide by more than 15% would be interpreted to be positive in peptide reactivity which is highly likely to cause skin sensitisation when the chemical is tested for local lymph node assay in mice.

Study acceptability criteria

The assay is accepted if the following criteria are met:

1) if the positive control (DEM) results in a peptide depletion ≥ 50%. Although the historical range is currently under development, it is expected that the positive control will result in ≥ 80% peptide depletion.

2) if the mean peptide depletion of the negative control tissues is < 10% (Gerberick et al., 2004).

3) if the standard deviation (SD) calculated from the individual peptide depletion of the 5 identically treated replicates are < 18% of mean value of the positive control.

Literature:

Jeong, Y.C. et al., in progress

Natsch, A. and Gfeller, H. (2008) LC-MS-based characterization of the peptide reactivity of chemicals to improve the in vitro prediction of the skin sensitization potential. Toxicol Sci 106, 464-478.

Gerberick, G.F., Vassallo, J.D., Bailey, R.E., Chaney, J.G., Morrall, S.W. and Lepoittevin, J.P. (2004) Development of a peptide reactivity assay for screening contact allergens. Toxicol Sci 81, 332-343.

Results and discussion

LLNA

Any other information on results incl. tables

The test substance was completely soluble in acetonitrile and was not precipitated by mixing with peptide solutions. After 24 h incubation, there was no colour change or a precipitate observed from the test substance.

The test article did not have any UV absorbance at 220 nm through entire HPLC chromatography and therefore there was no interference with HPLC-UV analyses for peptides. Furthermore, the test substance did not interfere with the MS detections used in the test system that were monitoring higher than 700.0 m/z.

Using the established calibration curve, the concentrations of free peptide were calculated for each sample (Table 1). Average peptide depletion by the test substance was 4.22 ± 1.84%. Negative and positive controls resulted in 4.83 ± 1.66% and 96.13 ± 0.21% peptide depletion, respectively. These results confirmed the assay was valid. Because there was no peptide depletion by the test substance, no further analysis was performed to measure dimerized- or oxidized-peptide by the test substance.

Table 1: Individual data from free peptide quantitation and average peptide depletion

Group	Replicate#	Analyte Peak Area (counts)	Peptide conc. (mM)	Peptide depletion (%)	Average depletion (%)
Test substance	1	15300000	98.03	1.97	4.22 ± 1.84
	2	15200000	97.37	2.63	
	3	14700000	94.07	5.93	
	4	14700000	94.07	5.93	
	5	14900000	95.39	4.61	
Negative control	1	14600000	93.40	6.60	4.83 ± 1.66
	2	14900000	95.39	4.61	
	3	15100000	96.71	3.29	
Positive control	1	1030000	3.78	96.22	96.13 ± 0.21
	2	1080000	4.11	95.89	
	3	1020000	3.71	96.29	

Applicant's summary and conclusion

Interpretation of results

other: Under the test conditions, there is no evidence that the test substance contains direct protein reactivity which would cause skin sensitisation.

Criteria used for interpretation of results

EU

Repeated dose toxicity

Endpoint summary: Repeated dose toxicity

Key value for chemical safety assessment

Repeated dose toxicity: oral

Effect level NOAEL 1000 mg/kg bw/day

Test type subacute

Species rat

Discussion

Repeated dose toxicity: oral

A reproduction/developmental toxicity screening test was performed with2 -amino-2 -(hydroxymethyl)-1,3 -propanediol(TRIS AMINO), according to OECD 421 (Ellis-Hutchings, 2012). Rats (12/sex/dose) were administered 100, 300 and 1000 mg/kg bw/day of the test substance, adjusted to pH 9, by gavage. Males were exposed for 29 days and females for up to 54 days (from 14 days before mating until day 4 of lactation). There was no mortality during the study period and no treatment-related

clinical signs or body weight changes were observed. There were no effects on organ weights. During the gross pathology, a thickened limiting ridge of the stomach was observed in all the males and females in the high-dose groups. Effects on the limiting ridge were also seen in the microscopic examination, as very slight to slight hyperplasia of the stratified squamous epithelium in all animals in the high-dose group. A few cases were also noted in the control or low- and mid-dose groups. A clear treatment-related effect was seen only in the cases classified as slight; with an increasing number of cases by increasing dose. The damage to the limiting ridge is most probably caused by local irritation of the test substance (adjusted to pH 9), which was administered in a relatively large volume. As humans do not have a limiting ridge and forestomach, the effect is considered to have limited toxicological relevance to humans. There were no treatment-related histopathological findings in the reproductive organs.
Therefore, the NOAEL systemic is considered to be≥1000 mg/kg bw/day for males and females in this study. Based on the local irritation effects the NOAEL local is set at 100 mg/kg bw/day.
Several publications on oral repeated dose toxicity studies performed with TRIS AMINO prior to the OECD guidelines are available. Darby and Anderson (1966) performed two studies on rats, recording mortality, clinical signs and performing gross pathology: In the 15-day study, the NOAEL was ≥ 2500 mg/kg bw/day, as no effects were seen up to and including the highest dose level. In the 31-day study, diarrhoea was observed at the highest dose level. Although this is a treatment-related effect, it is not likely to be an irreversible adverse effect. Therefore, the NOAEL is considered to be > 4000 mg/kg bw/day. In a subacute study, 10 rats/dose level were administered 1 or 2% (equivalent to approximately 1000 and 2000 mg/kg bw/day) of the test substance in the diet for 35 days (Giroux and Beaulaton, 1961). The mortality, clinical signs and body weight were recorded. At necropsy, gross pathology was performed and the stomachs of 5 animals from each group were subject to histopathological examination. A satellite group of 5 animals in each dose group was kept an additional 15 days. No effects were observed up to and including the highest dose level and there was no difference between the main treatment groups and satellite treatment groups.
In a subacute study dogs were administered 250, 1000 and 4000 mg/kg bw/day for 30 days (Darby and Anderson, 1966). Mortality and incidence of clinical signs was registered and gross pathology was performed on all animals, 12 in total. Vomiting and loose stools were noted occasionally in dogs administered 1000 mg/kg bw/day, and frequently in dogs administered 4000 mg/kg bw/day. These effects are similar to those observed in the rats and are considered to be treatment-related, though not irreversible adverse effects. The NOAEL is considered to be > 4000 mg/kg bw/day, the highest dose level tested.

Potential analogues for the target substance TRIS AMINO are other 2-amino-1,3-propanediols. Therefore, source substances are members of the aminopropanediol category: 2-amino-2-ethyl-1,3-propanediol (AEPD), 2-amino-2-methyl-1,3-propanediol (AMPD) and 2-amino-1,3-propanediol (APD). TRIS AMINO and the 2-amino-1,3-propanediols are expected to show comparable toxicokinetic characteristics, and it is anticipated that the absorbed amounts of all the aminopropanediols have limited systemic bioavailability and are rapidly eliminated by the kidneys. No relevant metabolism is expected, based on experimental data and on QSAR predictions. The modelling of potential metabolites using the OECD

QSAR toolbox v.2.0 (2010) did not predict relevant metabolites of TRIS AMINO or of any of the 2-amino-1,3-propanediols. Therefore, no metabolism by cytochrome P450 enzymes in-vivo is expected.
Available studies via the oral, dermal or intraperitoneal route on these substances caused no systemic toxicity. These include a combined oral repeated dose toxicity study and reproduction/developmental screening test performed with AEPD according to OECD 422 (Ishida, 2004). No effects on reproduction or fertility and no systemic toxicity were observed up to and including the highest dose level of 1000 mg/kg bw/day. Also, in a non-guideline developmental toxicity screening study performed in rats, AMPD did not shown any potential for causing developmental toxicity at doses of 1000 mg/kg bw/day (Rasoulpour and Andrus, 2011). The results of the acute studies, as well as the repeated dose studies, demonstrate that the main cause of toxicity was the intrinsic alkalinity of the respective test substances at the site of contact. Inhalation is of no concern, because the low vapour pressure of the pure substances means that exposure is unlikely to occur. In case of spray applications of technical products containing the neat substance, the concentration is very low (< 1%). The Cramer classification (related mainly to the oral route) also indicates a low toxicological concern for TRIS AMINO and the 2-amino-1,3-propanediols. Thus, both TRIS AMINO and 2-amino-1,3-propanediols are of low concern with regard to systemic toxicity.
Due to the structural similarity and the similar toxicological properties between TRIS AMINO and the 2-amino-1,3-propanediols, read-across using an analogue approach is justified.
In order to meet the standard information requirements according to Regulation (EC) 1907/2006 Annex IX, Column I, 8.6.2, a GLP-compliant subchronic toxicity study according to OECD 408 study is required. As no subchronic data are available with TRIS AMINO or 2-amino-1,3-propanediols, an oral 90-day repeated dose toxicity study in rats, following OECD 408 with extended reproduction parameters, is proposed. The test will be performed with 2-amino-1,3-propanediol (APD), and the results will be read-across to TRIS AMINO, based on an analogue approach. According to the information that APD represents the basic molecular structure compared with TRIS AMINO, AMPD and AEPD, which contain an additional hydroxyl, methyl or ethyl group, APD has been selected as the most appropriate test candidate. This is in accordance with Regulation (EC) 1907/2006, Annex XI, 1.5, which specifies that unnecessary tests should be avoided in terms of animal welfare.

Repeated dose toxicity: dermal
In a study report containing limited data, the potential dermal toxicity of TRIS AMINO in rabbits was evaluated (Machle et al., 1940). An unknown amount of the test substance was applied to the shaved skin for 4 hours without cover for 5 consecutive days. No local skin effects or clinical signs were observed and the body weight was not affected.

Repeated dose toxicity: other routes
Several intravenous and intraperitoneal repeated dose toxicity studies performed with TRIS AMINO prior to the OECD guidelines are available.
A study was performed in rabbits, in which 4 rabbits/dose/sex received an intravenous injection of 500 mg/kg bw/day TRIS AMINO, 5 days/week for 28 days (Thompson, 1965). The animals were observed for up to 20 days after the exposure ended. Local inflammatory lesions were observed in 7/8 rabbits around the injection

site on the ear. 2/4 rabbits in the recovery group had chronic interstitial nephritis, while 1/4 had peracute toxic nephrosis. These effects were considered to be treatment-related and caused by the alkalinity of the test substance. The LOAEL i.v. was considered to be 500 mg/kg bw/day.
In a study by Thompson (1965), 3-6 rats/group received daily doses of 0.3 M TRIS AMINO by intravenous injection (equivalent to 500 mg/kg bw/day) or intraperitoneal injection (equivalent to 1500 mg/kg bw/day) for 20 days, or received 10 days treatment per exposure route. Local effects observed at the i.v. and i.p. injection sites of approximately half the animals were attributed to the alkaline effect of the test substance. Peracute toxic nephrosis was observed in 5/6 rats in group 1 (i.v.); 6/6 in group 2 (i.p.); and 2/6 in group 3 (10 days i.v. and 10 days i.p.). Following a 20-day recovery period, only 5/6 rats in the satellite group 2 were affected. The LOAEL i.v. was considered to be 500 mg/mg bw/day, while the LOAEL i.p. was considered to be 1500 mg/kg bw/day.

Several additional studies were performed, in which very limited data was reported (Darby and Anderson, 1966; Mulinos, 1961; Roberts and Linn, 1961). The test substance was administered i.v. to rabbits, mice and dogs, or i.p. to dogs in doses from approximately 186 to 3000 mg/kg bw/day. The overall trend in these studies is that high doses (from approximately 1500 mg/kg bw/day) may cause adverse effects (convulsions, vomiting in dogs) or mortality in rabbits and dogs, while lower doses do not affect the animals. The observed effects were generally attributed by the authors to the alkalinity of the test substance.

Justification for classification or non-classification

The available data on the repeated dose toxicity of the test substance do not meet the criteria for classification according to Regulation (EC) 1272/2008 or Directive 67/548/EEC, and are therefore conclusive but not sufficient for classification.

Repeated dose toxicity: oral

Endpoint study record: Darby and Anderson, 1966, 30d, dog, RL4

Administrative Data

Purpose flag supporting study

Study result type experimental result

Reliability 4 (not assignable)

Rationale for reliability incl. deficiencies Original report not yet available

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	Darby, T. D. and Anderson, S. J.	1966	Tolerance and toxicity of THAM	Ann. Anesth. Franc. VII, 3, Juillet, Aout, Septembre 1966		DR-0017-3524-10			

Data access

data published

Cross-reference to same study

7.2.4 Darby and Anderson, 1966, iv, rat, RL4; 7.2.4 Darby and Anderson, 1966, ip, dog, RL2; 7.2.4 Darby and Anderson, 1966, iv, mouse, RL4; 7.5.1 Darby and Anderson, 1966, 31d, rat, RL4; 7.5.1 Darby and Anderson, 1966, 15d, rat, RL4; 7.5.4 Darby and Anderson, 1966, 3d, dog, RL2

Materials and methods

Test type

subacute

Limit test

no

Principles of method if other than guideline

Dogs were administered the test substance per oral route for 30 days. The mortality, clinical signs, urinary potassium levels and gross pathological findings were reported.

GLP compliance

no (performed prior to GLP)

Test materials

Identity of test material same as for substance defined in section 1 (if not read-across)

yes

Details on test material

- Name of test material (as cited in study report): THAM

Test animals

Species

dog

Strain

no data

Sex

no data

Administration / exposure

Route of administration

oral: unspecified

Vehicle

no data

Details on oral exposure

Dogs were administered 250, 1000 and 4000 mg/kg bw/day of the test substance for 30 days. Any additional doses were not specified, but the table gives the range 250-4000 mg/kg bw/day and the dose level 1000 mg/kg bw/day is mentioned in the text.

Analytical verification of doses or concentrations

no data

Duration of treatment / exposure

30 days

Doses/concentrations

250, 1000 and 4000 mg/kg bw/day

Basis no data

No. of animals per sex per dose

12 in total

Control animals

no data

Examinations

Observations and examinations performed and frequency

CAGE SIDE OBSERVATIONS: Yes. Animals were observed for mortality and clinical signs caused by exposure to the test substance.DETAILED CLINICAL OBSERVATIONS: No dataBODY WEIGHT: No dataFOOD CONSUMPTION: No dataFOOD EFFICIENCY: No dataWATER CONSUMPTION: No dataOPHTHALMOSCOPIC EXAMINATION: No dataHAEMATOLOGY: No dataCLINICAL CHEMISTRY: No dataURINALYSIS: The urinary potassium level was analysed.NEUROBEHAVIOURAL EXAMINATION: No data

Sacrifice and pathology

GROSS PATHOLOGY: Yes HISTOPATHOLOGY: No data

Results and discussions

Effect levels

Endpoint	Effect level	Based on	Sex	Basis for effect level / Remarks
NOAEL	4000 mg/kg bw/day (nominal)	test mat.	no data	

Results of examinations

Details on results

CLINICAL SIGNS AND MORTALITYThere was no mortality during the study period. The dogs administered 1000 mg/kg bw/day occasionally had loose stools and vomited. In the 4000 mg/kg bw/day dose group, vomiting and loose stools were frequently observed. These effects are treatment-related, but not considered to be irreversible adverse effects.URINALYSISAfter 15 days, the dogs administered doses equal to or higher than 1000 mg/kg bw/day had a reduction in urinary potassium levels. This is not considered to be an adverse effect, as there were no associated pathological findings. GROSS PATHOLOGYThere were no gross pathological findings.

Endpoint study record: Darby and Anderson, 1966, 15d, rat, RL4

Administrative Data

Purpose flag supporting study

Study result type experimental result

Reliability 4 (not assignable)

Rationale for reliability incl. deficiencies Original report is not yet available

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	Darby, T. D. and Anderson, S. J.	1966	Tolerance and toxicity of THAM	Ann. Anesth. Franc. VII, 3, Juillet, Aout, Septembre 1966		DR-0017-3524-10			

Data access

data published

Cross-reference to same study

7.2.4 Darby and Anderson, 1966, iv, rat, RL4; 7.2.4 Darby and Anderson, 1966, ip, dog, RL2; 7.2.4 Darby and Anderson, 1966, iv, mouse, RL4; 7.5.1 Darby and Anderson, 1966, 31d, rat, RL4; 7.5.1 Darby and Anderson, 1966, 30d, dog, RL4; 7.5.4 Darby and Anderson, 1966, 3d, dog, RL2

Materials and methods

Test type

subacute

Limit test

no

Principles of method if other than guideline

Rats were administered the test substance per oral route for 15 days. The mortality, clinical signs and gross pathological findings were reported.

GLP compliance

no (performed prior to GLP)

Test materials

Identity of test material same as for substance defined in section 1 (if not read-across)

yes

Details on test material

- Name of test material (as cited in study report): THAM

Test animals

Species

rat

Strain

no data

Sex

no data

Administration / exposure

Route of administration

oral: unspecified

Vehicle

no data

Details on oral exposure

Rats were administered 2500 mg/kg bw/day of the test substance for 15 days.

Analytical verification of doses or concentrations

no data

Duration of treatment / exposure

15 days

Doses/concentrations

2500 mg/kg bw/day

Basis no data

No. of animals per sex per dose

38 per dose

Control animals

no data

Examinations

Observations and examinations performed and frequency

CAGE SIDE OBSERVATIONS: Yes. Animals were observed for mortality and clinical signs caused by exposure to the test substance.DETAILED CLINICAL OBSERVATIONS: No dataBODY WEIGHT: No dataFOOD CONSUMPTION: No dataFOOD EFFICIENCY: No dataWATER CONSUMPTION: No dataOPHTHALMOSCOPIC EXAMINATION: No dataHAEMATOLOGY: No dataCLINICAL CHEMISTRY: No dataURINALYSIS: No dataNEUROBEHAVIOURAL EXAMINATION: No data

Sacrifice and pathology

GROSS PATHOLOGY: Yes HISTOPATHOLOGY: No data

Results and discussions

Effect levels

Endpoint	Effect level	Based on	Sex	Basis for effect level / Remarks
other: no effect level	2500 mg/kg bw/day (nominal)	test mat.	no data	

Results of examinations

Details on results

CLINICAL SIGNS AND MORTALITYThere was no mortality during the study. No clinical signs were observed.GROSS PATHOLOGYNo findings were reported.

Endpoint study record: Giroux and Beaulaton, 1961, 35d, rat, RL4

Administrative Data

Purpose flag supporting study

Study result type experimental result

Reliability 4 (not assignable)

Rationale for reliability incl. deficiencies Only secondary literature

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	Giroux, J.	1961	Recherches	Societe					

	and Beaulaton L.-S.		Pharmacologiques Sur Le THAM.	Pharmacie Montpellier. 21:206-217					

Data access

data published

Cross-reference to same study

7.2.1 Giroux and Beaulaton, 1961, mouse, RL2; 7.2.1 Giroux and Beaulaton, 1961, rat, RL2; 7.2.4 Giroux and Beaulaton, 1961, iv, dog, RL2; 7.2.4 Giroux and Beaulaton, 1961, iv, 1 and 4%, mouse, 7.2.4 Giroux and Beaulaton, 1961, iv, 1%, mouse, RL2; 7.2.4 Giroux and Beaulaton, 1961, iv, rabbit, RL2; 7.2.4 Giroux and Beaulaton, 1961, iv, rat, RL2; 7.2.4 Giroux and Beaulaton, 1961, subcutaneous, rat, RL2; 7.2.4 Giroux and Beaulaton, 1961, subcutaneous, mouse, RL2

Materials and methods

Test type

subacute

Limit test

no

Principles of method if other than guideline

To assess the subacute oral toxicity of the test substance, rats were exposed via the diet daily for 35 consecutive days. The mortality, clinical signs and body weight were recorded. At necropsy, gross pathology was performed and the stomachs of 5 animals from each group were subject to histopathological examination. Five animals in each group were kept an additional 15 days after exposure ended to evaluate any late effects of the test substance.

GLP compliance

no (performed prior to GLP)

Test materials

Identity of test material same as for substance defined in section 1 (if not read-across)

yes

Details on test material

- Name of test material (as cited in study report): Tris(hydroxymethyl)aminomethane, 2-amino-2-hydroxymethyl-1,3-propanediol, tromethamine, THAM- Physical state: White, crystalline substance

Test animals

Species

rat

Strain

no data

Sex

male

Details on test animals and environmental conditions

TEST ANIMALS- Source: The in-house animal laboratory- Age at study initiation: Young animals, age not further specified- Weight at study initiation: Approximately 60 gENVIRONMENTAL CONDITIONS- Temperature (°C): 20 ± 1

Administration / exposure

Route of administration

oral: feed

Vehicle

unchanged (no vehicle)

Details on oral exposure

PREPARATION OF DOSING SOLUTIONS:The test substance was mixed with the feed. The exact intake of the test substance is not known, as the food intake was not recorded.

Analytical verification of doses or concentrations

no

Duration of treatment / exposure

35 days

Frequency of treatment

Daily

Doses/concentrations

1% and 2% test solution in feed

Basis nominal in diet

1000 and 2000 mg/kg bw/day

Basis other: calculated based on an average feed intake of 10 g/day and average weight over the study period of 100g (OECD SERIES ON TESTING AND ASSESSMENT Number 32. Guidance Notes for Analysis and Evaluation of Repeat-Dose Toxicity Studies, 2002)

No. of animals per sex per dose

10 per dose

Control animals

yes, plain diet

Details on study design

- Dose selection rationale:Two pilot studies were performed to assess the effect of oral administration of the test substance. In the first, 3 dogs weighing 20 kgs were given a tablet with 1 g of the test product at 09.00 a.m., 14.00 and 18.00 (in total 3 g per day), daily for 8 days. The dogs were fed once daily, around 18.00 hrs. On the fifth day, one dog vomited in the morning and in the afternoon. No other effects on the gastrointestinal system were observed.In the second pilot study, 5 rabbits weighing approximately 2 kg were administered the test substance daily for 4 consecutive days. Some received 250 mg/kg bw/day and others 500 mg/kg bw/day of a 5% solution. The mortality, clinical signs and gross pathological effects were recorded. The test substance did not cause any systemic effect. Necrosis was observed in the tissue of the injection site, preventing the continuation of the study. No effects on the gastrointestinal tract system were observed.

Examinations

Observations and examinations performed and frequency

CAGE SIDE OBSERVATIONS: Yes. Five animals in each group were kept an additional 15 days for observation.- Time schedule: No dataBODY WEIGHT: Yes- Time schedule for examinations: The rats were weighed at unknown time points during the study periodFOOD CONSUMPTION AND COMPOUND INTAKE (if feeding study):- Food consumption for each animal determined and mean daily diet consumption calculated as g food/kg body weight/day: No- Compound intake calculated as time-weighted averages from the consumption and body weight gain data: NoFOOD EFFICIENCY:- Body weight gain in kg/food consumption in kg per unit time X 100 calculated as time-weighted averages from the consumption and body weight gain data: No OPHTHALMOSCOPIC EXAMINATION: NoHAEMATOLOGY: NoCLINICAL CHEMISTRY: NoURINALYSIS: NoNEUROBEHAVIOURAL EXAMINATION: No

Sacrifice and pathology

GROSS PATHOLOGY: Yes. 5 rats in each group were sacrificed and the major organs were examined and weighed (liver, spleen, kidneys, heart, testicles, adrenals). The stomach of several rats in each group were also examined. HISTOPATHOLOGY: The stomachs of several rats in each group were fixed and the histological sections examined microscopically.

Results and discussions

Effect levels

Endpoint	Effect level	Based on	Sex	Basis for effect level / Remarks
NOAEL	>= 2000 mg/kg bw/day, calculated	test mat.	male	calculated based on an average feed intake of 10 g/day and an average weight over the study period of 100g

Results of examinations

Details on results

CLINICAL SIGNS AND MORTALITYThere was no mortality during the study period. The rats that were observed for 15 days did not exhibit any adverse effects.BODY WEIGHT AND WEIGHT GAINThere was no significant difference in the body weight gain between the control and treatment groups.ORGAN WEIGHTSThe organ weights were comparable between the control and treatment groups.GROSS PATHOLOGYThere were no unusual findings.HISTOPATHOLOGYThe stomachs of the animals had no lesions or other adverse effects of the treatment.

Endpoint study record: Darby and Anderson, 1966, 31d, rat, RL4

Administrative Data

Purpose flag supporting study

Study result type experimental result

Reliability 4 (not assignable)

Rationale for reliability incl. deficiencies Original report not yet available

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date

publication	Darby, T. D. and Anderson, S. J.	1966	Tolerance and toxicity of THAM	Ann. Anesth. Franc. VII, 3, Juillet, Aout, Septembre 1966		DR-0017-3524-10			

Data access

data published

Cross-reference to same study

7.2.4 Darby and Anderson, 1966, iv, rat, RL4; 7.2.4 Darby and Anderson, 1966, ip, dog, RL2; 7.2.4 Darby and Anderson, 1966, iv, mouse, RL4; 7.5.1 Darby and Anderson, 1966, 30d, dog, RL4; 7.5.1 Darby and Anderson, 1966, 15d, rat, RL4; 7.5.4 Darby and Anderson, 1966, 3d, dog, RL2

Materials and methods

Test type

subacute

Limit test

no

Principles of method if other than guideline

Rats were administered the test substance per oral route for 31 days. The mortality, clinical signs and gross pathological findings were reported.

GLP compliance

no (performed prior to GLP)

Test materials

Identity of test material same as for substance defined in section 1 (if not read-across)

yes

Details on test material

- Name of test material (as cited in study report): THAM

Test animals

Species

rat

Strain

no data

Sex

no data

Administration / exposure

Route of administration

oral: unspecified

Vehicle

no data

Details on oral exposure

Rats were administered 250-4000 mg/kg bw/day of the test substance (any doses higher than 250 and lower than 4000 mg/kg bw/day were not specified) for 31 days.

Analytical verification of doses or concentrations

no data

Duration of treatment / exposure

31 days

Doses/concentrations

250-4000 mg/kg bw/day

Basis no data

No. of animals per sex per dose

36 in total

Control animals

no data

Examinations

Observations and examinations performed and frequency

CAGE SIDE OBSERVATIONS: Yes. Animals were observed for mortality and clinical signs caused by exposure to the test

substance.DETAILED CLINICAL OBSERVATIONS: No dataBODY WEIGHT: No dataFOOD CONSUMPTION: No dataFOOD EFFICIENCY: No dataWATER CONSUMPTION: No dataOPHTHALMOSCOPIC EXAMINATION: No dataHAEMATOLOGY: No dataCLINICAL CHEMISTRY: No dataURINALYSIS: No dataNEUROBEHAVIOURAL EXAMINATION: No data

Sacrifice and pathology

GROSS PATHOLOGY: Yes HISTOPATHOLOGY: No data

Results and discussions

Effect levels

Endpoint	Effect level	Based on	Sex	Basis for effect level / Remarks
NOAEL	4000 mg/kg bw/day (nominal)	test mat.	no data	

Results of examinations

Details on results

CLINICAL SIGNS AND MORTALITYThere was no mortality during the study. The highest dose level, 4000 mg/kg bw/day, caused moderate diarrhoea. This effect may be treatment-related, but is not considered to be an adverse effect.GROSS PATHOLOGYNo findings were reported.

Repeated dose toxicity: dermal

Endpoint study record: Machle et al, 1940, 5d, rabbit, RL4

Administrative Data

Purpose flag supporting study

Study result type experimental result

Reliability 4 (not assignable)

Rationale for reliability incl. deficiencies Original report not available and documentation is insufficient for assessment

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	Machle, W. et al.	1940	The physiological response of animals to some simple mononitroparaffins and to certain derivatives of these compounds	Journal of Industrial Hygiene and Toxicology, Oct. 1940; Vol. 22(3): 315-332					

Data access

data published

Cross-reference to same study

7.2.1 Machle et al, 1940, rabbit, RL4; 7.3.1 Machle et al, 1940, 5d, rabbit, RL4

Materials and methods

Test type

subacute

Limit test

no

Principles of method if other than guideline

To estimate the dermal toxicity of the test substance, an amount was applied to the shaved skin of rabbits and left to dry without covering. The test site was washed after 4 hours. The animals were exposed daily for 5 consecutive days. The skin effects, clinical signs and body weight were monitored during the study period.

GLP compliance

no (performed prior to GLP)

Test materials

Identity of test material same as for substance defined in section 1 (if not read-across)

yes

Details on test material

- Name of test material (as cited in study report): 2-amino-2-methylol-1,3-propanediol

Test animals

Species

rabbit

Strain

no data

Sex

no data

Details on test animals and environmental conditions

TEST ANIMALS- Housing: Animals were housed individually

Administration / exposure

Type of coverage

open

Vehicle

no data

Details on exposure

TEST SITEThe fur of the animals was clipped closely to the skin of the anterior abdominal wall. The animals were secured and test substance was pipetted onto the clipped skin area. The test substance was left to dry, exposed to the air. REMOVAL OF TEST SUBSTANCE- Washing (if done): the test site was washed with soap and water- Time after start of exposure: 4 hours

Analytical verification of doses or concentrations

no data

Duration of treatment / exposure

4 hours, for 5 consecutive days

Frequency of treatment

Daily

Doses/concentrations

The test substance was pipetted onto the skin in a 1/16th of an inch (0.16 cm) thick layer. The exact dose is not reported.

Basis no data

Control animals

no data

Examinations

Observations and examinations performed and frequency

CAGE SIDE OBSERVATIONS: Yes. Mortality and clinical signs. DETAILED CLINICAL OBSERVATIONS: NoDERMAL IRRITATION (if dermal study): Yes BODY WEIGHT: YesFOOD CONSUMPTION: NoFOOD EFFICIENCY: NoWATER CONSUMPTION: NoOPHTHALMOSCOPIC EXAMINATION: NoHAEMATOLOGY: NoCLINICAL CHEMISTRY: NoURINALYSIS: NoNEUROBEHAVIOURAL EXAMINATION: NoOTHER: No

Sacrifice and pathology

GROSS PATHOLOGY: NoHISTOPATHOLOGY: No

Results and discussions

Results of examinations

Details on results

CLINICAL SIGNS AND MORTALITYThere was no mortality. No clinical signs were observed.BODY WEIGHT AND WEIGHT GAINNo body weight changes were noted.OTHER FINDINGSThere were no local skin effects.

Repeated dose toxicity: other routes

Endpoint study record: Roberts and Linn, 1961, iv, 10d, rabbit, RL4

Administrative Data

Study result type experimental result

Reliability 4 (not assignable)

Rationale for reliability incl. deficiencies Original report is not available and documentation insufficient for assessment

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	Roberts, M. and Linn, S.	1961	Acute and subchronic toxicity of 2-amino-2-hydroxymethyl-1,3-propanediol	Annals of the New York Academy of Sciences, Vol. 92, Art. 2: 724-734					

Data access

data published

Cross-reference to same study

7.5.4 Roberts and Linn, 1961, iv, 10d, mouse, RL4; 7.5.4 Roberts and Linn, 1961, iv, 19d, rabbit, RL2;

Materials and methods

Test type

subacute

Limit test

no

Principles of method if other than guideline

Rabbits were exposed to the test substance under different conditions to assess the effect of rapid infusion and neutralising the test substance. The rabbits were injected rapidly (30 seconds) with 10 mL/kg bw 0.155M test substance once daily for 10 consecutive days. Mortality and clinical signs were reported, and gross pathology and histopathology was performed.

GLP compliance

no (performed prior to GLP)

Test materials

Identity of test material same as for substance defined in section 1 (if not read-across)

yes

Details on test material

- Name of test material (as cited in study report): 2-amino-2-hydroxymethyl-1,3-propanediol

Test animals

Species

rabbit

Strain

no data

Sex

no data

Administration / exposure

Route of administration

intravenous

Vehicle

no data

Details on exposure

Rabbits were exposed to the test substance under different conditions to assess the effect of rapid infusion and neutralising the test substance. The animals were injected rapidly (30 seconds) with 10 mL/kg bw/day 0.155M test substance and 10 mL/kg bw/day 0.155M adjusted to pH 5.5 (using HCl) once daily for 10 consecutive days. The dose is equivalent to app. 186 mg/kg bw/day.

Analytical verification of doses or concentrations

no data

Duration of treatment / exposure

10 days

Frequency of treatment

Daily

Doses / concentrations

10 mL 0.155M/kg bw/day

No. of animals per sex per dose

3 per dose

Control animals

no data

Examinations

Observations and examinations performed and frequency

CAGE SIDE OBSERVATIONS: Yes. Animals were observed for mortality and clinical signs.DETAILED CLINICAL OBSERVATIONS: NoBODY WEIGHT: NoFOOD CONSUMPTION: NoFOOD EFFICIENCY: NoWATER CONSUMPTION: NoOPHTHALMOSCOPIC EXAMINATION: NoHAEMATOLOGY: NoCLINICAL CHEMISTRY: NoURINALYSIS: NoNEUROBEHAVIOURAL EXAMINATION: No

Sacrifice and pathology

GROSS PATHOLOGY: Yes. Organs and tissues were examined for lesions. HISTOPATHOLOGY: Yes.

Results and discussions

Effect levels

Endpoint	Effect level	Based on	Sex	Basis for effect level / Remarks
NOAEL	186 mg/kg bw/day (nominal)	test mat.	no data	
NOAEL	186 mg/kg bw/day (nominal)	test mat.	no data	pH 5.5

Results of examinations

Details on results

CLINICAL SIGNS AND MORTALITYThere was no mortality and no clinical signs were observed.GROSS PATHOLOGYThere were no effects on gross pathology.HISTOPATHOLOGY: NON-NEOPLASTICThe histopathological examination did not reveal significant effects.

Endpoint study record: Thompson, 1965, iv-ip, 20d, rat, RL2

Administrative Data

Study result type experimental result

Reliability 2 (reliable with restrictions)

Rationale for reliability incl. deficiencies Basic data given

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report	Thompson, S. W.	1965	Toxicity studies with tris(hydroxymethyl)aminomethane		U.S. Army research and nutrition laboratory, Fitzsimons General Hospital, Denver, Colorado 80240, USA	DR-0017-3524-100			1965-03-01

Data access

other: Property of the U.S. Army Medical Research and Nutrition Laboratory, publicly available

Cross-reference to same study

7.2.4 key, Thompson, 1965, rat, RL2; 7.5.4 Thompson, 1965, iv, 20d, rabbit, RL2;

Materials and methods

Test type

subacute

Limit test

no

Principles of method if other than guideline

Rats were administered the test substance intravenously and/or intraperitoneally for 20 days. Some of the animals were observed for 24 hours or 7 days after the exposure ended. Gross pathology and histopathology of target organs was performed.

GLP compliance

no (performed prior to GLP)

Test materials

Identity of test material same as for substance defined in section 1 (if not read-across)

yes

Details on test material

- Name of test material (as cited in study report): Tris(hydroxymethyl)aminomethane, THAM- Physical state: White, crystalline solid from Abbott Laboratories

Test animals

Species

rat

Strain

Sprague-Dawley

Sex

male/female

Details on test animals and environmental conditions

TEST ANIMALS- Weight at study initiation: 200-300 g- Acclimation period: 14 days

Administration / exposure

Route of administration

other: intravenous and intraperitoneal

Vehicle

water

Details on exposure

All animals were administered 20 doses in total. Group 3 received 10 doses intravenously and then 10 doses intraperitoneally, while group 1 and 4 received all doses intravenously and group 2 and 5 received all doses intraperitoneally. The intravenous injections were given via the tail vein. The test solution was 'Sterile Anti-Acidosis Lyophilised' containing 36.3 g tris(hydroxymethyl)aminomethane, 1.75 g NaCl and 0.37 g KCl dissolved in 1000 mL water for injection.

Analytical verification of doses or concentrations

no

Duration of treatment / exposure

20 days

Frequency of treatment

Daily

Doses / concentrations

Group 1 (intravenous): 500 mg/kg bw 0.3M test substance, administered at 450 mg/kg/min Group 2 (intraperitoneal): 1500 mg/kg bw 0.3M test substance, administered as a single injection during 1-2 minutesGroup 3 (intravenous + intraperitoneal): 500 mg/kg bw 0.3M test substance. Intravenous doses administered at 450 mg/kg/min; intraperitoneal injections administered as a single injection during 1-2 minutes.Group 4 (control, intravenous): solution containing 9 g NaCl and 0.37 g KCl per liter of water. The rate of injection was 1 mL/kg/min.Group 5 (control, intraperitoneal): solution containing 9 g NaCl and 0.37 g KCl per liter of water

No. of animals per sex per dose

Group 1 and 2: 6Group 3: 3Group 4 and 5: 2

Control animals

yes, concurrent vehicle

Details on study design

- Dose selection rationale: an LD50 study was performed to determine the dose levels to be used in the subacute study. Two

groups of rats were dosed with 2000, 2500, 3000 and 3500 mg/kg bw of 0.6M; and 4000 and 4500 mg/kg bw of 0.9M test substance. The LD50 in the two groups was determined to be 3500 ± 100 mg/kg bw and 3600 ± 200 mg/kg bw, respectively.Half of the surviving rats of group 1 and 2 and all of the rats in group 3, 4 and 5 were observed for 24 hours after the 20th infusion, and were then sacrificed and necropsied. The remaining rats of groups 1 and 2 were observed for 7 days after exposure ended, before a scheduled necropsy.

Examinations

Observations and examinations performed and frequency

CAGE SIDE OBSERVATIONS: Yes, 24 hours (half the animals in group 1 and 2 and all the animals in group 3, 4 and 5) or 7 days (half the animals in group 1 and 2 only).DETAILED CLINICAL OBSERVATIONS: NoBODY WEIGHT: NoFOOD CONSUMPTION:- Food consumption for each animal determined and mean daily diet consumption calculated as g food/kg body weight/day: NoWATER CONSUMPTION: No OPHTHALMOSCOPIC EXAMINATION: No HAEMATOLOGY: No CLINICAL CHEMISTRY: No URINALYSIS: No NEUROBEHAVIOURAL EXAMINATION: No

Sacrifice and pathology

GROSS PATHOLOGY: Yes. Organs and tissues were examined for gross lesions.HISTOPATHOLOGY: Yes. Specimens of all organs and tissues were fixed in neutral buffered 10% formalin.

Results and discussions

Effect levels

Endpoint	Effect level	Based on	Sex	Basis for effect level / Remarks
LOAEL (i.v.)	1500 mg/kg bw/day	test mat.	no data	histopathology (kidneys, toxic nephropathy)

Results of examinations

Details on results

CLINICAL SIGNS AND MORTALITYThere was no mortality in any group. No clinical signs were reported.GROSS PATHOLOGYGroup 1 and 3 animals (intravenous exposure) had dry gangrene at the sites of the tail injections. Approximately half of the animals administered test substance intraperitoneally (group 2 and 3), exhibited 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. These were all local effects at the administration site and probably due to the alkalinity of the substance. None of the animals in group 1 or the control groups (4 and 5) had gross lesions.HISTOPATHOLOGY: NON-NEOPLASTICMicroscopic examination of tissues revealed chronic cellulitis at the injection sites. Peracute toxic nephrosis of the kidneys was observed in 5/6 rats in group 1 necropsied 24 hours after injection, but this adverse effect was not seen in animals allowed the 7 day recovery period. The same effect was noted in group 2, in 6/6 rats necropsied at 24 hours and 5/6 rats in the 7-day recovery group (see table 1). In all cases, the lesion was similar to that observed in the LD50 (pilot) study in rats administered 2000-3500 mg/kg bw test substance.

Any other information on results incl. tables

Table 1: Incidence of peracute toxic nephrosis in rats

Group	Administration route	Dosage	Number with peracute toxic nephrosis	Time of sacrifice
1	intravenous	500 mg/kg bw	5/6	24 hours after last infusion
			0/6	7 days after last infusion
2	intraperitoneal	1500 mg/kg bw	6/6	24 hours after last infusion
			5/6	7 days after last infusion
3	intravenous + intraperitoneal	500 mg/kg bw	2/6	24 hours after last infusion
			0/6	7 days after last infusion
4, control	intravenous	-	0/4	24 hours and 7 days after last infusion
5, control	intraperitoneal	-	0/4	24 hours and 7 days after last infusion

Endpoint study record: Mulinos, 1961, iv, 21d, dog, RL4

Administrative Data

Study result type experimental result

Reliability 4 (not assignable)

Rationale for reliability incl. deficiencies Original report not available and documentation insufficient for assessment

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
other company data	Mulinos, M. G.	1961	Toxic effects of Tris-amino buffer (Tris(hydroxymethyl)aminomethane)		The Industrial Bio-Test Laboratories, Northbrook, Illinois, USA	DR-0017-3524-100	ANGUS, a wholly owned subsidiary of The Dow Chemical Company		1961-12-05

Data access

data submitter is data owner

Cross-reference to same study

7.5.4 Mulinos, 1961, iv, 21d, rabbit, RL4

Materials and methods

Test type

subacute

Limit test

no

Principles of method if other than guideline

To assess the subacute intravenous effect of the test substance, dogs were exposed via an indwelling catheter for 21 days. The mortality and clinical signs were reported. Specified haematological and urinary parameters and blood glucose was measured, but the results not reported. Gross pathology and histopathology was performed.

GLP compliance

no (performed prior to GLP)

Test materials

Identity of test material same as for substance defined in section 1 (if not read-across)

yes

Details on test material

- Name of test material (as cited in study report): Tris(hydroxymethyl)aminomethane

Test animals

Species

dog

Strain

no data

Sex

no data

Administration / exposure

Route of administration

intravenous

Vehicle

other: Ringer's solution

Details on exposure

Dogs received the intravenous infusion via an indwelling catheter into the jugular vein close to the right atrium. The infusion rate was 0.5 mL/kg/minute of a 0.34M solution, which was shown previously to cause vomiting and convulsions in the dogs

sporadically and fairly infrequently, respectively. 2 animals were used for the control group.

Analytical verification of doses or concentrations

no data

Duration of treatment / exposure

21 days

Frequency of treatment

Daily

Doses / concentrations

1500 and 3000 mg/kg bw

No. of animals per sex per dose

5 per dose

Control animals

yes, concurrent vehicle

Examinations

Observations and examinations performed and frequency

CAGE SIDE OBSERVATIONS: YesDETAILED CLINICAL OBSERVATIONS: NoBODY WEIGHT: NoFOOD CONSUMPTION: NoWATER CONSUMPTION: NoOPHTHALMOSCOPIC EXAMINATION: No HAEMATOLOGY: Yes- Time schedule for collection of blood: prior to the study start and on the day following the final infusion- Anaesthetic used for blood collection: No data- Animals fasted: No data- Parameters examined: hemoglobin, hematocrit, white blood cell count, differential white blood cell countCLINICAL CHEMISTRY: Yes- Time schedule for collection of blood: Before and after infusion on 2 separate days- Parameters examined: GlucoseURINALYSIS: Yes- Time schedule for collection of urine: prior to the study start and on the day following the final infusion- Metabolism cages used for collection of urine: No data- Animals fasted: No data- Parameters examined: albumin, reducing substances, microscopic elements, sulfobromophthalein sodium clearance (BSP), nonprotein nitrogen (NPN)NEUROBEHAVIOURAL EXAMINATION: No

Sacrifice and pathology

GROSS PATHOLOGY: YesHISTOPATHOLOGY: Yes. The following organs and tissues were fixed in 10% formalin and stained with H&E: brain, heart, lungs, aorta, esophagus, stomach, small and large intestine, liver, spleen, kidneys, urinary bladder, genital organs, thyroid and adrenal glands.

Results and discussions

Results of examinations

Details on results

CLINICAL SIGNS AND MORTALITYOne dog in the 3000 mg/kg bw/day group died during the study period on day 4.The dogs in the 1500 and 3000 mg/kg bw/day groups exhibited convulsions and vomiting sporadically and fairly infrequently, respectively.HAEMATOLOGYThe hematology results were not included in the summary.CLINICAL CHEMISTRYThe glucose values were not included in the summary.URINALYSISThree dogs in the 1500 mg/kg bw/day group had an increased retention of BSP.GROSS PATHOLOGYIn 3 of the dogs receiving 1500 mg/kg bw/day, infarcts of the liver were observed. These were the same dogs that had increased retention of BSP.HISTOPATHOLOGY: NON-NEOPLASTICIn the same 3 dogs that had gross pathology effects in the liver (1500 mg/kg bw/day group), the histopathologic examination of the liver showed colonies of bacteria, acute inflammatory exudate and hypertrophy of the Kuppfer cells. The infarcts described grossly were multiple abcesses. This was attributed by the author to bacterial hepatitis and thus unrelated to the treatment. There were no other tissue changes observed.

Endpoint study record: Thompson, 1965, iv, 20d, rabbit, RL2

Administrative Data

Purpose flag	key study; robust study summary
Study result type	experimental result
Reliability	2 (reliable with restrictions)
Rationale for reliability incl. deficiencies	Acceptable, well documented study that meets basic scientific principles

Data source

Reference

Referenc e type	Author	Yea r	Title	Bibliographi c source	Testing laborator y	Repor t no.	Owner compan y	Compan y study no.	Repor t date
study report	Thompson , S. W.	196 5	Toxicity studies with tris(hydroxymethyl)aminometha		U.S. Army Research	DR-0017-			1965-03-01

			ne		and Nutrition Laboratory , Fitzsimons General Hospital, Denver, Colorado 80240, USA	3524-100			

Data access

other: Property of the U.S. Army Medical Research and Nutrition Laboratory, publicly available

Cross-reference to same study

7.2.4 key, Thompson, 1965, rat, RL2; 7.5.4 Thompson, 1965, iv-ip, 20d, rat, RL2

Materials and methods

Test type

subacute

Limit test

no

Principles of method if other than guideline

Rabbits were administered the test substance intravenously 5 days/week over 4 weeks. The animals were observed for 24 hours or 20 days after the exposure ended. The body weight and clinical signs were reported. Specified hematological and urinary parameters were examined and gross pathology and histopathology of target organs was performed.

GLP compliance

no (performed prior to GLP)

Test materials

Identity of test material same as for substance defined in section 1 (if not read-across)

yes

Details on test material

- Name of test material (as cited in study report): Tris(hydroxymethyl)aminomethane, THAM- Physical state: White, crystalline solid from Abbott Laboratories

Test animals

Species

rabbit

Strain

other: New Zealand

Sex

male/female

Details on test animals and environmental conditions

TEST ANIMALS- Weight at study initiation: 3.0-4.0 kg- Acclimation period: 14 days

Administration / exposure

Route of administration

intravenous

Vehicle

water

Details on exposure

The animals were administered 500 mg/kg bw/day of the test substance (0.3M) or saline to which 5 m Eq of KCl/L was added (control group). The dosage and rate of administration were equal in volume and time on a body weight basis; 500 mL/min. Intravenous infusions were given 5 days per week for 4 weeks. The test solution was 'Sterile Anti-Acidosis Lyophilised' containing 36.3 g tris(hydroxymethyl)aminomethane, 1.75 g NaCl and 0.37 g KCl dissolved in 1000 mL water for injection.

Analytical verification of doses or concentrations

no

Duration of treatment / exposure

28 days

Frequency of treatment

Daily, 5 days per week

Doses / concentrations

500 mg/kg bw/day

No. of animals per sex per dose

4

Control animals

yes, concurrent vehicle

Details on study design

Two males and 2 females from each group were sacrificed and necropsied 24 hours after the completion of the last treatment. The remaining animals in each group were observed for a total of 20 days after the last infusion, prior to necropsy.

Examinations

Observations and examinations performed and frequency

CAGE SIDE OBSERVATIONS: Yes - Time schedule: DailyDETAILED CLINICAL OBSERVATIONS: Yes. Rectal temperature was recorded twice daily during the acclimatisation and observation periods, and immediately before and hourly until temperatures returned to normal after all infusions given during the 28-day treatment period.BODY WEIGHT: Yes- Time schedule for examinations: DailyFOOD CONSUMPTION: Recorded dailyWATER CONSUMPTION: Yes- Time schedule for examinations: DailyOPHTHALMOSCOPIC EXAMINATION: NoHAEMATOLOGY: Yes- Time schedule for collection of blood: Heart blood samples were collected for BSP retention tests once a week during the acclimatisation and observation periods, and during the second week of the exposure period. Blood samples were collected from an ear vein once during the acclimatisation and observation periods and twice a week during the exposure period.- Anaesthetic used for blood collection: No data- Animals fasted: No data- How many animals: No data- Parameters examined: BSP retention (in heart blood), total serum proteins, A/G ratios, serum bilirubin, cephalin flocculation, serum transaminase (SGOT), hemoglobin, hematocrit, white blood cells, red blood cells, differential and platelet counts. CLINICAL CHEMISTRY: NoURINALYSIS: Yes- Time schedule for collection of urine: Daily. Urine output was recorded daily during the whole acclimatisation and study period. During the acclimatisation and observation periods, urinalysis was performed once per week on the first urine voided in the morning. During the exposure period, the urinalysis was performed on the first urine voided after infusion, on the first day of infusion and twice a week thereafter for a total of 3 weeks. On two days a week during the exposure period, a postinfusion urinary excretion of the test substance was measured on 4 (2 males and 2 females) rabbits in each group. The postinfusion time at which the specimen was collected varied between rabbits. - Metabolism cages used for collection of urine: No data- Animals fasted: No- Parameters examined: pH, albumin, glucose, benzidine test for hemoglobin on centrifuged sediment and supernate, and microscopic examination for red and white blood cells and casts. Method by Linn and Roberts (In vitro and in vivo effects of amino buffers. Annals N.Y. Acad. Sci. 1940; 92: 333-812) was used.NEUROBEHAVIOURAL EXAMINATION: No

Sacrifice and pathology

GROSS PATHOLOGY: Yes. Organs and tissues were examined for gross lesions.HISTOPATHOLOGY: Yes. Specimens of all organs and tissues were fixed in neutral buffered 10% formalin. Tissues from the liver and kidneys were fixed in formalin-calcium solution. From these specimens, paraffin embedded tissue sections were prepared and stained by Harris' hematoxylin and eosin technique. Histochemical methods to demonstrate the sites of activity of enzymes (alkaline phosphatase, acid phosphatase, esterase, peptidase, DPN diaphorase, TPN diaphorase) were performed using the frozen tissue sections of the rabbit liver and kidney specimens fixed in formalin-calcium.All histopathologic methods used were described by Thompson (Selected histochemical and histopathological methods, Springfield, Charles C. Thomas, 1965).

Results and discussions

Effect levels

Endpoint	Effect level	Based on	Sex	Basis for effect level / Remarks
LOAEL	500 mg/kg bw/day	test mat.	no data	histopathology (kidney lesions, chronic interstitial nephritis). Only one dose was used.

Results of examinations

Details on results

CLINICAL SIGNS AND MORTALITYNo animals died during the study period. 6/8 rabbits had temperatures in excess of 40.6 °C (105 °F) at some point during the exposure period, but the effect was not treatment-related. BODY WEIGHT AND WEIGHT GAINThe body weight of the animals fluctuated between 200 g and 400 g during the study in both groups, with no specific trend (no individual animal data reported). The effect is not considered to be treatment-related.FOOD CONSUMPTIONThe food consumption was similar between the treatment and control groups (no individual animal data reported).WATER CONSUMPTIONThe food consumption was similar between the treatment and control groups (no individual animal data reported).HAEMATOLOGYThe results of the weekly analyses on blood samples were within the normal ranges (those reported during the acclimatisation period) for the following parameters: total serum proteins, A/G ratio, serum bilirubin, cephalin flocculation, serum transaminase, RBC, differential counts, hemoglobin, hematocrit, and platelet counts. White blood cell counts in excess of 13,000 were seen in 5/8 rabbits in the treatment group. In all the cases, elevated WBC levels were noted in the animals with dry gangrene in the external ear.CLINICAL CHEMISTRYURINALYSISNo diuretic effect of the test substance was observed and no significant changes between the control and treatment groups were noted for any of the parameters tested during urinalysis. (No individual animal data reported).GROSS PATHOLOGYSeven of 8 rabbits receiving the test substance had inflammatory lesions of the external ear, around the infusion site. The lesions varied from swelling and redness to dry gangrene and erosion (no individual animal data reported). This is considered to be treatment-related local effect. The test substance is alkaline and is known to cause irritation at the site of exposure in an acute oral and a subcutaneous toxicity study. 2/4 treatment animals necropsied 20 days after the last treatment had visible infarcts in the kidneys. No gross lesions

were noted in any other organ or tissue in any other treated animal and no gross lesions were noted in the control animals.HISTOPATHOLOGY: NON-NEOPLASTICIn the 7 test substance treated animals that had gross lesions of the ear, there were microscopic lesions of chronic cellulites and necrosis in the subcutaneous tissues of the ear at the injection sites. The 2 animals with gross kidney lesions had histopathologic kidney lesions visible at the microscopic examination of the tissue samples. These animals also had chronic interstitial nephritis. Infiltrations of lymphocytes were observed in the tissue sections of the liver and kidney of 3 additional rabbits in the treatment group. The infiltrations were seen in animals that were sacrificed 20 days after the exposure ended, as well as in those sacrificed immediately following the final treatment. Peracute toxic nephrosis was observed in 1 rabbit sacrificed 20 days after exposure ended. Urolithiasis was also observed in this animal. Cases of peracute toxic nephrosis were also observed in rats treated with the test substance (Thompson, 1965). The kidney effects are probably related to the alkalinity of the substance, which is excreted primarily via the urine.The control animals had incidental lesions that were not related to the injections: periportal lymphocytic infiltration (1/8), bilateral chronic interstitial nephritis (1/8) and Encephalitozoonosis- compatible lesions in the brain (1/8).No difference in staining reaction was observed for the enzyme activity measured in liver and kidney tissue.

Endpoint study record: Roberts and Linn, 1961, iv, 10d, mouse, RL4

Administrative Data

Study result type experimental result

Reliability 4 (not assignable)

Rationale for reliability incl. deficiencies Original report not available and documentation insufficient for assessment

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	Roberts, M. and Linn, S.	1961	Acute and subchronic toxicity of 2-amino-2-hydroxymethyl-1,3-propanediol	Annals of the New York Academy of Sciences, Vol. 92, Art. 2: 724-734					

Data access

data published

Cross-reference to same study

7.5.4 Roberts and Linn, 1961, iv, 10d, rabbit, RL4; 7.5.4 Roberts and Linn, 1961, iv, 19d, rabbit, RL2

Materials and methods

Test type

subacute

Limit test

no

Principles of method if other than guideline

Mice were exposed to the test substance under different conditions to assess the effect of rapid infusion and neutralising the test substance. The mice were injected rapidly (30 seconds) with 50 mL/kg bw/day 0.155M test substance once daily for 10 consecutive days. Mortality and clinical signs were reported, and gross pathology and histopathology was performed.

GLP compliance

no (performed prior to GLP)

Test materials

Identity of test material same as for substance defined in section 1 (if not read-across)

yes

Details on test material

- Name of test material (as cited in study report): 2-amino-2-hydroxymethyl-1,3-propanediol

Test animals

Species

mouse

Strain

no data

Sex

no data

Administration / exposure

Route of administration

intravenous

Vehicle

no data

Details on exposure

Mice were exposed to the test substance under different conditions to assess the effect of rapid infusion and neutralising the test substance. The animals were injected rapidly (30 seconds) with 50 mL/kg bw/day 0.155M test substance and 50 mL/kg bw/day 0.155M adjusted to pH 5.5 (using HCl) once daily for 10 consecutive days. The dose is equivalent to app. 930 mg/kg bw/day.

Analytical verification of doses or concentrations

no data

Duration of treatment / exposure

10 days

Frequency of treatment

Daily

Doses / concentrations

50 mL/kg bw

No. of animals per sex per dose

3 per dose

Control animals

no data

Examinations

Observations and examinations performed and frequency

CAGE SIDE OBSERVATIONS: Yes. Animals were observed for mortality and clinical signs.DETAILED CLINICAL OBSERVATIONS: NoBODY WEIGHT: NoFOOD CONSUMPTION: NoFOOD EFFICIENCY: NoWATER CONSUMPTION: NoOPHTHALMOSCOPIC EXAMINATION: NoHAEMATOLOGY: NoCLINICAL CHEMISTRY: NoURINALYSIS: NoNEUROBEHAVIOURAL EXAMINATION: No

Sacrifice and pathology

GROSS PATHOLOGY: Yes. Organs and tissues were examined for lesions. HISTOPATHOLOGY: Yes.

Results and discussions

Effect levels

Endpoint	Effect level	Based on	Sex	Basis for effect level / Remarks
NOAEL	930 mg/kg bw/day (nominal)	test mat.	no data	
NOAEL	930 mg/kg bw/day (nominal)	test mat.	no data	pH 5.5

Results of examinations

Details on results

CLINICAL SIGNS AND MORTALITYThere was no mortality and no clinical signs were observed.GROSS PATHOLOGYThere were no effects on gross pathology.HISTOPATHOLOGY: NON-NEOPLASTICThe histopathological examination did not reveal significant effects.

Endpoint study record: Roberts and Linn, 1961, iv,19d, rabbit, RL4

Administrative Data

Study result type experimental result

Reliability 4 (not assignable)

Rationale for reliability incl. deficiencies Original report is not available and documentation insufficient for assessment

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	Roberts, M. and	1961	Acute and subchronic toxicity	Annals of the New York Academy of					

	Linn, S.		of 2-amino-2-hydroxymethyl-1,3-propanediol	Sciences, Vol. 92, Art. 2: 724-734					

Data access

data published

Cross-reference to same study

See chapter 7.2.4 Acute toxicity: other routes

Materials and methods

Test type

subacute

Limit test

no

Principles of method if other than guideline

Rabbits were exposed to the test substance under different conditions to assess the effect of slow infusion. Rabbits were injected slowly over 5 hours with 100 mL/kg bw 0.3M neutralised (pH 7.4 or 5.5) or non-neutralised test substance once daily for 19 consecutive days. The infusion rates varied. Mortality and clinical signs were reported, and gross pathology was performed.

GLP compliance

no (performed prior to GLP)

Test materials

Identity of test material same as for substance defined in section 1 (if not read-across)

yes

Details on test material

- Name of test material (as cited in study report): 2-amino-2-hydroxymethyl-1,3-propanediol

Test animals

Species

rabbit

Strain

no data

Sex

no data

Administration / exposure

Route of administration

intravenous

Vehicle

no data

Details on exposure

Rabbits were exposed to the test substance under different conditions to assess the effect of slow infusion. The rabbits were injected slowly over 5 hours with 100 mL/kg bw 0.3M neutralised (pH 7.4 or 5.5) or non-neutralised test substance once daily for 19 consecutive days. The infusion rates varied (see table 1).

Analytical verification of doses or concentrations

no data

Duration of treatment / exposure

19 days

Frequency of treatment

Daily

Doses / concentrations

Group 1: 79 and 100 mL 0.3M/kg bw Group 2: 95 and 125 mL 0.3M/kg, adjusted to pH 7.4Group 3: 90 and 100 mL 0.3M/kg, adjusted to pH 5.5

No. of animals per sex per dose

2 in group 1 and 3, 3 in group 2

Control animals

no data

Examinations

Observations and examinations performed and frequency

CAGE SIDE OBSERVATIONS: Yes. Animals were observed for mortality and clinical signs.DETAILED CLINICAL OBSERVATIONS: NoBODY WEIGHT: NoFOOD CONSUMPTION: NoFOOD EFFICIENCY: NoWATER CONSUMPTION: NoOPHTHALMOSCOPIC EXAMINATION: NoHAEMATOLOGY: YesTo assess the effect of the test substance on erythrocytes, a drop of rabbit blood was added to 0.3M of the test substance. After 1 hour the supernatant was examined for hemolysis.CLINICAL CHEMISTRY: NoURINALYSIS: NoNEUROBEHAVIOURAL EXAMINATION: No

Sacrifice and pathology

GROSS PATHOLOGY: Yes. Organs and tissues were examined for lesions. HISTOPATHOLOGY: Yes.

Other examinations

As necrosis was observed at most of the injection sites in rabbits injected with 0.3M, an additional test on the effect of pH on the severity of necrosis was performed. An intradermal injection of 0.1M was done and trypan blue injected intravenously. The irritation due to the test substance was assessed by observing the amount of extravasated dye.

Results and discussions

Effect levels

Endpoint	Effect level	Based on	Sex	Basis for effect level / Remarks
LOAEL	1470 mg/kg bw/day (nominal)	test mat.	no data	Mortality
LOAEL	1767 mg/kg bw/day (nominal)	test mat.	no data	Anorexia. pH adjusted to 7.4
LOAEL	1860 mg/kg bw/day (nominal)	test mat.	no data	Anorexia. pH adjusted to 5.5

Results of examinations

Details on results

CLINICAL SIGNS AND MORTALITYIn group 1, 2/2 rabbits died (day 2 and 3 after administration), in group 2 1/3 rabbits died (day 2 after administration). In group 3, there was no mortality due to test substance, but one rabbit was sacrificed on day 1 after administration due to a compound fracture. See table 1.Hemorrhage and convulsions were observed in animal 1 and 2, respectively, in group 1. In group 2, no clinical signs were noted in the animal that died. Anorexia and bloody urine, respectively, was observed in the 2 surviving animals. In group 3 anorexia was observed in the surviving animal. Irregular respiration was also observed in one or more unspecified animals. See table 1.HAEMATOLOGY0.3M of the test substance did not cause hemolysis.GROSS PATHOLOGYAbnormally red lungs, necrosis at the infusion site, bleached liver, darkened spleen, bloated stomach, and lesions in the heart and kidneys was observed in one or more animals. HISTOPATHOLOGY: NON-NEOPLASTICThe histopathological examination did not reveal significant effects.OTHER FINDINGSThe 0.3M solution was extremely irritating at the injection site. Neutralisation of the test substance with HCl reduced the irritation, indicating that the pH contributes to the irritation.

Any other information on results incl. tables

Table 1: Mortality and adverse effects of the test substance per dose level

Group	Dose (mg/kg bw)	Solution	Dose/day	Animal	Result
1	1470	0.155M	79 mL/kg bw	rabbit	Died, hemorrhage
1	1860	0.155M	100 mL/kg bw	rabbit	Died, convulsions
2	2325	0.155M, pH 7.4	125 mL/kg bw	rabbit	Died, no symptoms
2	1767	0.155M, pH 7.4	95 mL/kg bw	rabbit	Nonlethal, anorexia
2	2046	0.155M, pH 7.4	110 mL/kg bw	rabbit	Nonlethal, bloody urine
3	1674	0.155M, pH 5.5	90 mL/kg bw	rabbit	Sacrificed, compound fracture
3	1860	0.155M, pH 5.5	100 mL/kg bw	rabbit	Nonlethal, anorexia

Endpoint study record: Darby and Anderson, 1966, ip, 3d, dog, RL2

Administrative Data

Study result type experimental result

Reliability 2 (reliable with restrictions)

Rationale for reliability incl. deficiencies Basic data given

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	Darby, T. D. and Anderson, S. J.	1966	Tolerance and toxicity of THAM	Ann. Anesth. Franc. VII, 3, Juillet, Aout, Septembre 1966		DR-0017-3524-10			

Data access

data published

Cross-reference to same study

7.2.4 Darby and Anderson, 1966, iv, rat, RL4; 7.2.4 Darby and Anderson, 1966, ip, dog, RL2; 7.2.4 Darby and Anderson, 1966, iv, mouse, RL4; 7.5.1 Darby and Anderson, 1966, 31d, rat, RL4; 7.5.1 Darby and Anderson, 1966, 30d, dog, RL4; 7.5.1 Darby and Anderson, 1966, 15d, rat, RL4

Materials and methods

Test type

subacute

Limit test

no

Principles of method if other than guideline

Dogs were administered the test substance in Inpersol solution for dialysis intraperitoneally once to assess the acute toxicity or for 3 consecutive days during dialysis to assess the acute toxic effect in connection with dialysis. The mortality and clinical signs were recorded. The gross pathological effects were recorded at necropsy.

GLP compliance

no (performed prior to GLP)

Test materials

Identity of test material same as for substance defined in section 1 (if not read-across)

yes

Details on test material

- Name of test material (as cited in study report): THAM

Test animals

Species

dog

Strain

no data

Sex

no data

Administration / exposure

Route of administration

intraperitoneal

Vehicle

other: Inpersol dialysis solution

Details on exposure

3 dogs were dialyzed on 3 consecutive days. Each day, the dogs were anesthetized with pentobarbital i.v. and administered 30 mL/kg bw 0.075M test substance in Inpersol. A total of 6 exchanges were made. The time interval between exchanges was 30 minutes. The last instillation of 30 mL/kg bw was left in the peritoneal cavity, to ensure the maximum possible irritation.

Analytical verification of doses or concentrations

no data

Duration of treatment / exposure

3 days

Frequency of treatment

Daily

Doses / concentrations

30 mL/kg bw

No. of animals per sex per dose

3 per dose

Control animals

yes

Examinations

Observations and examinations performed and frequency

CAGE SIDE OBSERVATIONS: Yes. Animals were observed for 24 hoursDETAILED CLINICAL OBSERVATIONS: No dataBODY WEIGHT: NoFOOD CONSUMPTION: NoFOOD EFFICIENCY: NoWATER CONSUMPTION: NoOPHTHALMOSCOPIC EXAMINATION: No HAEMATOLOGY: NoCLINICAL CHEMISTRY: NoURINALYSIS: NoNEUROBEHAVIOURAL EXAMINATION: No

Sacrifice and pathology

GROSS PATHOLOGY: Yes HISTOPATHOLOGY: No

Results and discussions

Effect levels

Endpoint	Effect level	Based on	Sex	Basis for effect level / Remarks
other: adverse effect level	> 270 mg/kg bw/day (nominal)	test mat.	no data	

Results of examinations

Details on results

CLINICAL SIGNS AND MORTALITYOne dog died during the dialysis procedure on day 3. The dog had heartworm, which is not related to the treatment. No clinical signs were noted.GROSS PATHOLOGYIn the dog that died, dark spots on the lobes of the lungs were observed. 75 mL of fluid was found in the thoracic cavity. HISTOPATHOLOGY: NON-NEOPLASTICIn the dog that died, atelectasis (collapse of the lung) with emphysema was noted, and in many areas thickened alveolar walls with numerous invading lymphocytes. The findings suggest pulmonary congestion and pulmonary disease secondary to the long periods under anesthesia during the first 2 days of exposure. In the 2 other dogs, similar pulmonary pathology was seen. The changes were similar to those observed in control animals. Therefore, the effects are considered not to be treatment-related.

Endpoint study record: Mulinos, 1961, iv, 21d, rabbit, RL4

Administrative Data

Study result type experimental result

Reliability 4 (not assignable)

Rationale for reliability incl. deficiencies Original report not available and documentation insufficient for assessment

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
other company data	Mulinos, M. G.	1961	Toxic effects of Tris-amino buffer (Tris(hydroxymethyl)aminomethane)		The Industrial Bio-Test Laboratories, Northbrook, Illinois, USA	DR-0017-3524-100	ANGUS, a wholly owned subsidiary of The Dow Chemical Company		1961-12-05

Data access

data submitter is data owner

Cross-reference to same study

7.5.4 Mulinos, 1961, iv, 21d, dog, RL4

Materials and methods

Test type

subacute

Limit test

no

Principles of method if other than guideline

To assess the subacute intravenous effect of the test substance, rabbits were exposed via an indwelling catheter for 21 days. The mortality and clinical signs were reported. Specified haematological and urinary parameters and blood glucose was

measured, but the results not reported. Gross pathology and histopathology was performed.

GLP compliance

no (performed prior to GLP)

Test materials

Identity of test material same as for substance defined in section 1 (if not read-across)

yes

Details on test material

- Name of test material (as cited in study report): Tris(hydroxymethyl)aminomethane

Test animals

Species

rabbit

Strain

no data

Sex

no data

Administration / exposure

Route of administration

intravenous

Vehicle

other: Ringer's solution

Details on exposure

Rabbits received the intravenous infusion via an indwelling catheter into the jugular vein close to the right atrium. The infusion rate was 0.2 mL/kg/minute, which was shown previously not to cause the convulsions observed at higher infusion rates. Ringer's solution was used as the vehicle for the 0.34M solution. There were 2 animals in the control group.

Analytical verification of doses or concentrations

no data

Duration of treatment / exposure

21 days

Frequency of treatment

Daily

Doses / concentrations

1500 and 3000 mg/kg bw/day

No. of animals per sex per dose

5 per dose

Control animals

yes, concurrent vehicle

Examinations

Observations and examinations performed and frequency

CAGE SIDE OBSERVATIONS: YesDETAILED CLINICAL OBSERVATIONS: NoBODY WEIGHT: NoFOOD CONSUMPTION: NoWATER CONSUMPTION: NoOPHTHALMOSCOPIC EXAMINATION: No HAEMATOLOGY: Yes- Time schedule for collection of blood: prior to the study start and on the day following the final infusion- Anaesthetic used for blood collection: No data- Animals fasted: No data- Parameters examined: hemoglobin, hematocrit, white blood cell count, differential white blood cell count, glucoseCLINICAL CHEMISTRY: Yes- Time schedule for collection of blood: Before and after infusion on 2 separate days- Parameters examined: GlucoseURINALYSIS: Yes- Time schedule for collection of urine: prior to the study start and on the day following the final infusion- Metabolism cages used for collection of urine: No data- Animals fasted: No data- Parameters examined: albumin, reducing substances, microscopic elements, sulfobromophthalein sodium clearance (BSP), nonprotein nitrogen (NPN)NEUROBEHAVIOURAL EXAMINATION: No

Sacrifice and pathology

GROSS PATHOLOGY: YesHISTOPATHOLOGY: Yes. The following organs and tissues were fixed in 10% formalin and stained with H&E: brain, heart, lungs, aorta, esophagus, stomach, small and large intestine, liver, spleen, kidneys, urinary bladder, genital organs, thyroid and adrenal glands.

Results and discussions

Results of examinations

Details on results

CLINICAL SIGNS AND MORTALITYTwo rabbits in the 3000 mg/kg bw/day group died during the study period (day 6 and

12).The rabbits in both treatment groups exhibited rapid, shallow breathing during most of the infusion periods.HAEMATOLOGYThe hematology results were not included in the summary.CLINICAL CHEMISTRYThe glucose values were not included in the summary.URINALYSISThe results were not included in the summary.GROSS PATHOLOGYThe gross pathology results were not included in the summary.HISTOPATHOLOGY: NON-NEOPLASTICThere was evidence of hepatic and renal granulomatosis in both the control and treated animals. This was attributed by the author to parasites and thus unrelated to the treatment. There were no other tissue changes observed.

Genetic toxicity

Endpoint summary: Genetic toxicity

Key value for chemical safety assessment

Genetic toxicity

negative

Discussion

A bacterial mutagenicity test employing genetically modified E. coli was negative with 2-amino-2-(hydroxymethyl)-1,3-propanediol (TRIS AMINO) in the presence and the absence of a metabolic activation system (Hayes et al., 1984). The test results were obtained with one E.coli test strain that was transiently exposed to, and then removed from TRIS AMINO (1 mg/mL, pH 7.4) prior to the selection step for mutant cells.

There are no further data available on genetic toxicity of TRIS AMINO. However, there are reliable data for other substances considered suitable for read-across using the analogue approach. Potential analogues for TRIS AMINO are other 2-amino-1,3-propanediols, i.e. substances that share with the target substance a common propane backbone with amine group at 2-carbon position and primary alcohols at 1 and 3 positions: 2-amino-2-ethyl-1,3-propanediol (AEPD), 2-amino-2-methyl-1,3-propanediol (AMPD) and 2-amino-1,3-propanediol (APD). The only structural difference between TRIS AMINO and AEPD is a replacement of a hydroxyl group with a methyl group and two further analogues differ in the length of the alkyl side-chain at position 2: from 0 carbon atoms (APD) through 1 (AMPD) to 2 (AEPD). The target substance and the source substances share similar physico-chemical properties.
TRIS AMINO and the 2-amino-1,3-propanediols are expected to show comparable toxicokinetic characteristics, and it is anticipated that the absorbed amounts of all the aminopropanediols have limited systemic bioavailability and are rapidly eliminated by the kidneys. No relevant metabolism is expected, based on experimental data and on QSAR predictions. The modelling of potential metabolites using the OECD QSAR toolbox v.2.0 (2010) did not predict relevant metabolites of TRIS AMINO or of any of the 2-amino-1,3-propanediols. Therefore, no metabolism by cytochrome P450 enzymes in-vivo is expected.
A combined oral repeated dose toxicity study and reproduction/developmental screening test was performed with AEPD according to OECD 422 (Ishida, 2004). No effects on reproduction or fertility and no systemic toxicity were observed up to and including the highest dose level of 1000 mg/kg bw/day. In a non-guideline developmental toxicity screening study performed in rats, AMPD did not shown any potential for causing developmental toxicity at doses of 1000 mg/kg bw/day (Rasoulpour and Andrus, 2011). Furthermore, in an in-vitro developmental screen (limb bud assay) using AMPD, a lack of developmental toxicity (within the scope of the assay) was indicated (Ellis-Hutchings and Marshall, 2011). Available studies via the oral, dermal or intraperitoneal route on these substances also caused no systemic toxicity. The results of the acute studies, as well as the repeated dose studies, demonstrate that the main cause of toxicity was the intrinsic alkalinity of the respective test substances at the site of contact. Inhalation is of no concern, because the low vapour pressure of the pure substances means that exposure is unlikely to occur. In case of spray applications of technical products containing the neat substance, the concentration is very low (< 1%). The Cramer classification (related mainly to the oral route) also indicates a low toxicological concern for TRIS AMINO and the 2-amino-1,3-propanediols. Thus, both TRIS AMINO and 2-amino-1,3-propanediols are of low concern with regard to systemic toxicity.
With respect to molecular structures, no mutagenic potency is predicted using QSAR modelling and no structural alerts were detected for TRIS AMINO and 2-amino-1,3-propanediols. "H-acceptor-path3-

H-acceptor" is alerted in a large number of molecules and thus of practically no prediction value.
Due to the structural similarity and the similar toxicological properties between TRIS AMINO and the 2-amino-1,3-propanediols, read-across using an analogue approach is justified.
There are three in-vitro genetic toxicity studies available using the analogue substance AEPD. According to OECD guidelines a bacterial reverse mutation assay (Ames test), an in-vitro chromosomal aberration test in Chinese hamster lung cells and an in-vitro mammalian cell mutation assay in Chinese hamster ovary cells were performed with AEPD.
The in-vitro genetic toxicity of AEPD was investigated in a bacterial reverse mutation assay (Ames test) according to OECD 471 (Mochizuki, 2004). The preincubation method was conducted with S. typhimurium strains TA 1535, TA 1537, TA 98, TA 100 and E. coli WP2 uvrA at concentrations up to 5000 µg/plate. AEPD did not induce reversions in any of the S. typhimurium strains or in E. coli WP2 uvrA with or without metabolic activation. No cytotoxic effects were observed and all the positive controls were valid.
In a study conducted according to OECD 473, the potential of AEPD to induce chromosomal aberrations was tested in cultured Chinese hamster lung (CHL) cells (Sono, 2004). CHL cells were exposed to AEPD at concentrations up to 1200 µg/mL. No increase in chromosomal aberrations was observed in the experiments with short-term treatment (6 h) in the presence or absence of metabolic activation. No cytotoxic effects were observed and the positive controls were valid. Because of the negative results of the short-term treatment, an additional testing without metabolic activation was performed with continuous treatment (24 and 48 h). After continuous treatment, AEPD did not induce chromosomal aberrations in CHL cells.
AEPD was also tested for its potential to cause gene mutations in an in-vitro mammalian cell mutation assay according to OECD 476 (Indrani, 2011). Chinese hamster ovary (CHO) cells were treated with AEPD at concentrations of up to 1192 µg/mL for 4 h both with and without metabolic activation. After an expression time of 9 days in growth medium, cells were incubated for 10 days with 6 -thioguanine as selection agent for forward mutation at the HPRT locus. Both with and without metabolic activation, no increases in mutant frequency were observed in the initial and in the confirmatory gene mutation assay. At the highest tested concentration, AEPD caused cell growth inhibition, evaluated by relative cloning efficiency.
Taking into account all the available data, AEPD showed no evidence of a clastogenic and mutagenic potential with and without metabolic activation in in-vitro test systems. The results of AEPD will be read-across to TRIS AMINO, based on an analogue approach. This is in accordance with Regulation (EC) 1907/2006, Annex XI, which specifies that read-across of data from a suitable substance may be used to cover data gaps. Based on the analogue approach, it can be assumed that TRIS AMINO has no genotoxic potential.

Justification for classification or non-classification

Based on TRIS AMINO data and on read-across within an analogue approach, the available data on genetic toxicity do not meet the criteria for classification according to Regulation (EC) 1272/2008 or Directive 67/548/EEC, and are therefore conclusive but not sufficient for classification.

Genetic toxicity in vitro

Endpoint study record: key, RA-A, 115-70-8, Sono, 2004, CA, RL2

Administrative Data

Purpose flag	key study; robust study summary
Study result type	read-across from supporting substance (structural analogue or surrogate)
Reliability	2 (reliable with restrictions)
Rationale for reliability incl. deficiencies	GLP-guideline study, tested with the source substance 2-amino-2-ethyl-1,3-propanediol (CAS 115-70-8). In accordance to the ECHA guidance document "Practical guide 6: How to report read-across and categories (March 2010)", the reliability was changed from RL1 to RL2 to reflect the fact that this study was conducted on a read-across substance.

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report	Sono, A.	2004	Chromosomal aberration test of 2-Amino-2-ethyl-1,3-propanediol in cultured Chinese Hamster Cells.		Bozo Research Center 1284 Kamado, Gotemba-shi, Shizuoka, 412-0039, Japan	M-1129	Office of Chemical Safety Pharmaceutical and Food Safety Bureau Ministry of Health Labour and Welfare		2004-02-27

Data access

other: Japanese citizens, NGOs, NPOs, business, etc., are free to utilize and cite the results of the existing chemicals survey conducted by the three Ministries for any purpose. The results refer to existing chemicals survey conducted by the Japanese Government

Materials and methods

Type of genotoxicity

chromosome aberration

Type of study

in vitro mammalian chromosome aberration test

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 473 (In vitro Mammalian Chromosome Aberration Test)	no
according to	JAPAN: Guidelines for Screening Mutagenicity Testing Of Chemicals	no

GLP compliance

yes

Test materials

Identity of test material same as for substance defined in section 1 (if not read-across)

no

Test material identity

Identifier	Identity
EC name	2-amino-2-ethyl-1,3-propanediol

Details on test material

- Name of test material (as cited in study report): 2-amino-2-ethyl-1,3-propanediol- Analytical purity: 99.4%

Method

Target gene

not applicable

Species/strain

Species/strain mammalian cell line, other: Chinese hamster lung (CHL/IU) cells

Details on mammalian cell lines (if applicable) - Type and identity of media: Eagle's MEM with 10 vol.% calf serum (deactivated)

Additional strain characteristics no data

Metabolic activation with and without

Metabolic activation system cofactor supplemented post-mitochondrial fraction (S9-mix), prepared from the livers of rats treated with treated with phenobarbital and 5,6-benzoflavone

Test concentrations

without S9 mix(short-term treatment, continuous treatment 24 h and 48 h): 75, 150, 300, 600 and 1200 µg/mLwith S9 mix(short-term treatment): 75, 150, 300, 600 and 1200 µg/mL

Vehicle

- Vehicle(s)/solvent(s) used: physiol. saline

Controls

Negative controls yes (medium)

Solvent / vehicle controls yes (physiol. saline)

True negative controls no

Positive controls yes

Positive control substance other: without S9 mix: Mitomycin C (0.05 µg/mL), with S9 mix: Cyclophosphamide (15 µg/mL)

Details on test system and conditions

METHOD OF APPLICATION: in mediumDURATION- Exposure duration: 6 h (short time treatment), 24 and 48 h (continuous treatment)- Expression time (cells in growth medium): 18 h (for short time treatment)SPINDLE INHIBITOR (cytogenetic assays): colcemide (final concentration: 0.2 µg/mL)STAIN (for cytogenetic assays): Giemsa stain (2 vol.%)NUMBER OF REPLICATIONS: 2 plates per test, 1 experimentNUMBER OF CELLS EVALUATED: 200 metaphase images per dose (100 per plate)DETERMINATION OF CYTOTOXICITY- Method: The cell multiplication inhibiting action of the test substance was determined by measuring the cell density using a single-layer cultured cell densitometer and using the ratio of the cell density of the test group and of the negative group.

Evaluation criteria

The judgment was made according to the criteria of Ishidate et al. (1987). When the frequencies of appearance of the cells with chromosomal aberrations (CA) were < 5%, the results were considered negative; when the frequencies of CA were 5% or > 5%, the results were considered false positive; when the frequencies of CA were 10% or greater, the results were considered positive. Finally, in the cases in which dose dependence or reproducibility was observed the appearance of aberrant cells, the results were considered positive.

Results and discussions

Test results

Species/strain mammalian cell line, other: Chinese hamster lung (CHL/IU) cells

Metabolic activation with and without

Test system all strains/cell types tested

Genotoxicity negative

Cytotoxicity no

Vehicle controls valid yes

Negative controls valid yes

Positive controls valid yes

Additional information on results

RANGE-FINDING/SCREENING STUDIES:Based on the results of performing cell multiplication inhibition tests, it was decided to use a test concentration range of 75-1200 µg/mL.

Any other information on results incl. tables

No increase in chromosomal aberrations was observed in the test with either the short- term treatment (without S9 mix and with S9 mix) or continuous treatment.

Table 1: Results of experiments

Test item	Concentration	Cell growth ratio [mean in %]*	Aberrant cells in %	
	in µg/mL	in %	with gaps	without gaps

Exposure period 6-18 h, fixation time 24h, without S9 mix				
saline	0.9%	100	2.5	1.0
MMC	0.05	79	25	24
Test substance	75	93	2.5	1.0
	150	93	3.0	1.5
	300	86	2.5	0.5
	600	86	2.0	1.0
	1200	79	2.0	1.5
Exposure period 6-18 h, fixation time 24h, with S9 mix				
saline	0.9%	100	1.0	0
CP	15	93	79.0	79.0
Test substance	75	107	1.5	1.5
	150	93	1.0	0.5
	300	100	2.0	1.0
	600	93	2.0	1.0
	1200	85	2.5	1.5
Exposure period 24h, fixation time 24h, without S9 mix				
saline	0.9%	100	4.0	2.0
MMC	0.05	89	43.5	42.0
Test substance	75	89	3.0	0.5
	150	89	3.0	1.0
	300	111	2.5	1.0
	600	89	2.5	0.5
	1200	67	4.9	3.7
Exposure period 48h, fixation time 48h, without S9 mix				
saline	0.9%	100	3.0	0.5
MMC	0.05	94	68.0	66.5
Test substance	75	100	1.5	0
	150	106	1.5	0.5
	300	106	4.0	1.5
	600	100	1.5	0.5
	1200	50	4.8	3.6

MMC: Mitomycin C; CP: Cyclophosphamide (positive controls)
* The mean value showed as a growth ratio against the negative control value.

Applicant's summary and conclusion

Interpretation of results

negative

Endpoint study record: Hayes et al., 1984, forward selection assay, RL2

Administrative Data

Purpose flag	supporting study
Study result type	experimental result
Reliability	2 (reliable with restrictions)
Rationale for reliability incl. deficiencies	Basic data given. No information on analytical purity.

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	Hayes, S. et al.	1984	RK bacterial test for independently measuring chemical toxicity and mutagenicity: Short-term forward selection assay.	Mutation Research, 130:97-106					

Data access

data published

Materials and methods

Type of genotoxicity

gene mutation

Type of study

bacterial forward mutation assay

Principles of method if other than guideline

The RK test results were obtained with one E.coli assay strain that was transiently exposed to, and then removed from the test substance prior to the selection step for mutant cells.

GLP compliance

no data

Test materials

Identity of test material same as for substance defined in section 1 (if not read-across)

yes

Details on test material

- Analytical purity: no data- pH: 7.4

Method

Target gene

The E. coli selector strain CHY832 is deleted for bio-uvrB-chlA, was subsequently transduced for part of the bio operon and has integrated at attλ phage genes N-rex-cI[ind-ts857]-cro-cII-O-P.

Species/strain

Species/strain	E. coli, other: CHY832
Details on mammalian cell lines (if applicable)	The E. coli selector strain constructed for this study carries a small integrated fragment of the λ genome comprising genes N thru P. The active N, O and P genes on the fragment are negatively regulated by a temperature-sensitive 857 repressor protein coded by the included genecI. When the selector strain is transferred from 30 °C to 42 °C the cells are killed, with only rare spontaneous survivors able to form colonies at 42 °C.
Additional strain characteristics	no data
Metabolic activation	with and without
Metabolic activation system	S9-mix: Aroclor-1254-induced rat liver microsomal extract

Test concentrations

1 mg/mL (pH 7.4)

Vehicle

- Vehicle(s)/solvent(s) used: DMSO

Controls

Negative yes buffer (cell culture control)

controls	
Solvent / vehicle controls	yes (DMSO)
True negative controls	no
Positive controls	yes
Positive control substance	other: N-methylnitrosourea

Evaluation criteria

A substance is determined to be genotoxic if it is capable of increasing the forward mutation frequency for appearance of these mutant cells. Toxicity of the agent is independently evaluated by examining its effect on the viability of the selector strain at 30 °C, when the viral replication genes remain repressed.

Any other information on materials and methods incl. tables

In the RK test, the routine technique of transient exposure of the selector cells to the test substance, before the selection step for mutants, affords an important advantage in assaying the genotoxicity of highly toxic substance, since cell exposure to the test substance is minimised. The cells of the selector strain are killed upon shifting to 42 °C as a consequence of thermal derepression and subsequent expression of the replication genes from an integrated 10 -kilobase fragment of phageλ.

The hypothesis that pretreatment of the RK+ selector strain cells with a mutagen will increase the population of RK- survivor clones growing at 42 °C was tested.

Standard assay

The cells are pelleted and resuspended in either 0.01 M Na3 x Citrate (pH 4.0 -5.5), 0.01 M Na2 PO4 x HCl (pH 5.0 -7.2) or 0.01 M Tris x HCl (pH 7.4 -10.2) buffers containing 0.1 NaCl. The resuspended cells are mixed with either 0.2 mL of the equivalent buffer (untreated cell culture control), 0.2 mL DMSO or 0.2 mL DMSO containing the test substance (dissolved at 5x final concentration). The suspension are mixed, directly incubated 10 min at 30 °C, and microfuged for 1 min. The supernatant is decanted and pellet resuspended in 1 mL buffer. Each cell suspension is (i) directly spread on tryptone agar plates that are incubated 48 h at 42 °C, and (ii) diluted (10E-3 - 10E-5) in the same buffer and spread on tryptone plates incubated 48 h at 30 °C. Colonies arising upon the 42 °C plates are counted immediately upon removing from incubation (or after storage at 4 °C).

Activation assay (with S9 mix)

The assayed substance is dissolved in DMSO at 10x final concentration and incubated with 0.7 mL of S9 mix for 2 h at 37 °C. DMSO (1 mL) is then added along with 0.1 mL of 10x concentrated suspension of selector cells (= 3x10E9). The mixture is incubated 10 min at 37 °C and microfuged for 1 min. The cell pellet is resuspended in buffer, diluted and plated at 30 and 42 °C as in steps (i) and (ii) of the Standard assay.

Results and discussions

Test results

Species/strain E. coli, other: CHY832

Metabolic activation with and without

Test system all strains/cell types tested

Genotoxicity negative

Cytotoxicity yes

Vehicle controls valid yes

Negative controls valid yes

Positive controls valid yes

Any other information on results incl. tables

The test substance is toxic, but nonmutagenic.

Applicant's summary and conclusion

Interpretation of results

negative

Endpoint study record: key, RA-A, 115-70-8, Mochizuki, 2004, Ames, RL2

Administrative Data

Purpose flag key study; robust study summary

Study result type read-across from supporting substance (structural analogue or surrogate)

Reliability 2 (reliable with restrictions)

Rationale for reliability incl. deficiencies GLP-guideline study, tested with the source substance 2-amino-2-ethyl-1,3-propanediol (CAS 115-70-8). In accordance to the ECHA guidance document "Practical guide 6: How to report read-across and categories (March 2010)", the reliability was changed from RL1 to RL2 to reflect the fact that this study was conducted on a read-across substance.

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report	Mochizuki, H.	2004	Reverse mutation test of 2-Amino-2-Ethyl-1,3-Propanediol in bacteria.		Bozo Research Center 1284 Kamado, Gotemba-shi, Shizuoka, 412-0039, Japan	M-1128	Office of Chemical Safety Pharmaceutical and Food Safety Bureau Ministry of Health Labour and Welfare		2004-10-27

Data access

other: Japanese citizens, NGOs, NPOs, business, etc., are free to utilize and cite the results of the existing chemicals survey conducted by the three Ministries for any purpose. The results refer to existing chemicals survey conducted by the Japanese Government

Materials and methods

Type of genotoxicity

gene mutation

Type of study

bacterial reverse mutation assay (e.g. Ames test)

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 471 (Bacterial Reverse Mutation Assay)	no
according to	JAPAN: Guidelines for Screening Mutagenicity Testing Of Chemicals	no

GLP compliance

yes

Test materials

Identity of test material same as for substance defined in section 1 (if not read-across)

no

Test material identity

Identifier	Identity
EC name	2-amino-2-ethyl-1,3-propanediol

Details on test material

- Name of test material (as cited in study report): 2-amino-2-ethyl-1,3-propanediol- Analytical purity: 99.4%

Method

Target gene

his operon (for S. typhimurium strains) trp operon (for E. coli strains)

Species/strain

Species/strain S. typhimurium TA 1535, TA 1537, TA 98 and TA 100

Additional strain characteristics no data

Metabolic activation with and without

Metabolic activation system cofactor supplemented post-mitochondrial fraction (S9-mix), prepared from the livers of rats treated with phenobarbital and 5,6-benzoflavone

Species/strain E. coli WP2 uvr A

Additional strain characteristics no data

Metabolic activation with and without

Metabolic activation system cofactor supplemented post-mitochondrial fraction (S9-mix), prepared from the livers of rats treated with phenobarbital and 5,6-benzoflavone

Test concentrations

156, 313, 625, 1250, 2500 and 5000 µg/plate with and without metabolic activation

Vehicle

- Vehicle(s)/solvent(s) used: water

Controls

Negative controls no

Solvent / vehicle controls yes (water)

True negative controls no

Positive controls yes

Positive control substance other: without S9-mix

Remarks - S9-mix: 2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide (0.01 µg/plate) for TA100 and E.coli, (0.1 µg/plate) for TA 98; sodium azide (0.5 µg/plate for TA1535); 2-methoxy-6-chloro-9-[3-(2-chloroethyl)- aminopropylamino] acridine-2HCl for (1.0 µg/plate) TA1537

Negative controls no

Solvent / vehicle controls yes (water)

True negative controls no

Positive controls yes

Positive control substance other: with S9-mix

Remarks + S9-mix: Benzo[a]pyrene (5.0 µg/plate) for TA100, TA98 and TA1537; 2-aminoanthracene (2.0 µg/plate) for TA1535 and (10.0 µg/plate) for E.coli WP2 uvrA

Details on test system and conditions

METHOD OF APPLICATION: preincubationDURATION- Preincubation period: 20 min- Exposure duration: 48 hNUMBER OF REPLICATIONS: 3 plates for each concentrationDETERMINATION OF CYTOTOXICITY- Method: The inhibition of growth of the test bacterial strains by the test substance was observed with a stereoscopic microscope.

Evaluation criteria

When the number of reverse mutation colonies increased by almost twice the solvent control or more, and reproducibility or dependence on the dose of the test substance was observed, the result was considered positive.

Any other information on materials and methods incl. tables

The positive controls 2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide, 2-methoxy-6-chloro-9-[3-(2-chloroethyl)- aminopropylamino] acridine-2HCl, benzo[a]pyrene and 2-aminoanthracene were dissolved in dimethylsulfoxide. The positive control sodium azide was dissolved in water.

Results and discussions

Test results

Species/strain S. typhimurium TA 1535, TA 1537, TA 98 and TA 100

Metabolic activation with and without

Test system all strains/cell types tested

Genotoxicity negative

Cytotoxicity no

Vehicle controls valid yes

Negative controls valid not examined

Positive controls valid yes

Species/strain E. coli WP2 uvr A

Metabolic activation with and without

Test system all strains/cell types tested

Genotoxicity negative

Cytotoxicity no

Vehicle controls valid yes

Negative controls valid not examined

Positive controls valid yes

Additional information on results

TEST-SPECIFIC CONFOUNDING FACTORS- Precipitation: Precipitation of a white crystalline substance was observed in the test substance treatment groups of 2500 µg/plate or higher in the presence of the metabolic activation system, regardless of the kind of bacterial strain used. RANGE-FINDING/SCREENING STUDIES: In the range finding study, 8 treatment doses of the test solution were used in the range of 0.305 - 5000 µg/plate. Precipitation of a white crystalline substance was observed at the highest dose of 5000 µg/plate in the presence of a metabolic activation system, regardless of the kind of bacterial strain used. No inhibition of growth of bacterial strains was observed, regardless of the kind of strain and the presence or absence of metabolic activation.ADDITIONAL INFORMATION ON CYTOTOXICITY:No inhibition of growth of bacterial strains was observed, regardless of the kind of strain and the presence or absence of metabolic activation (data not shown).

Any other information on results incl. tables

Table 1: Results of the main test

With or without S9-mix	Test substance concentration (µg/plate)	Mean number of revertant colonies per plate (average of 3 plates ± standard deviation)				
		Base-pair substitution type			Frameshift type	
		TA 100	TA 1535	E.coli WP2 uvrA	TA 98	TA 1537
-	0	113 ± 21.4	10 ± 1.0	29 ± 3.5	17 ± 3.1	12 ± 1.5
-	156	110 ± 12.4	8 ± 1.2	28 ± 2.6	12 ± 2.1	9 ± 1.2
-	313	108 ± 13.1	8 ± 2.3	26 ± 3.8	13 ± 4.0	13 ± 2.0
-	625	107 ± 4.7	7 ± 1.0	27 ± 0.6	16 ± 1.7	10 ± 1.0
-	1250	114 ± 7.1	7 ± 2.1	27 ± 4.6	13 ± 3.1	10 ± 2.1
-	2500	115 ± 2.3	9 ± 4.2	28 ± 4.4	15 ± 3.0	10 ± 2.0
-	5000	114 ± 7.4	7 ± 1.7	19 ± 3.1	12 ± 2.5	10 ± 1.7
Positive controls, -S9	**Name**	**AF-2**	**SAZ**	**AF-2**	**AF-2**	**ICR-191**
	Concentrations (µg/plate)	0.01	0.50	0.01	0.10	1.0
	Mean No. of colonies/plate (average of 3 ± SD)	469 ± 18.1	176 ± 34.7	235 ± 29.5	363 ± 9.8	1194 ± 138.0
		TA 100	**TA 1535**	**E.coli WP2 uvrA**	**TA 98**	**TA 1537**
+	0	126 ± 5.9	13 ± 0.6	29 ± 2.9	20 ± 2.0	12 ± 2.6
+	156	123 ± 2.5	8 ± 0.6	27 ± 2.7	19 ± 0.6	14 ± 1.7
+	313	127 ± 12.2	8 ± 0.6	26 ± 4.4	20 ± 1.5	13 ± 2.0
+	625	106 ± 6.0	9 ± 1.5	25 ± 2.5	19 ± 2.1	11 ± 1.2
+	1250	125 ± 10.2	10 ± 2.1	22 ± 3.1	23 ± 2.1	12 ± 1.5
+	2500	126 ± 3.2	8 ± 2.1	19 ± 2.1	18 ± 2.5	12 ± 3.0
+	5000	135 ± 9.0	7 ± 2.1	21 ± 3.8	14 ± 2.9	10 ± 2.3
Positive controls, +S9	**Name**	**B[a]P**	**2AA**	**2AA**	**B[a]P**	**B[a]P**
	Concentrations (µg/plate)	5.00	2.00	10.0	5.00	5.00
	Mean No. of colonies/plate (average of 3 ± SD)	621 ± 23.8	117 ± 16.7	407 ± 12.1	247 ± 5.5	111 ± 6.7

AZ: Sodium azide
F-2: 2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide
CR-191: 2-Methoxy-6-chloro-9-[3-(2-chloroethyl)-aminopropylamino]acridine*2HCl
[a]P: Benzo[a]pyrene
AA: 2-Aminoanthracene

Applicant's summary and conclusion

Interpretation of results

negative

Endpoint study record: key, RA-A, 115-70-8, Indrani, 2011, HPRT, RL2

Administrative Data

Purpose flag	key study; robust study summary		
Study result type	read-across from supporting substance (structural analogue or surrogate)	**Study period**	14 Feb - 06 June 2011
Reliability	2 (reliable with restrictions)		
Rationale for reliability incl. deficiencies	GLP-guideline study, tested with the source substance 2-amino-2-ethyl-1,3-propanediol (CAS 115-70-8). In accordance to the ECHA guidance document "Practical guide 6: How to report read-across and categories (March 2010)", the reliability was changed from RL1 to RL2 to reflect the fact that this study was conducted on a read-across substance.		

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report	Indrani, B.K.	2011	XU-12399.00: IN VITRO MAMMALIAN CELL GENE MUTATION TEST IN CHO CELLS		Department of Safety Assessment, Advinus Therapeutics Limited, Post box No. 5813, Plot Nos. 21 & 22, Peenya II phase, Bangalore 560 058, India	G7698	ANGUS, a wholly owned subsidiary of The Dow Chemical Company	DR-0439-6069-004	2011-07-12

Data access

data submitter is data owner

Materials and methods

Type of genotoxicity

gene mutation

Type of study

mammalian cell gene mutation assay

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 476 (In vitro Mammalian Cell Gene Mutation Test)	no
according to	EU Method B.17 (Mutagenicity - In Vitro Mammalian Cell Gene Mutation Test)	no
according to	EPA OPPTS 870.5300 - In vitro Mammalian Cell Gene Mutation Test	no

GLP compliance

yes (incl. certificate) (CLP Certificate Germany; CLP Certificate The Netherlands; CLP Certificate India)

Test materials

Identity of test material same as for substance defined in section 1 (if not read-across)

no

Test material identity

Identifier	Identity
EC name	2-amino-2-ethyl-1,3-propanediol

Details on test material

- Name of test material (as cited in study report): XU-12399.00; 2-Amino-2-ethyl-1,3-propanediol- Physical state: yellow viscous liquid to semi solid- Analytical purity: 99.2% ± 0.06% (w/w) (by gas chromatography with identification by gas chromatography mass spectrometry and nuclear magnetic resonance (Megregian and Pell, 2010); water content determined to be 0.68% (w/w) by Coulometric Karl Fischer.- Impurities (identity and concentrations): no process impurities were detected at ≥ 0.1%- Lot No.: 201000687-21- Storage condition of test material: ambient (+ 18 °C to + 36 °C)- Other: The identity of the test article was provided by the study sponsor by a Certificate of Analysis. The responsibility for the correct identity and purity rests with the sponsor.

Method

Target gene

hprt locus

Species/strain

Species/strain Chinese hamster Ovary (CHO)

Details on Type and identity of media: - basic medium: Ham's F-12 medium supplemented with sodium bicarbonate,

mammalian cell lines (if applicable)	antibiotics and L-glutamine- basic medium supplemented with 10% fetal bovine serum was the complete medium and was used for the growth and multiplication of cells as well as in detaching and diluting the cells- basic medium without serum was the treatment medium used for target cell exposure to the test article and controls- cloning medium was basic medium supplemented with 20% fetal bovine serum and was used for determination of cell viability or plating/cloning efficiency- selective medium was basic medium supplemented with 20% fetal bovine serum and the selective agent 6-Thioguanine (6-TG) at 35 µM and was used for the selection of mutants.
Metabolic activation	with and without
Metabolic activation system	cofactor supplemented post-mitochondrial fraction (S9-mix), prepared from the livers of rats treated with Aroclor 1254

Test concentrations

initial gene mutation test: 12, 38, 119, 337, 1192 µg/mL with and without metabolic activationconfirmatory gene mutation test: 15, 44, 132, 397, 1192 µg/mL with and without metabolic activation

Vehicle

- Vehicle(s)/solvent(s) used: sterile distilled water (SDW)

Controls

Negative controls	no
Solvent / vehicle controls	yes
True negative controls	no
Positive controls	yes
Positive control substance	other: with S9: 3-Methylcholanthrene at 8 µg/mL; without S9: ethyl methanesulphonate at 600 µg/mL

Details on test system and conditions

DURATION- Exposure duration: 4 h- Expression time (cells in growth medium): 9 days- Selection time (if incubation with a selection agent): 10 daysSELECTION AGENT (mutation assays): 6-ThioguanineNUMBER OF REPLICATIONS: duplicates; two independent experiments (initial gene mutation test and confirmatory gene mutation test: each with and without metabolic activation)DETERMINATION OF CYTOTOXICITY- Method: relative cloning efficiency (RCE: Effect of the test item on cell multiplication was estimated by expressing cytotoxicity relative to the solvent control cultures.)

Evaluation criteria

Criteria for determining a positive result: concentration related or reproducible increases in mutant frequencies.Criteria for data acceptability: - The cloning efficiency of the solvent controls should not be less than 60%.- The mean mutant frequency of the solvent controls in each experiment should fall within a range of 0 to 20 mutants per 1E+06 clonable cells. - The positive controls must induce a statistically significant response.

Results and discussions

Test results

Species/strain	Chinese hamster Ovary (CHO)
Metabolic activation	with and without
Test system	all strains/cell types tested
Genotoxicity	negative
Cytotoxicity	yes (at 1192 µg/mL)
Vehicle controls valid	yes
Negative controls valid	not examined
Positive controls valid	yes

Additional information on results

TEST-SPECIFIC CONFOUNDING FACTORS- Effects of pH: The test item altered the pH of the test solutions at and above 298 µg/mL with metabolic activation and at and above 149 µg/mL without metabolic activation, therefore, the pH of the test solutions at these concentrations was adjusted to neutrality before exposure to the cells. - Effects of osmolality: The test

substance did not cause any appreciable changes in the osmolarity of the test solutions at the end of 4-h exposure with or without metabolic activation.- Water solubility: 859-879 g/L- Precipitation: The test substance did not precipitate in the medium up to the highest tested concentration with or without metabolic activation.RANGE-FINDING/SCREENING STUDIES: A preliminary cytotoxicity test was performed. COMPARISON WITH HISTORICAL CONTROL DATA: The frequencies of mutants of the positive controls and of the solvent control were within laboratory historical controls.ADDITIONAL INFORMATION ON CYTOTOXICITY:Initial gene mutation assay: There was no evidence of excessive cytotoxicity (i.e. <10% RCE) at any of the tested concentrations either with or without metabolic activation. The RCE values in the presence of metabolic activation, ranged from 38.4 to 81.3% while in the absence of metabolic activation, ranged from 44.6 to 82.3% compared to the solvent control.Confirmatory gene mutation assay: There was no evidence of excessive cytotoxicity (i.e. <10% RCE) at any of the tested concentrations either with or without metabolic activation. The RCE values in the presence of metabolic activation, ranged from 43.3 to 87.0% while in the absence of metabolic activation, ranged from 58.7 to 77.9% compared to the solvent control.

Any other information on results incl. tables

Table 1: Initial gene mutation assay with metabolic activation

Concentration[µg/mL]	Toxicity assay: Relative Cloning Efficiency [%]	Mutation assay: total TG^rcolonies per 2E+06 cells	Mutation assay: ACE at selection [%]	TG^rmutants per 1E+06 clonable cells	Mean TG^rmutants per 1E+06 clonable cells	Mutation factor
0 (SDW)	98.8	25	94.5	13.2	12.1	1.0
0 (SDW)	101.2	20	91.0	11.0		
12	81.3	15	89.3	8.4	9.1	0.8
12	79.0	17	86.5	9.8		
38	69.1	16	82.8	9.7	8.3	0.7
38	70.2	12	87.8	6.8		
119	61.5	18	69.5	12.9	14.9	1.2
119	57.7	22	65.2	16.9		
377	45.1	21	61.5	17.1	17.7	1.5
377	43.2	23	63.0	18.3		
1192	38.4	24	66.5	18.0	19.8	1.6
1192	41.1	29	67.2	21.6		
3-MCA	39.6	284	82.2	172.8	169.2	14.0
3-MCA	41.5	294	88.8	165.5		

3-MCA 3-Methylcholanthrene
TG^r: 6-Thioguanine resistant
SDW: sterile distilled water

Table 2: Initial gene mutation assay without metabolic activation

Concentration[µg/mL]	Toxicity assay: Relative Cloning Efficiency [%]	Mutation assay: total TG^rcolonies per 2E+06 cells	Mutation assay: ACE at selection [%]	TG^rmutants per 1E+06 clonable cells	Mean TG^rmutants per 1E+06 clonable cells	Mutation factor
0 (SDW)	100.4	19	93.8	10.1	9.9	1.0
0 (SDW)	99.6	18	93.8	9.6		
12	82.3	23	93.0	12.4	11.5	1.2
12	79.5	19	89.7	10.6		
38	75.2	28	87.5	16.0	14.8	1.5
38	78.6	24	88.7	13.5		
119	67.1	30	82.5	18.2	16.1	1.6
119	67.6	24	86.3	13.9		
377	66.1	25	86.8	14.4	13.8	1.4
377	64.9	23	87.5	13.1		

1192	51.3	23	81.3	14.1	14.4	1.5
1192	44.6	23	78.8	14.6		
EMS	43.5	25	86.7	144.2	159.2	16.1
EMS	48.1	281	80.7	174.2		

EMS:Ethyl methane sulphonate
TG^r: 6-Thioguanine resistant
SDW: sterile distilled water

Table 3: Confirmatory gene mutation assay with metabolic activation

Concentration[µg/mL]	Toxicity assay: Relative Cloning Efficiency [%]	Mutation assay: total TG^rcolonies per 2E+06 cells	Mutation assay: ACE at selection [%]	TG^rmutants per 1E+06 clonable cells	Mean TG^rmutants per 1E+06 clonable cells	Mutation factor
0 (SDW)	102.6	29	93.7	15.5	11.9	1.0
0 (SDW)	97.4	15	91.3	8.2		
15	87.0	17	88.5	9.6	9.6	0.8
15	83.9	16	84.3	9.5		
44	68.0	26	80.8	16.1	14.3	1.2
44	67.3	20	80.0	12.5		
132	71.1	12	81.7	7.3	10.0	0.8
132	64.0	19	75.3	12.6		
397	52.7	24	78.8	15.2	12.0	1.0
397	54.5	15	85.2	8.8		
1192	43.3	20	74.2	13.5	12.9	1.0
1192	43.7	21	85.2	12.3		
3-MCA	45.3	285	85.8	166.0	175.0	14.7
3-MCA	46.1	304	82.7	183.9		

3-MCA 3-Methylcholanthrene
TG^r: 6-Thioguanine resistant
SDW: sterile distilled water

Table 4: Confirmatory gene mutation assay without metabolic activation

Concentration[µg/mL]	Toxicity assay: Relative Cloning Efficiency [%]	Mutation assay: total TG^rcolonies per 2E+06 cells	Mutation assay: ACE at selection [%]	TG^rmutants per 1E+06 clonable cells	Mean TG^rmutants per 1E+06 clonable cells	Mutation factor
0 (SDW)	99.4	12	88.3	6.8	8.4	1.0
0 (SDW)	100.6	18	89.8	10.0		
15	77.9	20	91.3	10.9	10.9	1.3
15	73.8	18	83.7	10.8		
44	65.6	21	80.2	13.1	11.7	1.4
44	65.0	17	83.2	10.2		
132	62.4	13	79.8	8.1	6.8	0.8
132	60.9	9	82.2	5.5		
397	68.6	16	81.7	9.8	8.1	1.0
397	68.8	10	77.8	6.4		
1192	66.9	13	72.0	9.0	11.4	1.4
1192	58.7	21	76.2	13.8		
EMS	39.4	299	76.2	196.3	198.1	23.6
EMS	40.9	283	70.8	199.8		

EMS Ethyl methanesulphonate

TG^r: 6-Thioguanine resistant
SDW: sterile distilled water

Applicant's summary and conclusion

Interpretation of results

negative

Carcinogenicity

Endpoint summary: Carcinogenicity

Discussion

TRIS AMINO did not induce tumors when tested in male Syrian golden hamsters receiving 0.2 mL of a mixture of TRIS-based buffer and 0.9% NaCl intratracheally into the lungs once per week for life (Ketkar et al., 1979).

There are no standard animal data available on carcinogenicity or on chronic toxicity of2-amino-2-(hydroxymethyl)-1,3-propanediol (TRIS AMINO).
However, it can be assumed that TRIS AMINO has no carcinogenic potential based on the fact that the substance was not shown to be mutagenic or clastogenic in the available genetic toxicity studies and bears no structural similarity to known carcinogens. Furthermore, TRIS AMINO has no functional groups associated with carcinogenicity and did not produce any evidence of neoplasia in the available repeated dose toxicity studies. The Cramer classification (related mainly to the oral route) also indicates a low toxicological concern for TRIS AMINO.
In addition, TRIS AMINO is also used in clinical applications e.g. to correct acute or respiratory acidosis or it can be used to alkalinise body fluids and promote diuresis in order to enhance the elimination of salts of weak acids such as salicylate or barbiturate (Nahas, 1962, 1963). Due to its therapeutic indication, TRIS AMINO is well investigated in animals and humans. There was no evidence of a carcinogenicity of TRIS AMINO in pharmacokinetic studies.
Therefore, it is concluded that TRIS AMINO does not have a carcinogenic potential.
Literature not cited in the IUCLID
Nahas, G.G. (1962) The pharmacology of tris(hydroxymethyl)aminomethane during CO2 load. Am. J. Physiol. 204:113-118
Nahas, G.G. (1963) The clinical pharmacology of THAM (tris(hydroxymethyl)-aminomethane).Clin. Pharmacol. Ther. 4:784-803

Justification for classification or non-classification

Based on all available data of TRIS AMINO within the analogue approach, no carcinogenic potential is expected. The available data do not meet the criteria for classification according to Regulation (EC) 1272/2008 or Directive 67/548/EEC, and therefore are conclusive but not sufficient for classification.

Endpoint study record: Ketkar, M. et al., 1979, hamster, intratracheal instillation, RL2

Administrative Data

Study result type	experimental result
Reliability	2 (reliable with restrictions)
Rationale for reliability incl. deficiencies	Acceptable, well documented publication which meets basic scientific principles.

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	Ketkar, M. et al.	1979	Investigations on the carcinogenic burden by air pollution in man. Intratracheal instillation studies with benzo[a]pyrene in a mixture of Tris buffer and saline in syrian golden hamsters.	Cancer Letters, 6: 279-284					

Data access

data published

Materials and methods

Limit test

no

Principles of method if other than guideline

Male Syrian golden hamsters received 0.2 mL of a mixture of Tris-based buffer and 0.9% NaCl intratracheally into the lungs once weekly for life.

GLP compliance

no

Test materials

Identity of test material same as for substance defined in section 1 (if not read-across)

yes

Details on test material

- Analytical purity: not data

Test animals

Species

hamster, Syrian

Strain

other: Syrian golden hamster

Sex

male

Details on test animals and environmental conditions

TEST ANIMALS- Source: Central Institute for the Breeding of Laboratory Animals, TNO, Zeist, The Netherlands- Age at study initiation: 8-week-old- Housing: 5 males per cage (Makrolon cages Type II)- Diet (e.g. ad libitum): pelleted diet (RMH-TMB; Hope Farms, Woerden, The Netherlands); ad libitum- Water (e.g. ad libitum): ad libitumENVIRONMENTAL CONDITIONS- Temperature (°C): 22 ± 2- Humidity (%): 55 ± 5- Air changes (per hr): 20

Administration / exposure

Route of administration

intratracheal

Vehicle

physiol. saline

Details on exposure

PREPARATION OF DOSING SOLUTIONS:mixture of Tris buffer and 0.9% NaCl (physiol. saline)

Analytical verification of doses or concentrations

no

Duration of treatment / exposure

for life

Frequency of treatment

weekly

Post exposure period

not applicable

Doses / concentrations

Basis other: 0.2 mL mixture of Tris buffer and 0.9% NaCl

No. of animals per sex per dose

28 animals in the test group (Tris buffer and NaCl)29 animals in the untreated group29 animals per dose in the positive control groups

Control animals

yes

Positive control

benzo[a]pyrene (dose in mg): 0.125, 0.25, 0.50, 1.00

Examinations

Statistics

The statistical analyses were calculated according to SPSS computer programs. Differences in tumor incidences were analysed according to Chi-square test and the differences in survival times were evaluated by U-test. Correlations between dose, body weight, survival times and tumors in the trachea, larynx and lungs were tested with correlation coefficients of Spearman. Since a possible dose-response relationship with survival times could have an effect on the tumor incidences in the different groups, partial correlation coefficients controlling for survival time were computed between the other parameters.

Any other information on materials and methods incl. tables

Animals were observed for life or sacrificed when moribund. Body weight was recorded. Complete autopsies were performed and the organs fixed in formalin. Paraplast 6 µm thick sections were stained with hematoxylin and eosin.

Results and discussions

Results of examinations

Any other information on results incl. tables

The average survival time of untreated hamsters was 88 ± 22 weeks and the average body weight at death was 116 ± 10 g. In comparison, hamsters treated with Tris buffer and NaCl survived on average78 ± 25 weeks and revealed an average body weight at death of 114 ± 6 g . No tumors of the respiratory tract were induced by Tris buffer and NaCl.

Positive control: With increasing dose levels above 0.25 mg benzo[a]pyrene, survival times, average body weight and tumour incidences decreased. Papillary polyps, squamous cell papillomas and carcinomas developed in both the larynx and trachea. In addition, bronchiogenic adenomas, adenocarcinomas and squamous cell carcinomas were induced in the lung. The highest incidence of respiratory tract tumors (83%) were seen in hamsters receiving 0.25 mg benzo[a]pyrene.

Toxicity to reproduction

Endpoint summary: Toxicity to reproduction

Effects on fertility

Key value for chemical safety assessment

Effect level for fertility (oral exposure) NOAEL 1000 mg/kg bw/day

Discussion

A reproduction/developmental toxicity screening test was performed with 2-amino-2-(hydroxymethyl)-1,3-propanediol (TRIS AMINO) according to OECD 421, to assess the potential reproductive and fertility effects of TRIS AMINO (Ellis-Hutchings, 2012). Doses of 100, 300 and 1000 mg/kg bw/day of the test substance were administered by gavage to rats during pre-mating, mating, gestation and until lactation day 4. The males were exposed for 29 days and the females for up to 54 days. The test substance was adjusted to pH 9 due to animal welfare considerations. As no systemic effects were observed up to and including the highest dose level, the NOAEL for systemic toxicity is considered to be ≥ 1000 mg/kg bw/day. Irritation effects on the limiting ridge of the forestomach were attributed to the local irritating effect of TRIS AMINO. This effect is not relevant to humans, as they do not have a forestomach. However, the observation leads to a NOAEL for local toxicity of 100 mg/kg bw/day.

In the parental generation, the reproduction parameters (including mating index, fertility index, gestation period, delivery index, and number of live pups) were not affected by treatment with the test substance up to and including the highest dose level of 1000 mg/kg bw/day. The NOAEL for reproduction is therefore considered to be ≥ 1000 mg/kg bw/day.

In the pups (F1-generation), no effects on viability were observed. The body weight of male pups in the 250 mg/kg bw/day group was significantly increased, compared to that of the control group, at the time of birth. As it was not dose-related, only observed in one sex and at one time point, it is considered to have no biological significance. No treatment-related histopathological findings were noted at study termination. A statistically significant difference in the sex ratio (No. males/No. liveborn pups = 0.64) was observed in the control group only and is considered to be incidental. Therefore, the NOAEL for teratogenicity was considered to be ≥ 1000 mg/kg bw/day.

Potential analogues for the target substance TRIS AMINO are other 2-amino-1,3-propanediols. Therefore, source substances are members of the aminopropanediol category: 2-amino-2-ethyl-1,3-propanediol (AEPD), 2-amino-2-methyl-1,3-propanediol (AMPD) and 2-amino-1,3-propanediol (APD). TRIS AMINO and the 2-amino-1,3-propanediols are expected to show comparable toxicokinetic characteristics, and it is anticipated that the absorbed amounts of all the aminopropanediols have limited systemic bioavailability and are rapidly eliminated by the kidneys. No relevant metabolism is expected, based on experimental data and on QSAR predictions. The modelling of potential metabolites using the OECD QSAR toolbox v.2.0 (2010) did not predict relevant metabolites of TRIS AMINO or of any of the 2-amino-1,3-propanediols. Therefore, no metabolism by cytochrome P450 enzymes in-vivo is expected.
Available studies via the oral, dermal or intraperitoneal route on these substances caused no systemic toxicity. The results of the acute studies, as well as the repeated dose studies, demonstrate that the main cause of toxicity was the intrinsic alkalinity of the respective test substances at the site of contact. Inhalation is of no concern, because the low vapour pressure of the pure substances means that exposure is unlikely to occur. In case of spray applications of technical products containing the neat substance, the concentration is very low (< 1%). The Cramer classification (related mainly to the oral route) also indicates a low toxicological concern for TRIS

AMINO and the 2-amino-1,3-propanediols. Thus, both TRIS AMINO and 2-amino-1,3-propanediols are of low concern with regard to systemic toxicity.
Due to the structural similarity and the similar toxicological properties between TRIS AMINO and the 2-amino-1,3-propanediols, read-across using an analogue approach is justified.

A combined oral repeated dose toxicity study and reproduction/developmental toxicity screening test was also performed with AEPD, according to OECD 422 (Ishida, 2004). No effects on reproduction or fertility and no systemic toxicity were observed up to and including the highest dose level of 1000 mg/kg bw/day. This result supports the hypothesis that aminopropanediols show no toxicity to reproduction, and is in line with Regulation (EC) 1907/2006, Annex XI regarding the use of data on similar or surrogate substances as part of a weight of evidence approach to cover an endpoint.

According to Regulation (EC) 1907/2006, Annex IX, a tiered approach should be applied to determine if further testing is required for the endpoint toxicity to reproduction. If effects on reproduction parameters are noted in a subacute or subchronic repeated dose toxicity study, a 2-generation reproduction toxicity study should be performed.
For the endpoint repeated dose toxicity, a testing proposal for a 90-day repeated dose toxicity study following OECD 408 with extended reproduction parameters (additional sperm motility parameter and extra attention for reproductive organs/tissues in all groups) has been proposed with APD, as the most appropriate test candidate. As soon as the data is available, the results will be read across. The outcome of this subchronic toxicity study will determine the subsequent testing procedure. This is in accordance with Regulation (EC) 1907/2006, which specifies that unnecessary tests should be avoided in terms of animal welfare.

Literature not cited in IUCLID
Ishida, 2004, 2-Amino-2-ethyl-1,3-propanediol toxicity tests for Acute oral, Repeated dose and Reproductive/Developmental Toxicity and Genetic Toxicity. Report no. R-870

Developmental toxicity / teratogenicity

Key value for chemical safety assessment

Effect level for developmental toxicity (oral exposure)	NOAEL 1000 mg/kg bw/day

Discussion

There are no animal data available that assesses the potential developmental toxicity of 2-amino-2-(hydroxymethyl)-1,3-propanediol (TRIS AMINO) alone. However, a reproduction/developmental toxicity screening test was performed with TRIS AMINO according to OECD 421, to assess the potential reproductive and fertility effects of TRIS AMINO (Ellis-Hutchings, 2012). No adverse effects on developmental parameters (including number of live pups, viability, body weight, sex ratio) in the F1-generation were observed following treatment with doses of TRIS AMINO up to and including 1000 mg/kg bw/day.

In addition, there are reliable data for other substances considered suitable for read-across using the analogue approach. Potential analogues for the target substance TRIS AMINO are other 2-amino-1,3-propanediols. Therefore, source substances are members of the aminopropanediol category: 2-amino-2-ethyl-1,3-propanediol (AEPD), 2-amino-2-methyl-1,3-propanediol (AMPD) and 2-amino-1,3-propanediol (APD). TRIS AMINO and the 2-amino-1,3-propanediols are expected to show comparable toxicokinetic characteristics, and it is anticipated that the absorbed amounts of all the aminopropanediols have limited systemic bioavailability and are rapidly eliminated by the kidneys. No relevant metabolism is expected, based on experimental data and on QSAR predictions. The modelling of potential metabolites using the OECD QSAR toolbox v.2.0 (2010) did not predict relevant metabolites of TRIS AMINO or of any of the 2-amino-1,3-propanediols. Therefore, no metabolism by cytochrome P450 enzymes in-vivo is expected.
Available studies via the oral, dermal or intraperitoneal route on these substances also caused no systemic toxicity. The results of the acute studies, as well as the repeated dose studies, demonstrate that the main cause of toxicity was the intrinsic alkalinity of the respective test substances at the site of contact. Inhalation is of no concern, because the low vapour pressure of the pure substances means that exposure is unlikely to occur. In case of spray applications of technical products containing the neat substance, the concentration is very low (< 1%). The Cramer classification (related mainly to the oral route) also indicates a low toxicological concern for TRIS AMINO and the 2-amino-1,3-propanediols. Thus, both TRIS AMINO and 2-amino-1,3-propanediols are of low concern with regard to systemic toxicity
Due to the structural similarity and the similar toxicological properties between TRIS AMINO and the 2-amino-1,3-propanediols, read-across using an analogue approach is justified.
In a combined oral repeated dose toxicity and reproduction/developmental toxicity screening study according to OECD 422, no adverse effects on developmental parameters (including number of live pups, viability, body weight, sex ratio) in the F1-generation were observed following treatment with doses of AEPD up to and including 1000 mg/kg bw/day (Ishida, 2004).
In addition, in a non-guideline reproduction/developmental toxicity screening study, the potential of AMPD to cause reproductive and developmental toxicity in rats was investigated (Rasoulpour and Andrus, 2011). Female rats were administered AMPD from before mating until gestation day 14. During the pre-mating period the groups were administered either increasing doses from 100 up to 1000 mg/kg bw/day (34 days) or 1000 mg/kg bw/day, the limit dose (6 days). The rats were sacrificed on gestation day 14 and examined. No differences were observed between the control group and the treatment groups regarding numbers of corpora lutea, implantation rates and implantation position, resorption rates, pre-implantation loss, post-implantation loss, pup viability and number of normal embryos per litter. The examination of the reproductive tract did not reveal treatment-related effects.
The potential of AMPD to cause embryotoxicity was assessed in an in-vitro limb bud micromass assay by exposing undifferentiated rat embryo limb bud mesenchymal cells to AMPD concentrations of 0.01 to 1000 µM in the cell culture medium (Ellis-Hutchings and Marshall, 2011). Following cell differentiation into chondrocytes, specific parameters (cell differentiation, cell viability, cell number, neutral red uptake, cell growth) were determined. Under the scope of the assay, the results predict a

lack of developmental toxicity for AMPD.
The studies performed with TRIS AMINO, AMPD and AEPD support the hypothesis that aminopropanediols have the same toxicity profile with respect to systemic toxicity and, specifically, developmental toxicity. In line with Regulation (EC) 1907/2006, Annex XI, the available data on these substances are used as part of a weight of evidence approach to show that no toxicity to reproduction is likely to occur.
According to Regulation (EC) 1907/2006 Annex IX, Column I, 8.7.2, a GLP-compliant prenatal development toxicity study is mandatory to meet the standard information requirements. To meet this requirement, a prenatal developmental toxicity study following OECD 414 in rats via the oral route is proposed. The test will be performed with 2-amino-1,3-propanediol (APD), and the results will be read-across to TRIS AMINO, based on an analogue approach. In line with the information that APD represents the basic molecular structure compared with TRIS AMINO, AMPD and AEPD, which contain an additional hydroxyl, methyl or ethyl group, APD has been selected as the most appropriate test candidate. This is in accordance with Regulation (EC) 1907/2006, Annex XI, which specifies that read-across of data from a suitable substance may be used to avoid unnecessary tests for animal welfare reasons.
Literature not cited in IUCLID
Ellis-Hutchins and Marshall, 2011, ANGUS MOLECULES (DMAMP, AMPD AND 3-AB): IN VITRO EMBRYOTOXICITY SCREENING STUDY IN THE RAT EMBRYO LIMB BUD MICROMASS ASSAY, Report no. 100118, Company study no. DR-0050-0118-010

Rasoulpour and Andrus, 2011, ANGUS MOLECULES (DMAMP, AMPD AND 3-AB): IN VIVO DEVELOPMENTAL TOXICITY SCREENING STUDY IN Crl:CD(SD) RATS, Report no. 100176, Company study no. DR-0050-0118-011

Ishida, 2004, 2-Amino-2-ethyl-1,3-propanediol toxicity tests for Acute oral, Repeated dose and Reproductive/Developmental Toxicity and Genetic Toxicity. Report no. R-870

Justification for classification or non-classification

The available data on the reproduction, fertility and teratogenic toxicity, and on effects on or via lactation of the test substance do not meet the criteria for classification according to Regulation (EC) 1272/2008 or Directive 67/548/EEC, and are therefore conclusive but not sufficient for classification.

Toxicity to reproduction

Endpoint study record: key, Ellis-Hutchings, 2012, OECD 421, RL1

Administrative Data

Purpose flag	key study; robust study summary
Study result type	experimental result **Study period** 17 May - 08 Jul 2011

Reliability 1 (reliable without restriction)

Rationale for reliability incl. deficiencies GLP-guideline study

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report	Ellis-Hutchings, R.G. et al.	2012	Tris(hydroxymethyl)aminomethane: Reproduction/developmental toxicity screening test in Crl:CD(SD) rats		Toxicology & Environmental Research and Consulting, The Dow Chemical Company Midland, Michigan 48674, USA	111044	ANGUS, a wholly owned subsidiary of The Dow Chemical Company	K-018227-007	2012-01-31

Data access

data submitter is data owner

Materials and methods

Test type

screening

Limit test

no

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 421 (Reproduction / Developmental Toxicity Screening Test)	no
according to	other guideline: EPA OPPTS 870.3550, Reproductive/Developmental Toxicity Screening Test. July, 2000	no

GLP compliance

yes

Test materials

Identity of test material same as for substance defined in section 1 (if not read-across)

yes

Details on test material

- Name of test material (as cited in study report): tris(hydroxymethyl)aminomethane, 2-amino-2-(hydroxymethyl)-1,3-propanediol, tromethamine- Analytical purity: 99.9% (with 0.018% wt. water in batch XK0731LA1C and 0.021% wt. water in batch XK1231LA1C)- Purity test date: 2011- Lot/batch No.: XK0731LA1C and XK1231LA1C- Storage condition of test material: at room temperature

Test animals

Species

rat

Strain

other: Crl:CD(SD)

Sex

male/female

Details on test animals and environmental conditions

TEST ANIMALS- Source: Charles River Laboratories Inc., Portage, Michigan, USA- Age at study initiation: (P) approximately 8 weeks- Weight at study initiation: (P) Males: 255.2 ± 4.9-256.6 ± 6.2 g; Females: 190.6 ± 8.6- 194.6 ± 8.5 g (mean ± SD) - Fasting period before study: no- Housing: animals were housed individually in stainless steel cages, except during breeding and during the littering phases; during breeding, one male and one female were housed per cage; during littering, dams and their litters were housed in plastic cages provided with ground corncob nesting material from approximately GD 19 until termination. Cages had wire mesh floors and were suspended above catch pans. Non-woven gauze was placed in the cages to provide a cushion from the flooring for rodent feet and also provided environmental enrichment. In order to better visualize copulation and plugs, gauze was not placed in cages during the breeding phase. - Diet: LabDiet Certified Rodent Diet #5002 (PMI Nutrition International, St. Louis, Missouri) in meal form, ad libitum- Water: tap water, ad libitum- Acclimation period: at least 7 daysENVIRONMENTAL CONDITIONS- Temperature (°C): 22 ± 1 (maximum permissible excursion of ± 3 °C)- Humidity

(%): 40-70- Air changes (per hr): 12-15, on average- Photoperiod (hrs dark / hrs light): 12/12IN-LIFE DATES: From: 17 May 2011 To: 15 Jun 2011 (males); 30 Jun and 08 Jul 2011 (females)

Administration / exposure

Route of administration

oral: gavage

Vehicle

water

Details on exposure

PREPARATION OF DOSING SOLUTIONS: The test material was mixed with deionised water to reach a dose volume of 4 mL/kg bw; calculated according to the latest body weight, and the solution was adjusted to pH 9. Dose solutions were prepared periodically during the study period based on stability data.VEHICLE- Concentration in vehicle: 25, 75 and 250 mg/mL- Amount of vehicle (if gavage): 4 mL/kg bw

Details on mating procedure

- M/F ratio per cage: 1/1- Length of cohabitation: until pregnancy was confirmed or up to two weeks- Proof of pregnancy: vaginal plug or sperm in vaginal smear referred to as day 0 of pregnancy- After successful mating each pregnant female was caged (how): individually

Analytical verification of doses or concentrations

yes

Details on analytical verification of doses or concentrations

Analyses by HPLC/ELSD were performed to determine the concentration of the test material of all dosing solutions from the first mix of the main study prior to the start of dosing. The low- and high-dose solutions from the first mix of the main study were analyzed to confirm homogeneous distribution of the test material concurrent with dose confirmation. The stability was measured on Day 0, 1, 6, 15 and 22. The stability results were 93.4-112.9% of target concentration with RDS up to 18.1% on Day 0; and 81.7-109.4% of target concentration with RDS up to 13.9% on Day 22. The concentration and homogeneity of the dose solutions was 91.4-109.1% of target concentration, with RSD of up to 12.5% (N = 6). Quantitation was performed using external standard calibration; the accuracy of the standards was 94.6-110% (mean) of the nominal values. The large RSDs were explained by the use of an external standard calibration and the ELSD, which both tend to give a larger RSD.

Duration of treatment / exposure

(P) Males: 29 days (14 days prior to mating, 14 days during mating, up to and including Day 29) (P) Females: up to 54 days (14 days prior to mating, up to 14 days during mating, approximately 22 days during gestation, 4 days during lactation)

Frequency of treatment

Daily, 7 days/week

Details on study schedule

- Age at mating of the mated animals in the study: approximately 10 weeks

Doses / concentrations

100, 300, 1000 mg/kg bw/day

Basis other: nominal dose

No. of animals per sex per dose

10

Control animals

yes, concurrent vehicle

Further details on study design

- Dose selection rationale: a range-finding study was performed in which 3 rats/sex/dose were administered 0, 250, 500, 750 or 1000 mg/kg bw/day via gavage for 14 days. All the animals survived to scheduled termination and there were no treatment-related clinical signs. The body weights and feed consumption were comparable between the control and treatment groups, and no effects were noted on organ weights or during gross necropsy. As no effects were observed at the highest dose level of 1000 mg/kg bw/day and this is the limit dose defined in the EPA OPPTS 870.3550 guideline, dose levels of 100, 300 and 1000 mg/kg bw/d were selected for the main study. - Other: the pH of the test solution was adjusted to 9 prior to dosing

Examinations

Parental animals: Observations and examinations

CAGE SIDE OBSERVATIONS: Yes- Time schedule: at least twice daily during the study period for all animals, including dams and their litters- Cage side observations included, but were not limited to: morbidity, mortality, decreased/increased activity, repetitive behaviour, vocalization, incoordination/limping, injury, neuromuscular function (convulsion, fasciculation, tremor, twitches), altered respiration, blue/pale skin and mucous membranes, severe eye injury (rupture), alterations in fecal consistency, and fecal/urinary quantity DETAILED CLINICAL OBSERVATIONS: Yes- Time schedule: daily. Animals were observed for abnormalities in the eyes, urine, feces, gastrointestinal tract, extremities, movement, posture, reproductive system, respiration, skin/hair-coat, and mucous membranes, as well as an assessment of general behaviour, injuries, or palpable mass/swellings. Females were observed for signs of parturition from around gestation day 20.BODY WEIGHT: Yes- Time schedule for examinations: for all animals, at least once during the pre-exposure period and on the first day of dosing (Day 0). Males were also weighed weekly during the study period. Females were weighed weekly during the pre-mating and mating period; on gestation day (GD) 0, 7, 14, 17 and 20; females that delivered litters were weighed on lactation day 1 and 4;

females that failed to mate or deliver a litter were weighed at least weekly until termination.FOOD CONSUMPTION:- Food consumption for each animal determined and mean daily diet consumption calculated as g food/kg body weight/day: Yes. Feed consumed was determined weekly during the two week pre-breeding period for males and females by weighing feed crocks at the start and end of a measurement cycle. Feed consumption was not measured for males or females due to co-housing during breeding. Following breeding, feed consumption was not measured for males. For females during gestation, feed consumption was measured on GD 0, 7, 14, and 20. After parturition, feed consumption was measured on lactation day 1 and 4. Feed consumption was not recorded for females that failed to mate or deliver a litter. - Compound intake calculated as time-weighted averages from the consumption and body weight gain data: NoWATER CONSUMPTION: No

Sperm parameters (Parental animals)

Parameters examined in P male parental generations:- all males: testis weight, epididymis weight

Litter observations

STANDARDISATION OF LITTERS- Performed on day 4 postpartum: no, pups were terminated on lactation day 4PARAMETERS EXAMINEDThe following parameters were examined in F1 offspring:number and sex of pups, stillbirths, live births, presence of gross anomalies, body weight (lactation day 1 and 4), physical or behavioural abnormalities, number of live and dead pups (lactation day 0, 1 and 4), clinical observations (daily) GROSS EXAMINATION OF DEAD PUPS:yes, for external abnormalities; possible cause of death was not determined for pups born or found dead.

Postmortem examinations (Parental animals)

SACRIFICE- Male animals: all surviving animals were sacrificed after 29 days of exposure, in a fasted state- Maternal animals: all surviving animals were sacrificed between lactation day 5 and 8, or at least 24 days after the end of the mating period for females not producing a litter, in a fasted stateGROSS NECROPSY- Gross necropsy consisted of external examination of tissues and orifices, in situ examination of the eyes, and internal examinations including the cervical, thoracic, and abdominal viscera.Samples of the following tissues/organs were collected from all the animals and preserved in neutral, phosphate-buffered 10% formalin: kidneys, liver, pituitary, gross lesions, cervix, coagulating glands, mammary gland (females), ovaries, oviducts, prostate, seminal vehicles, uterus, vagina. The testes and epididymides were fixed in Bouin's. Transponders were removed and placed in jars with the tissues. The uteri of all females in control and treatment groups were stained with an aqueous solution of 10% sodium sulfide stain (Kopf et al., 1964) for approximately one minute and were examined for the presence and number of implantation sites, prior to preservation. HISTOPATHOLOGY / ORGAN WEIGHTSThe weights of the epididymides, kidneys, liver, and testes were recorded for all the animals in the control and treatment groups. Histological examinations were performed on the preserved tissues/organs from the animals in the control and high-dose groups. A qualitative assessment of the stages of spermatogenesis was made of the testes in control and high-dose males by examining the relationship between spermatogonia, spermatocytes, spermatids, and spermatozoa (cycle of spermatogenesis) as seen in cross sections of the seminiferous tubules by microscopic evaluation; and by examination of sections of both testes for the presence of degenerative changes (e.g., vacuolation of the germinal epithelium, a preponderance of Sertoli cells, sperm stasis, inflammatory changes, mineralization, and fibrosis)

Postmortem examinations (Offspring)

SACRIFICE- The F1 offspring was sacrificed at 4 days of ageGROSS NECROPSY- Gross necropsy consisted of external examinations of the pupsHISTOPATHOLOGY / ORGAN WEIGTHSNo histopathological examinations were performed; no organs weights were recorded

Statistics

Parental body weights and gestation and lactation body weight gains, litter mean body weights, feed consumption, and organ weights (absolute and relative) was first evaluated by Bartlett's test (alpha = 0.01; Winer, 1971) for equality of variances. Based upon the outcome of Bartlett's test, either a parametric (Steel and Torrie, 1960) or non-parametric (Hollander and Wolfe, 1973) analysis of variance (ANOVA) was performed. If the ANOVA was significant at alpha = 0.05, a Dunnett's test (alpha = 0.05; Winer, 1971) or the Wilcoxon Rank-Sum (alpha = 0.05; Hollander and Wolfe, 1973) test with Bonferroni's correction (Miller, 1966) was performed. Feed consumption values were excluded from analysis if the feed is spilled or scratched. Gestation length, average time to mating, and litter size were analyzed using a nonparametric ANOVA. If the ANOVA was significant, the Wilcoxon Rank-Sum test with Bonferroni's correction was performed. Statistical outliers (alpha = 0.02) were identified by the sequential method of Grubbs (1969) and only excluded from analysis for documented, scientifically sound reasons. The mating, conception, fertility and gestation indices were analyzed by the Fisher exact probability test (alpha = 0.05; Siegel, 1956) with Bonferroni's correction. Evaluation of the neonatal sex ratio on postnatal day 1 was performed by the binomial distribution test (alpha = 0.05; Steel and Torrie, 1960). Gender of pups found dead on postnatal day 0 was included in sex ratio calculations. Survival indices, post-implantation loss, and other incidence data among neonates was analyzed using the litter as the experimental unit by the censored Wilcoxon test (alpha = 0.05; Hollander and Wolfe, 1973) as modified by Haseman and Hoel (1974) with Bonferroni's correction. Non-pregnant females, females with resorptions only, or females found to be pregnant after staining of their uteri were excluded from the appropriate analyses.

Reproductive indices

Calculated for all doses levels:Female mating index = (No. females with evidence of mating/No. paired) x 100Male mating index = (No. males with evidence of mating/No. paired) x 100Female conception index = (No. females with evidence of pregnancy/No. mated) x 100Male conception index = (No. males siring a litter/No. mated) x 100Female fertility index = (No. females with evidence of pregnancy/No. paired) x 100Male fertility index = (No. males siring a litter/No. paired) x 100Gestation index = (No. females delivering a viable litter/No. females delivering a litter) x 100Post-implantation loss = (No. implants – No. viable offspring)/(No. implants) x 100

Offspring viability indices

Calculated for all dose levels:Gestation survival index = percentage of delivered pups alive at birthDay 1 or 4 pup survival index = (No. viable pups on day 1 or 4/No. born live) x 100

Any other information on materials and methods incl. tables

The stability of the test material in the vehicle was determined prior to the start of

dosing at concentrations ranging from 0.25 – 250 mg/mL. The samples were kept at ambient temperature and were exposed to light. Analysis was performed by high performance liquid chromatography with evaporative light scattering detection (HPLC/ELSD). Quantitation was performed using external standard calibration.

Results and discussions

Effect levels

Endpoint	Generation	Sex	Effect level	Based on	Basis for effect level / Remarks
NOAEL (reproduction)	P	male/female	>= 1000 mg/kg bw/day (nominal)	test mat.	No effects observed up to and including the highest dose level
NOAEL (systemic)	P	male/female	>= 1000 mg/kg bw/day (nominal)	test mat.	No effects observed up to and including the highest dose level
NOAEL (local)	P	male/female	100 mg/kg bw/day (nominal)	test mat.	histopathology; hyperplasia of the limiting ridge, inflammation of the submucosa of the glandular stomach. This effect is not relevant to humans.

Results of examinations: parental animals

Clinical signs (parental animals)

no effects

Body weight and food consumption (parental animals)

no effects

Test substance intake (parental animals)

not examined

Reproductive function: estrous cycle (parental animals)

not examined

Reproductive function: sperm measures (parental animals)

no effects

Reproductive performance (parental animals)

no effects

Organ weights (parental animals)

no effects

Gross pathology (parental animals)

yes (1000 mg/kg bw/day: thickening of the limiting ridge, local irritation effect)

Histopathology (parental animals)

yes (300 mg/kg bw/day: hyperplasia of the epithelium of the limiting ridge (males), inflammation of the submucosa, glandular stomach, local irritation effect)

Details on results (parental animals)

CLINICAL SIGNS AND MORTALITY (PARENTAL ANIMALS)There was no mortality during the study period. No treatment-related clinical signs were observed during the study period.BODY WEIGHT AND FOOD CONSUMPTION (PARENTAL ANIMALS)No significant differences in body weight or body weight gain were observed between the male control and treatment groups. In females, no significant differences in body weight gain were noted between the control and treatment groups. A statistically significant increase in body weight gain was observed in the female mid-dose group during lactation day 1-4, compared to the control group. This effect is considered to be incidental, as no effect was noted in the female high-dose group. There were no significant differences in feed consumption between the control and treatment groups during the study period. TEST SUBSTANCE INTAKE (PARENTAL ANIMALS)The test substance was administered by gavage daily, with doses based on the body weight, ensuring an accurate dosing of the animals.REPRODUCTIVE FUNCTION: SPERM MEASURES (PARENTAL ANIMALS)There were no effects on the testis weight or epididymis weight in the control or treatment groups.REPRODUCTIVE PERFORMANCE (PARENTAL ANIMALS)No treatment-related effects were noted on the male/female mating index, male/female conception index, male/female fertility index, gestation index, time to mating, gestation period, and post-implantation loss (see Table 1). ORGAN WEIGHTS (PARENTAL ANIMALS)There was no significant difference in organ weights between the control and treatment groups.GROSS PATHOLOGY (PARENTAL ANIMALS)10/10 females and 10/10 males in the high-dose groups had a thickened limiting ridge of the stomach (see Table 2). The limiting ridge is a thin protrusion from the forestomach that demarcates the forestomach from the glandular stomach in rats. The effects are considered to be caused by a local irritation effect of the test substance and are therefore treatment-related. However, as humans do not have a forestomach and limiting ridge, the findings are of limited relevance to humans. The gross pathology examinations did not reveal any treatment-related findings in the reproductive organs.HISTOPATHOLOGY (PARENTAL ANIMALS)Very slight to slight hyperplasia of the stratified squamous epithelium was observed at the limiting ridge of the stomach of 1, 0, 4 and 10 male, and in 2, 0, 2 and 10 female rats in the control, low-, mid- and high-dose group, respectively (see Table 2). In addition, subacute to chronic inflammation of the submucosa of the glandular stomach was noted in at least 1

animal in all groups; classified as very slight or slight. A clear treatment-related effect was seen only in the cases classified as slight; where 0, 0, 3 and 9 males, and 0, 0, 1 and 4 females in the control, low-, mid- and high-dose group, respectively, were affected. This is considered to be a local irritating effect of the test substance, which was administered in a relatively large volume. As humans do not have a limiting ridge and forestomach, the effect is considered to have limited toxicological relevance to humans. There were no treatment-related histopathological findings in the reproductive organs.

Results of examinations: offspring

Viability (offspring)

no effects

Clinical signs (offspring)

no effects

Body weight (offspring)

no effects

Sexual maturation (offspring)

not examined

Organ weights (offspring)

not examined

Gross pathology (offspring)

no effects

Histopathology (offspring)

not examined

Details on results (offspring)

VIABILITY (OFFSPRING)There were no significant differences in gestation survival index or viability between the control and treatment groups (see Table 1). Due to the death of several pups in high-dose litters, the survival index for this dose group was slightly lower than the survival index for the remaining groups, at 99.3% on lactation day 1 and 97.8% on lactation day 4, compared to 100% for all other groups on lactation day 1 and 4. However, it remained within the historical data range (97.7-100.0%, see Table 3).CLINICAL SIGNS (OFFSPRING)No treatment-related clinical signs were observed in the control or treatment groups.BODY WEIGHT (OFFSPRING)There were no significant differences in body weight between the control and treatment groups on lactation day 1 and 4. GROSS PATHOLOGY (OFFSPRING)No treatment-related effects were noted during the gross pathological examination in any group.OTHER FINDINGS (OFFSPRING)The sex ratio was statistically significantly changed in the control group, compared to all the treatment groups. As only the control group was affected, this is considered to be an incidental observation (see Table 1).

Any other information on results incl. tables

Table 1: Reproduction performance and developmental parameters

Observations	Dose (mg/kg bw/day)			
	Control	100	300	1000
Pairs started (N)	10	10	10	10
Male mating index, % (N)	100.0 (10/10)	100.0 (10/10)	100.0 (10/10)	100.0 (10/10)
Female mating index, % (N)	100.0 (10/10)	100.0 (10/10)	100.0 (10/10)	100.0 (10/10)
Time to mating, days (mean ± SD)	2.0 ± 0.9	3.7 ± 3.2	2.7 ± 1.8	2.9 ± 1.1
Male fertility index, % (N)	80.0 (8/10)	100.0 (10/10)	90.0 (9/10)	100.0 (10/10)
Female fertility index, % (N)	80.0 (8/10)	100.0 (10/10)	90.0 (9/10)	100.0 (10/10)
Post-implantation loss, % (mean ± SD)	8.88 ± 14.93	9.79 ± 13.64	4.18 ± 4.44	4.89 ± 5.25
Gestation length, days (mean ± SD)	21.6 ± 0.5	21.5 ± 0.5	21.4 ± 0.5	22.0 ± 0.5
Gestation index, % (N)	100.0 (8/8)	100.0 (10/10)	100.0 (9/9)	100.0 (10/10)
Gestation survival index, % (N)	100.0 (110/110)	100.0 (138/138)	99.3 (138/139)	100.0 (138/138)
Day 1 survival index, % (N)	100.0 (110/110)	100.0 (138/138)	100.0 (138/138)	99.3 (137/138)
Day 4 survival index, % (N)	100.0 (110/110)	100.0 (138/138)	100.0 (138/138)	97.8 (135/138)

Live pups/dam at birth (mean ± SD)	13.8 ± 2.5	13.8 ± 2.8	15.3 ± 1.4	13.8 ± 3.5
Live pups/dam lactation day 1 (mean ± SD)	13.8 ± 2.5	13.8 ± 2.8	15.3 ± 1.4	13.7 ± 3.6
Live pups/dam lactation day 4 (mean ± SD)	13.8 ± 2.5	13.8 ± 2.8	15.3 ± 1.4	13.5 ± 3.5
Pups born dead	0/110	0/110	1/139	0/138
Sex ratio Day 1, male:females	64:36*	49:51	47:53	50:50
Pup body weight lactation day 1, g (mean ± SD) - male - female	 7.6 ± 0.7 7.1 ± 0.7	 7.5 ± 0.8 7.1 ± 0.7	 7.1 ± 0.6 6.6 ± 0.6	 7.8 ± 1.0 7.4 ± 1.0
Pup body weight lactation day 4, g (mean ± SD) - male - female	 10.8 ± 1.3 10.2 ± 1.2	 10.7 ± 1.3 10.2 ± 1.3	 10.2 ± 0.8 9.6 ± 0.8	 11.2 ± 2.0 10.7 ± 2.1

* p = 0.05

Table 2: Results of clinical parameters

Sex	Males				Females			
Dose (mg/kg bw/day)	0	100	300	1000	0	100	300	1000
Stomach, No. examined	10	10	10	10	10	10	10	10
Hyperplasia, with inflammation, epithelium, limiting ridge - Very slight	1	0	4	3	2	0	2	5
- Slight	0	0	0	7	0	0	0	5
Subacute to chronic inflammation, glandular submucosa, multifocal - Very slight	3	2	3	1	2	1	6	5
- Slight	0	0	3	9	0	0	1	4

Table 3: Historical control data, pup survival index (%) post-natal day 4

Study No.	1	2	3	4	5	6	7	8	9
Year reported	2007	2007	2008	2009	2010	2010	2010	2011	2011
Post-natal day 4	99.4	98.8	97.7	99.4	95.4	99.4	99.4	97.7	100.0

Applicant's summary and conclusion

Conclusions

2-amino-2-(hydroxymethyl)propane-1,3-diol had no effect on reproductive performance

Developmental toxicity / teratogenicity

Exposure related observations in humans

Direct observations: clinical cases, poisoning incidents and other

Endpoint study record: WoE, Brasch et al., 1982, pharmacokinetics, RL2

Administrative Data

EU: REACH

Purpose flag weight of evidence

Study result type experimental result

Reliability 2 (reliable with restrictions)

Rationale for reliability incl. deficiencies Acceptable, well documented publication which meets basic scientific principles.

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	Brasch, H. et al.	1982	Pharmacokinetics of TRIS(hydroxymethyl-)aminomethane in healthy subjects and in patients with metabolic acidosis.	Eur. J. Clin. Pharmacol. 22:257-264					

Data access

data published

Materials and methods

Study type

other: study with healthy subjects and patients with metabolic acidosis

Endpoint addressed

basic toxicokinetics

Principles of method if other than guideline

The study was carried out to investigate the pharmacokinetics in healthy subjects and in patients with metabolic acidosis.

GLP compliance

no data

Test materials

Identity of test material same as for substance defined in section 1 (if not read-across)

yes

Details on test material

- Analytical purity: analytical grade

Method

Type of population

no data

Subjects

The subjects were 6 healthy volunteers (5 males and 1 female, aged 27 -37 years, weighing 50 -90 kg) and 20 patients in a surgical intensive care unit.

Ethical approval

no data

Route of exposure

other: intravenous

Reason of exposure

other: intentional and accidental

Exposure assessment

no data

Details on exposure

Healthy volunteers:The volunteers received TRIS 121 mg/kg (=1 mmol/kg). The 0.3 mol/L solution, titrated to pH 7.4 with HCl, was infused in 30 min in an antebrachial vein. Venous blood samples (2 mL) were collected from the other arm. The blood was centrifuged immediately and plasma and erythrocytes frozen separately until analysed. Urine was also collected.

Examinations

- Tissues and body fluids sampled: urine, blood, haemodialysis/haemofiltration fluid, gastric juice, bile- Time and frequency of sampling: - blood (healthy volunteers): 10, 20, 30 min after starting the infusion; 5, 10, 20 and 40 min, 1, 2, 4, 8, 12, 20 and 24 after ending of infusion- urine (healthy volunteers): from beginning of infusion until 30 min after its end; from 30 min to 4 h; from 4 h to 8 h; from 8 h to 24 h- blood (patients): 20, 40, 60, 80, 100, 120 min after starting the infusion; 10, 20,40 min, 1, 2, 4, 8, 12, 18, 24 and 36 h after ending of infusion; in some patients the observation period was extended up to 72 h with further samples taken at 12 h intervals.- urine (patients): Urine was collected for 24 h and in some cases for 1 and 2 subsequent 24 h periods.

Medical treatment

Patients:TRIS therapy was indicated because of metabolic acidosis with a base excess of more than 3 mmol/L. Additional medication included cardiac glycosides, sympathomimetics, low dose heparin, cimetidine, antibiotics and continuous infusions of glucose (20% or 50%) and aminoacid solutions. Generally 40 mL/kg fluid was given in 24 h. Electrolytes were administered individually according to the ionogram. The additional medication of the patients did not usually interfere with this assay, but in three patients unidentified peaks with the same retention time as TRIS appeared in the chromatogram. These cases had to be discarded from the study.The amount of TRIS required to correct acidosis was calculated from the equation: amount of buffer (mmol) = 0.3 x base excess x body weight (kg). A 3 mol/L solution (pH 10.9) was infused via an indwelling catheter in the jugular or subclavian vein, in 2 h. Blood (1 mL) and urine samples were collected.In cases of acute anuria haemodialysis or haemofiltration was performed once or twice a day to keep the plasma creatinine level between 300 -400 µmol/L. In a single session plasma creatinine was reduced by about 1000 µmol/L. The earliest haemodialysis was carried out 4 h after the end of the TRIS infusion.In 2 patients gastric juice was drained continuously via a plastic tube and was collected for 48 h for TRIS analysis. In one case a T-drain had been implanted in the common bile duct during choledocholithotomy, and the drained bile was collected quantitatively over two 24 h periods.

Any other information on materials and methods incl. tables

Method typp for identification:

The acid-base status of patients was determined from arterial blood by a Technicon blood-gas analyser. Creatinine concentrations in blood and urine were determined by the colorimetric method of Jaffe and the endogenous creatinine clearance was calculated. Tris concentrations in plasma, erythrocytes and other samples were determined via GC (modified from Hulshoff and Kostenbauder, 1978). Hewlett Packard 5710 A GC equipped with a FID.

Results and discussions

Results of examinations

Healthy volunteers:At the end of the infusion, the TRIS concentration in plasma averaged 565 µg/mL. There was a biexponential decline of plasma TRIS levels and after 24 h the level was only 3.8 µg/mL. TRIS concentration in erythrocytes rose more slowly, with a maximum 20 min after the end of infusion. After 2 h TRIS levels in erythrocytes were about 1.5 times those in plasma, and they remained well above the corresponding plasma levels during the rest of the observation period.Pharmacokinetic parameters were calculated from the individual concentration-time curves using a two-compartment model with elimination from the central compartment. The volume of the central compartment was 225-279 mL/kg and the final volume of distribution was 647-1140 mL/kg. The half-life of TRIS was 3.9-9.3 h.TRIS is mainly excreted by the kidney. Already 30 min after the end of infusion, 25% of the administered dose was found in urine and after 24 h 82% had been eliminated in this way. This was less than the total elimination of TRIS which, from the area under the curves, was calculated to be 97% during 24 h.

Effectivity of medical treatment

Anuric patients:A monoexponential decline of plasma TRIS levels was found in all 6 subjects. Drug concentration in erythrocytes was usually higher than in plasma. The half-life ranged between 15 and 58 h, and the volume of distribution (418 - 604 mL/kg) was only slightly larger than the volume of the central compartment in patients with two compartment kinetics (187 - 563 mL/kg). 25 -66% of the infused TRIS dose left the plasma during the first 24 h, and the clearance averaged 16.7 mL/kg*h. In 5 patients repeated (2 -4) periods of haemodialysis or haemofiltration had no influence on plasma TRIS level.Haemodialysis fluid could not be collected and TRIS concentration in haemofiltration fluid could not be determined for technical reasons (unidentified substances were eluted from the Amicon filters and produced large peaks with almost the same retention times as TRIS and the internal standard). Therefore, the amount of TRIS eliminated by these procedures is not known.Normuric patients:A biexponential decline of TRIS concentration in plasma was seen in 9 of 11 patients, and drug concentration in erythrocytes increased to about 1.5 -times the plasma level. The volume of the central compartment was 187 -563 mL/kg and the final volume of distribution was 658 -5247 mL/kg. The half-life of TRIS was 16 -45 h. In 2 patients with poor renal function (creatinine clearance: 15 mL/kg*h) TRIS plasma levels declined monoexponentially.Up to 72% of the dose of TRIS was found in the urine after 24 h. An additional 2 -5% was excreted during the next 24 h and even in the urine of a third collection period (48 -72 h after infusion) a small amount of TRIS could be detected (1.5% of the dose). The amount of TRIS eliminated from the body, as calculated from the area under the curves, was always considerably larger than the quantity recovered from urine collection over the same time period. On average, the total body clearance of TRIS exceeded the endogenous creatinine clearance by 17.7 mL/kg*h, and there was a positive correlation between the two parameters. In 2 patients less than 0.2% of the infused dose was found during 24 h either in gastric juice or bile.

Any other information on results incl. tables

11 patients produced normal 24 h urine volumes (610 -3480 mL) and are termed normuric in contrast to the anuric patients. However, also patients with poor renal

function were included in the investigation.

It is apparent that the half-life of TRIS was much longer in the normuric patients than in healthy subjects, and that the volumes of distribution, especially the final volume of distribution, were much larger. In 2 patients of the normuric group with poor renal function (creatinine clearance: 15 mL/kg*h) TRIS plasma levels declined monoexponentially. This resembled the results obtained from the anuric patients. In anuric patients, TRIS concentration in erythrocytes was usually higher than in plasma, but the difference was not as marked as in healthy subjects or nurmucic patients.

The biexponential decline of TRIS plasma levels in healthy subjects may indicate distribution to two different body compartments, one probably being extracellular water, as the volume of the central compartment equalled the volume of the extracellular space. TRIS is then taken up and accumulated inside cells, since the final volume of distribution is larger than the volume of total body water.

Sensitisation data (humans)

Endpoint study record: WoE, RA-A, 115-70-8, Greier et al., 2003, RL2

Administrative Data

EU: REACH

Purpose flag	weight of evidence
Study result type	read-across from supporting substance (structural analogue or surrogate)
Reliability	2 (reliable with restrictions)
Rationale for reliability incl. deficiencies	Acceptable, well documented publication which meets basic scientific principles.

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
publication	Greier, J. et al.	2003	Patch testing with components of water-based metalworking fluids.	Contact Dermatitis, 49:86-90					

Data access

data published

Materials and methods

Type of sensitisation studied

skin

Study type

study with volunteers

Principles of method if other than guideline

A clinical trial of 233 dermatitis patients with present or past occupational exposure to metalworking fluids (MWF) were patch tested with MWF including 2-amino-2-ethyl-1,3-propanediol (AEPD).

GLP compliance

no data

Test materials

Identity of test material same as for substance defined in section 1 (if not read-across)

no

Test material identity

Identifier	Identity
EC name	2-amino-2-ethyl-1,3-propanediol

Details on test material

- Name of test material (as cited in study report): 2-amino-2-ethyl-1,3-propanediol (AEPD)- Analytical purity: 85%

Method

Type of population

occupational

Ethical approval

no data

Subjects

- Number of subjects exposed: 233; for AEPD: 160 patients were tested (no further data).- Sex: male- Age: 19 to 75 years (mean age of 39.3 years; median: 38 years)

Controls

MWF allergens (i. a. fragrance mix, colophonium, Balsam of Peru, monoethanolamine, diethanolamine, etc.)

Route of administration

dermal

Details on study design

TYPE OF TEST(S) USED: patch test (epicutaneous test) ADMINISTRATION- Description of patch: Patch tests were performed and read according to international guidelines modified by the German Contact Dermatitis Research Group (Schnuch et al., 2001, Leitlinien der Deutschen Dermatologischen Gesellschaft (DDG) zur Durchführung des Epikutantests mit Kontaktallergenen. Hautarzt, 52:864-866).- Vehicle / solvent: water- Concentrations: 1% (The test preparation was made from an aqueous stock solution containing the stated concentration of the chemical (85%).)EXAMINATIONS- Statistical analysis: Data were analysed at the Information Network of Departments of Dermatology data centre at the University of Göttingen, using the statistical program system SAS 8.2 (SAS Institute, Cary, NC, USA).

Any other information on materials and methods incl. tables

The study period was from April 2000 to July 2002, including 5 departments of dermatology. Patch testing with the study panel was not restricted to metalworkers with contact dermatitis who are currently exposed to MWF. In order not to miss previously acquired sensitisations to MWF components, also dermatitis patients with previous MWF exposure were tested.

Among the 233 tested patients, patch test application time was 1 day in 2 centres (37%), and 2 days in 3 centres (62.2%), in line with the routine patch test procedures in the respective clinics. All tests were read at least until Day 3. For data analysis, only reactions at Day 3 were selected.

Results and discussions

Results of examinations

NO. OF PERSONS WITH/OUT REACTIONS COMPARED TO STUDY POPULATION- Number of subjects with positive reactions: 1- Number of subjects with negative reactions: 159

Any other information on results incl. tables

Only 1 of 160 patients reacted positively to AEPD. This patient did not react to other MWF components, in particular not to monoethanolamine and diethanolamine. Hence, no clinical relevance of the positive test reaction to AEPD could be found. However, not all the MWFs previously used by this patient could be identified. Therefore, previous occupational exposure and relevance could be regarded as possible.

The patient reacted positively to AEPD belongs to a group of metalworkers currently exposed to MWF with work-related hand dermatitis. At the time of patch testing, this patient had been working in the metalworking industry for 2 -20 years.

The importance of AEPD as MWF allergen still remains to be established.

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

TO: F. Alan Andersen, Ph.D.
Director - COSMETIC INGREDIENT REVIEW (CIR)

FROM: Halyna Breslawec, Ph.D.
Industry Liaison to the CIR Expert Panel

DATE: September 24, 2012

SUBJECT: Concentration of Use by FDA Product Category: Tromethamine

1101 17th Street, N.W., Suite 300 | Washington, D.C. 20036-4702 | 202.331.1770 | 202.331.1969 (fax) | www.personalcarecouncil.org

Concentration of Use by FDA Product Category - Tromethamine

Product Category	FDA Code†	Maximum Concentration of Use
Eye brow pencil	03A	2%
Eye liner	03B	1-2%
Eye shadow	03C	0.8%
Eye lotion	03D	2%
Mascara	03F	2%
Perfumes	04B	0.02%
Powders (dusting and talcum)	04C	0.0002-0.05%
Other fragrance preparations	04E	0.2%
Hair conditioners	05A	0.01%
Shampoos (noncoloring)	05F	0.001%
Tonics, dressings and other hair grooming aids	05G	0.8%
Blushers (all types)	07A	2%
Face powders	07B	0.05%
Foundations	07C	0.3-2%
Lipstick	07E	0.3%
Makeup bases	07F	0.4%
Nail creams and lotions	08C	4%
Dentifrices (aerosol, liquid, pastes and powders)	09A	0.002%
Bath soaps and detergents	10A	0.00009%
Aftershave lotions	11A	2%
Shaving cream (aerosol, brushless and lather)	11E	1%
Skin cleansing (cold creams, cleansing lotions, liquids and pads)	12A	0.5%
Face and neck creams, lotions and powders	12C	

not spray		1-2%
Body and hand creams, lotions and powders not spray	12D	0.05-2%
Moisturizing creams, lotions and powders not spray	12F	1%
Paste masks and mud packs	12H	4%
Skin fresheners	12I	0.3%
Other skin care preparations rinse-off	12J	2%
Suntan gels, creams and liquids not spray	13A	0.2-2%
Other suntan preparations	13C	0.5%

†Product category codes used by FDA

Information collected in 2012
Table prepared September 24, 2012

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

TO: F. Alan Andersen, Ph.D.
Director - COSMETIC INGREDIENT REVIEW (CIR)

FROM: Halyna Breslawec, Ph.D.
Industry Liaison to the CIR Expert Panel

DATE: January 4, 2013

SUBJECT: Comments on the Scientific Literature Review on Tromethamine

Key Issue

As suggested by Dow Chemical Company, please remove the information about Aminomethyl Propanol from the report on Tromethamine. The analogue approach provided by Dow should be summarized in the report and information on Aminomethyl Propanediol (previously reviewed by CIR) and Aminoethyl Propanediol (INCI - but not yet reviewed by CIR) should be added to the report.

Additional Comments

p.1 - The Cosmetic Use section should also note that Europe considers Tromethamine to be regulated under Annex III entry 61 Monoalkylamines, monoalkanolamines and their salts, which limits secondary amine content in the ingredient to 0.5%.

p.2 - The title of reference 15 suggests that THAM citrate was studied. This is not mentioned in the Absorption, Distribution, Metabolism and Excretion section.

p.2 - Please correct: "resulted in alkalinizes", "tromenamine", "strain not provided; not provided"

p.2 - In the last paragraph, both THAM and Tris are used as abbreviations. After explaining that both may be used to represent Tromethamine, please only use one abbreviation through the rest of the report.

p.3 - Although the references are in Table 3, it would also be helpful to include the references in the text of the Acute Exposure section.

p.4 - What effects were observed in Sprague-Dawley rats fed Tromethamine for 3 months (reference 40)?

p.4 - What was the route of exposure used in the study described in reference 43? The first paragraph says "i.v.", the second paragraph says "i.p.".

p.4, 5, 8 - The title of reference 44 is "Intratracheal instillation studies with benzo[a]pyrene in a mixture of Tris buffer and saline in Syrian golden hamsters". Benzo[a]pyrene is not a drug as indicated in the description of this study on p.4 where it states: "vehicle control in an experiment for a drug". What was the dose volume used in reference 44? On p.4 it says "2 mL", on p.5 it says "0.2 ml", p.8 it says "0.2 ml". Providing the dosing volume without any

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information about the concentration of Tromethamine in the dosing solution provides no information about the dose that was used.

p.5 - Intradermal injection should not be included in the dermal exposure section.

p.5 - Please complete the following: "He was administered (30 g in water: 10%) over 1 h."

p.8 - In the Summary, please provide the FDA product category in which a concentration of 4% was reported.

p.10, Table 3 - The subcutaneous exposure studies should not be presented under dermal exposure.