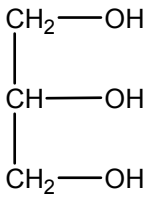
GLYCEROL

SYNONYMS

1,2,3-Propanetriol
Glyceritol
Glycerole
Glycyl alcohol
1,2,3-Trihydroxypropane

CHEMICAL STRUCTURE



CHEMICAL FORMULA

$C_3H_8O_3$

IDENTIFIER DETAILS

CAS Number	:	56-81-5
CoE Number	:	-
FEMA	:	2525
EINECS Number	:	200-289-5
E Number	:	422

CLP CLASSIFICATION

Ingredient CLP Classification: No

Endpoint	Classification	Category
Acute Oral Toxicity	-	-
Acute Dermal Toxicity	-	-
Acute Inhalation Toxicity	-	-
Skin Corrosive/irritant	-	-
Eye Damage/Irritation	-	-
Respiratory Sensitisation	-	-
Skin Sensitisation	-	-
Mutagenicity/Genotoxicity	-	-
Carcinogenicity	-	-
Reproductive Toxicity	-	-
Specific Target Organ Toxicity	-	-
Aspiration Toxicity	-	-

SPECIFICATIONS

Melting Point: 17.8°C

Boiling point: 290°C [Decomposes, NTP, 2003]

Octanol/Water Partition Coefficient: -1.76 [HSDB, 2003]

PURPOSE

Humectant; solvent.

STATUS IN FOOD AND DRUG LAWS

CoE limits:

Beverages (mg/kg)	Food (mg/kg)	Exceptions (mg/kg)
-	-	-

Acceptable Daily Intake:

ADI (mg/kg)	ADI Set by	Date Set	Comments
Not specified	JECFA	2002	To be finalised

FDA Status: [CFR21]

Section Number	Comments
182.1320	Glycerol

HUMAN EXPOSURE

Natural Occurrence: Glycerol is reportedly found naturally occurring in apple, cider, beer sour cherries, peach and wine [Fenaroli, 2005]. The two major sources of glycerol in the human diet are as a food ingredient and predominantly the major source of glycerol is the glycerol contained in dietary fat. Simple lipids consist of triglycerides, which are esters of fatty acids with glycerol and are metabolised in the body [Bowman *et al.,* 1990].

Glycerole is commonly added to food because of its humectant and plasticiser properties, to keep foods moist and with a soft texture. Glycerol is also used as a solvent for food colorings and agents and as a bodying agent in baked goods [Winter, 1978].

Reported Uses: Glycerol is reportedly used in baked goods at 34.04 ppm, fats and oils at 0.21 ppm, milk products at 83.59 ppm, frozen dairy at 0.46 ppm, fruit juice at 0.07 ppm, meat products at 1.76 ppm, soft candy at 3.75 ppm, confection frosting at 69.74 ppm, jam and jelly at 54.0 ppm, sweet sauce at 74.02 ppm, gelatin pudding at 42.75 ppm, snack foods at 23.50 ppm,

non-alcoholic beverages at 1.39 ppm, alcoholic beverages at 0.92 ppm, hard candy at 22.49 ppm, and chewing gum at 18.55 ppm [Fenaroli, 2005].

TOXICITY DATA

This ingredient has been registered under REACH. Under REACH, registrants have an obligation to provide information on substances they manufacture or import. This information includes data on hazardous properties (covering various toxicological endpoints), guidance on safe use and classification and labelling. The European Chemicals Agency (ECHA) makes this information publicly available on its website: http://echa.europa.eu/.

Carmines (2002), Rustemeier *et al*. (2002), Roemer *et al*. (2002) and Vanscheeuwijck *et al.* (2002) reported on a testing program designed to evaluate the potential effects of 333 ingredients added to typical commercial blended test cigarettes on selected biological and chemical endpoints. The studies performed included a bacterial mutagenicity screen [Ames assay] a mammalian cell cytotoxicity assay [neutral red uptake], determination of smoke chemical constituents and a 90-day rat inhalation study. Based on the findings of these studies, the authors concluded that the addition of the combined ingredients, including Glycerol at levels up to 42048 ppm, “did not increase the overall toxicity of cigarette smoke”, [Carmines, 2002].

Carmines *et al., (*2005) conducted the following studies with glycerol, pyrolysis studies, smoke chemistry and biological activity studies (bacterial mutagenicity, cytotoxicity, in vivo micronucleus, and sub-chronic rodent inhalation) with mainstream smoke, or mainstream smoke preparations from cigarettes containing various target levels (5 %, 10 %, and 15 %) of glycerol. The actual levels of glycerol in the respective test cigarettes were determined to be 3.2 %, 6.2 % and 8.4 % after cigarette production. At simulated tobacco burning temperatures up to 900˚C glycerin did not pyrolyze extensively suggesting that glycerol would transfer intact to the mainstream smoke (smoke was not analyzed for glycerol in this study). On a tar basis, nicotine in smoke was significantly decreased at 10 % and 15 % glycerol while water was increased at all addition levels. Addition of 10 % or 15 % glycerol also resulted in a statistically significant increase in acrolein (9 %) and a decrease in acetaldehyde, propionaldehyde, aromatic amines, nitrogen oxides, tobacco specific nitrosamines, and phenols. Addition of 5 % glycerol produced the same decrease in smoke constituents as the 10 % and 15 % groups but there was no concomitant increase in acrolein. Biological tests indicated no relevant differences in the genotoxic or cytotoxic potential of either mainstream smoke (or smoke preparations) from cigarettes with added glycerol compared to control cigarettes. Cigarette smoke atmosphere dilution, coupled with the lower nicotine delivery in the test cigarettes that contained glycerol resulted in a lower nicotine delivery to the glycerol cigarette smoke exposed rats of the 90-day inhalation study. Smoke atmosphere acrolein was also reduced in a concentration-related manner [Carmines *et al.,* 2005].

McAdam *et al*., (2011) reports chemical, biological and human exposure data related to experimental cigarettes containing up to 60% of a novel glycerol containing "tobacco-substitute" sheet. Analysis of mainstream smoke from experimental cigarettes showed reductions in yields of most measured constituents, other than some volatile species. In vitro toxicological tests showed reductions in the activity of smoke particulates in proportion to their glycerol content. Human exposure to nicotine was reduced by a mean of 18% as determined by filter studies and by 14% using 24h urinary biomarker analysis. Smoke particulate exposures were reduced by a mean of 29% in filter studies and NNK exposure by similar amounts based on urinary 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol concentrations [McAdam *et al*., 2011].

In Vivo Toxicity Status

LD_{50}	rat	oral	4.42-27.5 g/kg/bw	(Deichmann 1941, Smyth *et al.,* 1941, Hine *et al.,* 1943, Uche *et al.,* 1987)
LD_{50}	rat	*i.p*.	8.3 g/kg/ bw	(Price 2000)
LD_{50}	rat	*i.p*.	4.4 g/kg/ bw	[RTECS, 2003]
LD_{50}	rat	*i.v.*	5.6 g/kg/ bw	(Price 2000)
LD_{50}	rat	*s.c.*	100 mg/ kg	[RTECS, 2003].
LDL_0	rat	*i.m.*	10 mg/ kg	[RTECS, 2003].
LD_{50}	rabbit	oral	17.6 g/kg/ bw	(Deichmann 1941)
LD_{50}	rabbit	oral	27.g/kg/ bw	[RTECS, 2003]
LD_{50}	rabbit	*i.v.*	53 g/ kg	[RTECS, 2003].
LD_{50}	rabbit	skin.	> 10 g/ kg	[RTECS, 2003].
LD_{50}	mouse	oral	19.3-26.1g/kg/ bw	(Kudo *et al.,* 1972, Latven 1939)
LD_{50}	mouse	oral.	4.1g/ kg	[RTECS, 2003].
LD_{50}	mouse	s.c.	91 mg/ kg	[RTECS, 2003].
LD_{50}	mouse	*i.p*.	8.98 g./kg/bw	(Price 2000).
LD_{50}	mouse	*i.v.*	6.20 g/kg/bw	(Price 2000)
LD_{50}	mouse	i.v.	4.2g/ kg	[RTECS, 2003].
LD_{50}	guinea pig_	oral	7.75-11.5 g/kg/bw	(Hine *et al.,* 1953, Smyth et al 1941)
LC_{50}	rat	ihl	> 570 mg/m^3/1H	[RTECS, 2003]
TDL_0	Human	oral	1428 mg/kg [Behaviour (headache) and G. I. effects [Nausea or vomiting)]	[RTECS, 2003]

Multiple Dose Toxicity Data:

TDL_0	rat	oral	16800 mg/kg [28 day continuous treatment] Endocrine effects observed [RTECS, 2003]
TDL_0	rat	oral	96 g/kg (30 day intermittent treatment) Blood and enzyme effects observed [RTECS, 2003]

TDL_0	mouse	oral	560 g/kg (8 wk continuous treatment) Effects observed in lungs, thorax, or respiration [RTECS, 2003]

In the Swiss albino rat, the acute oral toxicity was associated with increased relative weights of the heart, kidney and liver. Histopathological examination revealed congestion of the blood vessels of the lungs, the accumulation of blood in the kidney renal tubules, neuronal degeneration and necrosis [Uche *et al.,* 1987].

Rats injected intravenously with 10 % glycerol for nine consecutive days, developed an increase in succinate dehydrogenase and acid phosphatase activity in kidney and degeneration of renal tubular epithelium ultrastructure one and four days after the last injection. By 19 days after the last treatment the kidneys were similar to those of control animals [Nowak *et al.,* 1979]. Injection of glycerol at a 50 % concentration at 10 ml/kg to rats produced proximal tubular damage and acute renal failure. At 48 and 72 hours after dose administration there was increase in cytochrome P-450 was significantly increased in both the kidney and spleen. The authors suggest that glycerol evoked pathological changes in both tissue and may affect the metabolism of xenobiotics and endogenous liver and kidney hormones [Kertai *et al,* 2000].

Rats received 10 mL/kg, i.p., hypertonic glycerol solution (50% v/v) or saline (NaCl 0.85 g %) followed by 24 h water deprivation. Rats were killed 24 h later. Creatinine levels and the activity of creatine kinase (CK) and lactate dehydrogenase (LDH) were determined in the plasma. In addition, CK, pyruvate kinase and LDH activity and oxidative stress parameters (free radical formation, lipid peroxidation and protein carbonylation) were measured in renal tissue. 3. Glycerol did not alter plasma creatinine kinase (CK) activity and increased plasma creatinine levels, which suggested renal insufficiency and the absence of rhabdomyolysis. Renal CK and pyruvate kinase activities were decreased, suggesting diminution of energy homeostasis in the kidney. Plasma and renal LDH activity was decreased, whereas the formation of free radicals, lipid peroxidation and protein carbonylation were increased, suggesting oxidative stress. 4. It was suggested that glycerol may provoke renal lesions by mechanisms other than those induced by rhabdomyolysis [Rieger *et al*., 2008].

Carcinogenicity and mutagenicity

Two studies in which glycerol was administered to rats [Sprague-Dawley and Long-Evans] at concentrations of 0, 2500, 5000 or 10000 mg/kg/bw day for a period of 2 years was reported to have no treatment related effects. The reported NOEL was 10000 mg/kg/bw/day for both studies. A similar study in which glycerol was administered in the diet at 0, 5000, 10000 or 20000 mg/kg bw per day) for a period of 50 weeks was again reported to be without effect. Therefore this study had a reported NOEL of 20000 mg/kg bw day [JECFA, 2002].

Glycerol has been shown to promote lung tumorigenesis induced by 4-nitroquinone 1 oxide [4NQO] in several studies [BIBRA, 1993, Yano *et al.,* 1992, 1994] treated ddY male mice to 5 % glycerol in the drinking water. The authors have suggested that the tumour promoting effect of glycerol is further enhanced by high levels of dietary iron, and postulate that oxidative stress was modulating the observed effect [Yano *et al.,* 1992, 1994].

Nagahara *et al.,* (1990) hypothesised the glycerol modulated pulmonary tumourigenesis through metabolic activation in bronchiolar cells. Male ddY mice were given 1 mg/g urethane and/or a 5 % glycerol solution. The authors reported that glycerol did not affect urethane induced pulmonary tumourigenesis, but did however, modify the metabolism of urethane in the bronchiolar epithelium, [Nagahra *et al.,* 1990].

Mice of various strains [A/J, SWR, MaMYJ, BALB/cByJ, 129J and C57BL/6J] were exposed to three carcinogens, 3-methylcholanthrene, 4NQO, urethane and then exposed to drinking water containing 1 or 5 % glycerol for up to 4 months. The authors failed to find any effect of chronic glycerol exposure, and glycerol failed to influence the number or incidence of lung tumours in the mouse strains used. There was no evidence that glycerol affected liver tumour development [Witschi *et al.,* 1989].

Wilson *et al.,* (1978) reported that on adding glycerol to tobacco smoke condensate dissolved in acetone, the topical carcinogenicity and the ability to produce epithelial hyperplasia was reduced. These studies suggested that there was a sequential process linking the progression of normal skin to, to hyperplasia, to benign neoplasia to malignant neoplasia. Glycerol, they suggested, impeded the natural process of progression, [Wilson *et al.,* 1978].

Dermal toxicity

The application of neat glycerol was not found to be irritating to the skin of rabbits in both: single, repeated, occluded and non occluded patch tests [Hayakaya 1988]. Repeated uncovered or covered patch tests with neat glycerol at a concentration of 10 % were reported to cause skin thickening in guinea pigs [Hayakawa, 1988]. Gad *et al.,* (1986) reported no irritation following application of glycerol to the shaved skin of mice [BIBRA, 1993].

In humans application of glycerol at 5 or 15% aqueous solution and as a 10 % solution in a cosmetic product, [BIBRA, 1993] reported a decrease in transdermal water loss across the skin, which is indicative of irritation. In ‘several thousand’ dermatitis patients exposed to a 50 % aqueous solution of glycerol in covered patches for up to 24 hours , the solution was reported as non irritating [Hannuksela, 1979].

A group of 15 guinea pigs were administered a neat intradermal injection of glycerol, followed by a skin test challenge at the same site no animals responded to that and subsequent challenges. A similar result was obtained for mice when glycerol was to the stomach skin for four days; on a subsequent challenge there was no evidence of sensitisation [BIBRA, 1993].

Glycerol dropped on the human eye causes a strong stinging and burning sensation, with tearing and dilation of the conjunctival vessels, but with no obvious injury [Grant, 1986].

When introduced into the anterior chamber of the rabbit eye, glycerol causes an inflammatory reaction and edema of the cornea with wrinkling of the posterior surface and damage of endothelial cells. [Grant, 1986]. Aqueous 50% glycerol in the anterior chamber of rabbits causes significantly less reaction, though within 5 min it visibly dehydrates the lens, causing its capsule to become wrinkled. Glycerol was not found to be toxic when 0.1 ml of neat glycerol was applied to the eyes of cats [BIBRA, 1993]

Eye-rabbit	126 mg Mild irritant
Eye Rabbit	500 mg/24 Hour Mild irritant [NTP, 2003].

In humans application of neat glycerol to 50 volunteers failed to produce any evidence of sensitisation [BIBRA, 1993].

Reproductive and developmental studies

TDL_0	rat	oral	100 mg/kg (1D male) [RTECS, 2003] [Effects on post-implantation mortality]
TDL_0	rat	itt	280 mg/kg (2D male) [RTECS, 2003] [Effects on paternal spermatogenesis]
TDL_0	rat	itt	1600 mg/kg (1D male) [RTECS, 2003] [Effects on male fertility]
TDL_0	rat	itt	862 mg/kg (1D male) [RTECS, 2003] [Effects on paternal spermatogenesis]
TDL_0	Monkey	itt	119 mg/kg (1D male) [RTECS, 2003] [Effects on paternal spermatogenesis]

Note: itt- Intertesticular and 1D and 2D refers to the total number of days administration prior to mating].

No effects of feeding glycerol have been reported in various studies conducted by the FDRL (1973) in rats at the highest dose levels of 1310 mg/kg/day mice at 1,280 mg/kg and rabbits at 1,180 mg/kg. Wegener (1953), found no teratogenic effects in 10 male and female rats dosed at 2.5 mg/kg bw day by gavage for more than 12 weeks [Wegener, 1953].

In a limited study, 15g/kg of glycerol administered to rats in the diet, led to a decrease in mean pup weight for seven successive generations. Dietary administration at 5g/kg to six successive generations of rats failed to produce any teratogenic effects [Guerrant *et al.,* 1947].

Inhalation studies

A recent study investigated the effect of cigarettes, containing various additives in three combinations, in a 90 day nose-only smoke inhalation study

in rats [Vanscheeuwijck *et al.,* 2002]. These ingredients included glycerol at 42048 ppm, a level described as a multiple of its typical use in a US cigarette. The data from this study along with that from a number of other biological and chemical studies indicate that the addition of the combined ingredients "did not increase the inhalation toxicity of the smoke, even at the exaggerated levels used" [Vanscheeuwijck *et al.,* 2002].

In Greenspan *et al.,* (1988) and Renne *et al.,* (1992) rats were exposed to glycerol at 0, 1, 2, or 4 g/m^3 for two weeks. There was no evidence of effects of treatment, on blood parameters, food consumption, organ weights, there was, however, a decrease in weight gains for rats dosed above 1 g/m^3. In the 13-week study Sprague Dawley rate were dosed at 0.033, 0.165, 0.660 g/m^3 for 13 weeks [Renee *et al.,* 1992]. In both the 2 and 13 week studies a number of effects were seen, only the development of squamous metaplasia of the epithelium lining the base of the epiglottis was considered to be of biological significance. This minimal change was considered to be an adaptive response to the mild irritant effect of inhaled glycerol [Greenspan *et al.,* (1988), Renne *et al.,* (1992)].

Incorporation of glycerol at target levels up to 15 % did not produce any adverse effects in rats exposed for 90-days. The major observation in the study was a reduced biological activity of the smoke as indicated by a reduction in the severity and/or incidence of focal macrophage accumulation in the lungs and goblet cell hyperplasia/hypertrophy in the nose (level 1), and goblet cell staining depletion in the nose (level 1). The results of these studies with glycerol applied to cigarette tobacco suggested that adding glycerol to cigarette tobacco at typical use levels does not adversely alter the smoke chemistry or biological effects normally associated with exposure to mainstream cigarette smoke [Carmines *et al.,* 2005].

Heck *et al.,* (2002) examined the role of Glycerol (CAS no. 56-81-5) and propylene glycol (CAS no. 57-55-6) which are commonly used as humectant ingredients in manufactured cigarettes to control and maintain the moisture content of the cut tobacco filler, the potential of these added humectants, to affect the toxicity of cigarette smoke in a sub chronic nose-only smoke inhalation study in rats. Filtered test cigarettes were prepared from an American-style tobacco blend containing either glycerol added at 5.1 % w/w tobacco, propylene glycol at 2.2 % w/w tobacco, or combinations of these humectants totalling 2.3 %, 3.9 %, and 7.2 % w/w tobacco. Other groups of animals were exposed similarly to the smoke of reference cigarettes without added humectants, or to filtered air (sham control). Smoke exposures were conducted for 1 h/day, 5 days/wk for 13 wk, at target smoke particulate concentrations of 350 mg/m^3. All smoke-exposed groups had equivalent increases in blood carboxyhemoglobin, serum nicotine, and serum cotinine relative to the air controls. Smoke-associated reductions in body weights and occasional increases in heart and lung weights were generally similar among the various exposure conditions at necropsy. Assessments of respiration conducted after 3, 6, 9, and 12 wk of smoke exposure indicated an initial smoke-related but not humectant-related decrease in respiratory rate, tidal volume, and minute volume during the first 20 min of each smoke exposure.

Heck *et al.,* (2002) reported that the respiratory-tract histopathology was consistent across sexes and smoke groups, comprising (1) diffuse and focal alveolar pigmented macrophages and chronic interstitial inflammation in the lung, (2) laryngeal epithelial hyperplasia, squamous metaplasia, and scab formation, and (3) epithelial hyperplasia in the anterior nose. The smoke-related histopathology resolved substantially during a 6-wk postexposure recovery period. It was concluded that the addition of the tested humectants to cigarettes, singly or in combination, had no meaningful effect on the site, occurrence, or severity of respiratory-tract changes or on the measured indices of pulmonary function. It was concluded that the addition of glycerol and propylene glycol to cigarettes does not significantly affect the biological activity of inhaled cigarette smoke in the rat model [Heck *et al.,* 2002].

The addition of glycerol at 70,000 ppm to reference cigarettes, used in a 90 day-sub-chronic inhalation exposure in rats, led to a series of pathological changes to smoke exposure that were indistinguishable from those changes caused by the control cigarettes. This indicated that addition of glycerol to a reference cigarette had no discernable effect upon the type or severity of the treatment related pathological changes associated with tobacco smoke exposure [Baker *et al.,* 2004].

Other relevant studies

Following oral administration in humans , glycerol is rapidly absorbed from the gastro-intestinal tract, and peak serum concentrations occurred with 60-90 min. Increased intraocular pressure begins to decline with 10-30 min following oral administration of glycerol,]. Glycerol is rapidly absorbed and undergoes further metabolism [McEvoy, 1993].

Glycerol can exert systemic toxic effects when given orally or parenterally in very large doses. Major toxic effects [haemolysis, hemoglobinuria, & renal failure] are a function of concentration and the route of administration. Systemic effects do not follow copious application to skin [Gilman *et al.,* 1980]

Glycerol is reported to be endogenous in humans and not excreted, but metabolised completely. Glycerol is metabolised by the glycolytic pathway after conversion (in the liver) to glycerol-3-phosphate. Glycerol-3-phosphate undergoes oxidation yielding dihydroxyacetone phosphate which is subsequently isomerised to glyceraldehydes-3-phosphate, eventually yielding pyruvic acid. Pyruvic acid then follows two routes of metabolism. In aerobic conditions it is converted to acetyl coenzyme A and enters the citric acid cycle. However, in anaerobic conditions pyruvic acid is reduced by lactic dehydrogenase to lactic acid (occurs primarily in muscle in periods of excessive exercise). The lactic acid produced diffuses through muscle tissue and is reported to be transported to the liver where it is converted to glucose via gluconeogenesis. However, lactic acid may be further catabolised in the lactic acid cycle, [JECFA, 2002]. Previous studies have reported 7-14 % of an orally administered dose to be excreted unchanged in the urine, (within 2 ½ hours) with most reportedly being incorporated into body fat [HSDB, 2003].

Following absorption from the G.I. tract glycerol is reported to be distributed throughout the blood. Although it does not usually appear in the ocular fluids, it may enter the orbital sac if the eye is inflamed this may subsequently lead to a reduction is osmotic effect [HSDB, 2003].

Behavioural data:
No data identified

In Vitro Toxicity Status

Carcinogenicity and mutagenicity
Additional information concerning the *in vitro* mutagenicity of this material may be found in "An Interim report on data originating from Imperial Tobacco Limited's Genotoxicity testing programme September 2003" or "An updated report on data originating from Imperial Tobacco Limited's external Genotoxicity testing programme – Round 2 August 2007".

Glycerol has been found to be negative in the Ames strains TA97, TA98, TA100, TA 102, TA1535, TA1537 and TA1538 at concentrations up to 5 mg/plate both with and without metabolic activation [Shimizu *et al.,* 1985].

Doolittle *et al.,* (1988) found negative results when testing glycerol in the Ames strains TA98, TA100, TA1535, TA1537 and TA1538; in the rat hepatocyte unscheduled DNA synthesis (UDS)assay; in the Chinese Hamster Ovary [CHO] chromosome aberration assay; the CHO mammalian mutagenicity assay and the CHO sister chromatid exchange assay. The above assays except for the rat hepatocyte UDS assay were conducted with and without metabolic activation. The authors conclude that neither glycerol nor any of it is metabolites showed any genotoxic activity in any of the tests performed.

Glycerol has also been found to be negative in the *E. coli*. WP2 UVRA assay, both with and without metabolic activation up to 5mg plate (5000 ug/plate) [Shimizu *et al.,* 1985].

Roemer *et al.* (2002) reported on a study in which cigarettes containing various additives in three different combinations were produced. Smoke condensates prepared from these cigarettes were then tested in two different *in vitro* assays. The mutagenicity of the smoke condensate was assayed in the Salmonella plate incorporation [Ames] assay with tester strains TA98, TA100, TA102, TA1535 and TA1537 in the presence and absence of an S9 metabolic activation system. The cytotoxicity of the gas/vapour phase and the particulate phase was determined in the neutral red uptake assay with mouse embryo BALB/c 3T3 cells. The authors concluded that the *in vitro* mutagenicity and cytotoxicity of the cigarette smoke was not increased by the addition of the ingredients which included Glycerol at levels up to 42048 ppm (a multiple of its typical use in a US cigarette) [Roemer *et al.,* 2002].

The mutagenicity of the smoke condensate was assayed in the *Salmonella* plate incorporation [Ames] assay with the tester strain TA98 in the presence of

an S9 metabolic activation system. The cytotoxicity of the cigarette condensate was determined in the neutral red uptake assay and the (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H tetrazolium, inner salt assay (MTS assay) with the human hepatocellular liver carcinoma cell line, HEP-G2. It was concluded that the *in vitro* mutagenicity and cytotoxicity of the cigarette smoke was not increased by the addition of the ingredients, which included *glycerol* at levels up to 50000 ppm.

Baker *et al.,* [2004], examined the effects of the addition of 482 tobacco ingredients upon the biological activity and chemistry of mainstream smoke. The ingredients, essentially different groups of flavourings and casings, were added in different combinations to reference cigarettes. The addition of glycerol at 70,000 ppm was determined not to have affected the mutagenicity of the total particulate matter (TPM) of the smoke in either the Ames, *in vitro* micronucleus assay or the neutral red assay when compared with that of the control cigarettes [Baker *et al.,* 2004].

The cigarette ingredients cocoa powder, glycerol, and saccharose were investigated regarding their potential effect on the resulting mainstream smoke, i.e., smoke chemistry (Hoffmann analytes), mammalian cell cytotoxicity (Neutral Red Uptake assay), and bacterial mutagenicity (Ames assay). Each ingredient was added at three concentrations to the tobacco of a 6 mg and 10 mg 'tar' yield experimental American blend filter cigarette (obtained under ISO/FTC smoking regime). The lowest application concentration was equivalent to the normal approximate use level of the ingredients; the highest application level was up to 5-fold higher. The resulting data were compared with the respective control cigarettes without addition of the ingredients. The addition of glycerol resulted in a decrease in the delivery of several smoke constituents (generally around 20%), e.g. aldehydes, phenolics, and N-nitrosamines. Water in the particulate phase (TPM) was distinctly increased (up to +150%). The cytotoxicity of the TPM was decreased (approx. -15%). Mutagenicity was not affected. The results are in agreement with currently available literature. Based on the total evidence, it can be concluded that the three ingredients added at their current use levels do not increase the inherent toxicity of the cigarette smoke [Roemer *et al*., 2010].

Reproductive and developmental toxicity

Glycerol was examined In the rodent whole embryo culture (WEC), as a possible alternative vehicle for insoluble compounds. Other carrier solutions tried contained bovine serum albumin (BSA) and glycerol as well as the solvents, formamide, dimethylformamide (DMF), dimethyl sulfoxide (DMSO) and ethanol, for the relative teratogenicity and delivery of the insoluble teratogen, all-trans retinoic acid (RA). At a concentration of ≤ 0.04 %, formamide and DMF exhibited no significant toxicity to cultured rat embryos and were effective at delivering RA to the embryo. The BSA and glycerol carrier solutions were not teratogenic, although both inhibited robust formation of yolk sac vasculature. Both solutions delivered RA to the cultured rat embryos at higher doses. It was concluded that all four solvents/solutions may

have utility as vehicles dependent upon the chemical properties of the compound to be solubilised [Augustine-Rauch *et al.,* 2004].

Other relevant studies

Glycerol has been shown to have low neurotoxicity [EC_{50} >2000 g/ml], and low cytotoxicity in a number of cell lines [Williams *et al.,* 1994].

PYROLYSIS AND TRANSFER STUDIES

Information relating to the pyrolysis and/or transfer of glycerol is detailed in the Report on Thermochemical Properties of Ingredients document. In the aforementioned document, the term 'pyrolysis' means the heating of an ingredient in isolation under controlled conditions in an analytical device to examine its degradation potential. The expression 'transfer data' on the other hand is used to describe the fate of an ingredient in qualitative and quantitative terms following the smoking of a tobacco product to which it has been applied.

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