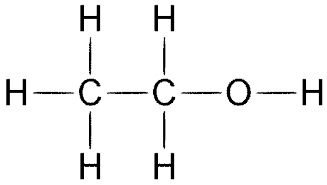
Ethanol

Ingredient synonym names

Ethyl alcohol
Alcohol dehydrated
Cologne spirit
Ethanol 200 proof
Ethanol absolute
Ethyl hydroxide
Grain alcohol
Jaysol
Methyl carbinol
Potato alcohol
Spirits of wine
Synasol
Tecsol
Wine distillate
Wine, burgandy

IDENTIFIER DETAILS

Ingredient chemical structure



CAS Number	FEMA Number	Additive Number
64-17-5	2419	-

Ingredient EC Number	FL Number	CoE Number
200-578-6	02.078	11891

Chemical formula C2H5OH

Ingredient CLP Classification

Ingredient REACH Registration Number

01-2119457610-43

Acute Oral Toxicity	Eye Damage/Irritation	Carcinogenity
0	0	0

Acute Dermal Toxicity	Respiratory Sensitisation	Reproductive Toxicity
0	0	0

Acute Inhalation Toxicity	Skin Sensitisation	Aspiration Toxicity
0	0	0

Skin Corrosive/Irritant	Mutagenicity/ Genotoxicity	Specific Target Organ Toxicity

Ethanol

0	0	0

SPECIFICATIONS

Melting Point	-114.1°C	Boiling Point	78.3°C

STATUS IN FOOD AND DRUG LAWS

Acceptable Daily Intake (ADI, mg/kg)	Limited by GMP (JECFA1970)
Acceptable Daily Intake (ADI) comments	Ethanol posed no safety concern at its current level of intake when ethyl esters are used as flavouring agents
FDA Status	[CFR21] 184.129 3Ethyl alcohol

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

HUMAN EXPOSURE

Ingredient Natural Occurence (if applicable)

Ethanol is reported to be found in apple, apple aroma, apple essence, apple juice, bacon fat, beef extract, blackberry, blackcurrant, bread, Brussels sprouts, cabbage, carrot root, cauliflower, bleu cheese, cheddar cheese, Swiss cheese, cocoa bean, cherry, coffee, cream, cucumber and many other sources [Fenaroli, 1995]. Ethanol is present as an endogenous substance in the blood of man, being produced probably in the intestinal tract, at an average level of 1.5 mg/l [Baselt, 1988].

References - Ingredient Natural Occurence

Baselt, (1988). In Biological Monitoring Methods for Industrial Chemicals. 2nd Edition. Littleton, MA: PSG Publishing Co., Inc. 140.

Ingredient Reported Uses

Ethanol is reportedly used in baked goods at 1610.0 ppm, milk products at 150.0 ppm, frozen dairy at 2179.0 ppm, condiment relish at 1200.0 ppm, soft candy at 5460.0 ppm, gelatin pudding at 1510.0 ppm, non-alcoholic beverages at 2420.0 ppm, gravies at 5000.0 ppm, hard candy at 746.3 ppm, and chewing gum at 1691 ppm [Fenaroli, 1995].

References - Ingredient Reported Uses

Fenaroli, (1995). Fenaroli’s Handbook of flavour ingredients Volume 2 3rd Edition CRC Press London.

TOXICITY DATA

In Vivo Data

Acute Toxicity Data

9488mg/kg bw, Mouse, oral - Spector, 1956
8285 mg/kg bw, mouse, s.c. - Spector, 1956
4700 mg/kg bw, mouse , s.c. - Browning, 1965
1973 mg/kg bw, mouse, i.v. - Spector, 1956

7060-13660 mg/kg bw, rat, oral - Spector,1956;Lewis 2000
5000 - 6060 mg/kg bw, rat, i.p, - Spector,1956;Lewis, 2000

20000 ppm/10 h , rat, inhalation - Lewis, 2000
1440 mg/kg bw, rat, i.v. - Lewis, 2000
5560 mg/kg bw, Guinea-pig, oral - Lewis, 2000
5560 mg/kg bw, guinea-pig, i.p. - Spector, 1956

6300 -9500 mg/kg bw, rabbit, oral - Spector,1956;Lewis, 2000

JECFA (1970). FAO/WHO Expert committee on food additives Fourteenth report.
Spector, WS, (1956). In Handbook of Toxicology, W. B. Saunders & Co.
Lewis (2000). Sax's dangerous properties of industrial materials. Vol 2, 10th Edition. John Wiley & Sons Inc. New York.
Browning, (1965). In Toxicity and Metabolism of Industrial Solvents, Elsevier, Amsterdam.

In Vivo Carcinogenicity/Mutagenicity

Ethanol had no effect on micronucleus incidence in the bone marrow of rats exposed to 5% ethanol in the drinking water for 10-30 days [Balansky et al., 1993]; or at 10 and 20% in rats exposed for up to 7 weeks [Tates et al., 1980]; or administered to mice at up to 40% for 27 days [Chaubey et al., 1977].

No chromosomal aberrations were found in the bone marrow or peripheral lymphocytes of rats exposed to ethanol in the drinking water at 10 or 20 % for 3-6 weeks [Tates et al., 1980]. In Chinese hamsters, there was no effect of ethanol at 10% in the drinking water for 9 weeks or 20 % in the drinking water for 12 Weeks [Korte et al., 1979; Korte et al., 1981].

Sister chromatid exchanges [SCE] have been reported to be induced in the majority of reported in vivo studies, although the effects have also been reported to have been small, that is a less than doubling of the back ground frequency rate (Phillips et al., 2001). SCE was induced in the bone marrow of mice exposed to ethanol given in the drinking water at 10 or 20% for 3 16 weeks [Obe et al., 1979] or in mice injected intraperitonealy at 0.6 2.4 g/kg [Pinna Calva et al., 1993]. An increase in the SCE of peripheral lymphocytes and not bone marrow was seen for rats exposed to ethanol at 10 or 20% for 3-6 weeks [Tates et al., 1980].

The effects of ethanol on the incidence and histology of gastric cancers induced by N-methyl-N'-nitro-N-nitrosoguanidine were investigated in Wistar rats. Rats received alternate-day i.p. injections of 2.5 ml/kg body wt of 20 % ethanol in 0.9 % sodium chloride solution. After 20 wk of oral treatment with N-methyl-N'-nitro-N-nitrosoguanidine, prolonged administration of ethanol resulted in a significant increase in the number and incidence of gastric cancers of the glandular stomach in wk 52. However, ethanol was found not to influence the histological types of the gastric cancers [Iishi et al., 1989].

In hamsters given ethanol in drinking water and also given the known pancreatic carcinogen N-nitrosobis[2-oxopropyl]amine [20 mg/kg, s.c.], no neoplastic lesions were observed at the end of the experiment, whereas 11 out of 14 control hamsters given the carcinogen alone had neoplastic lesions. The lipase activity was reduced in hamsters given the carcinogen alone but was normal in the ethanol and carcinogen treated animals [Tweedie et al.,

1981].

Intraperitoneal administration of ethanol [0.7-1.8 mg/g/day for 7 days] to mice at [10% in drinking water, 2-8 wk] initially decreased the level of hepatic microsomal cytochrome p450, arylhydrocarbon hydroxylase,and protein. Oral ethanol decreased the microsomal protein content, then increased it; cytochrome P450 increased and arylhydrocarbon hydroxylase decreased by ther en d of the study. In mice pre-treated both orally and i.p. with ethanol, the binding of benzo[a]pyrene [B[a]P] to DNA increased. More tumours developed in pre-treated mice given B[a]P than in controls given only B[a]P. Pretreated mice had muscle tumours, and the controls mice had mammary tumors [Capel et al., 1978].

The modifying effect of ethanol on aflatoxin B1 [AFB1]-induced hepatocarcinogenesis was examined in male ACI/N rats by chronic treatment at the post initiation phase. Rats received an i.p. injection of AFB1 [1.5 mg/kg] twice a wk for 10 wk [a total of 20 doses]. Following a week of acclimation, they were given 10% ethanol as drinking water for 56 wk. The effects of ethanol on hepatocarcinogenesis were evaluated in terms of the incidence of altered hepatocellular foci and neoplasms at the end of the experiment. Exposure to AFB1 alone induced a substantial number of altered foci [6.98 iron excluding foci/sq cm] in rats. The number of altered liver cell foci in rats receiving AFB1 followed by ethanol was significantly increased [26.39 iron excluding foci/sq cm]. In the rats given ethanol after AFB1, the total area and mean diameter of both iron excluding foci and altered foci identified in haematoxylin and eosin-stained sections were significantly higher than in the rats exposed to AFB1 alone. The incidence of liver cell tumours of the group given AFB1 and ethanol [3/15, 20%] was higher than that of the group treated with AFB1 alone [0/14, 0%]. The treatment of rats with ethanol alone for 56 wk did not induce either [Tanaka et al., 1989].

Ethyl alcohol was administered in the drinking water of Sprague Dawley rats at a concentration of 0 or 10 % ad libitum. Treatment started at 39 Weeks of age for breeders 7 days prior to breeding of from embryo life [offspring] until spontaneous death. Ethyl alcohol was demonstrated to cause carcinogenic for various organs and tissue. Ethanol was considered to be a multipotential carcinogenic agent. In addition to causing tumours ethyl alcohol induced malignant tumours of the oral cavity, lips and tongue [Soffriti et al., 2002].

110 Sprague Dawley rats (10wks old) were split into five groups. Group A (20 rats) no treatment, Group B (20 rats) receiving an ethanol supplement of 1.23 g/kg bw/day in drinking water, Group C (30 rats) received 18 weekly doses of dimethylhydrazine (DMH) (21 mg/kg bw from beginning of study), Group D (20 rats) received ethylen-diamin-tetracetic acid (EDTA) solution (18 weeks), group E (20 rats) received ethanol plus DMH injections (at the doses stated above). Results indicated no further induction or modification of colorectal carcinogenesis with ethanol supplementation [Perez-Holanda et al., 2005].

References - In Vivo Carcinogenicity/Mutagenicity

Chaubey, (1977). Evaluation of the effect of ethanol on the frequency of micornuclei in the bone marrow of Swiss mice. Mutat Res. 43: 441-444.

Korte et al., (1979). The influence of ethanol treatment on the cytogenetic effects in bone marrow cells of Chinese hamsters by cyclophosphamide, aflatoxibn B1 and patulin Toxicology 12: 53-61.

Korte et al., (1981). Influence of chronic ethanol uptake and acute acetaldehyde treatment on the chromosomes of bone marrow cells and pripheral lymphocytes of Chinese hamsters Mutat Res. 88: 389-395.

Pina Calva et al., (1993). SCE frequencies induced by ethanol, tequila and brandy in mouse bone marrow cells in vivo. Toxicol Lett. 66: 1-5.

Phillips et al., (2001). Is ethanol genotoxic? A review of the published data. Mutagenesis 16: 91-101.

Obe et al., (1979). Induction of chromosome aberrations in peripheral lymphocytes of human blood in vitro and SCEs in Bone marrow cells of mice in vivo by ethanol and its metabolite acetaldehyde. Mutat Res 68: 291-294.

Tates et al., (1980). Cytogenetic effects in hepatocytes, bone-marrow cells and blood lymphocytes of rats exposed to ethanol in the drinking water Mutat Res 79: 285-288.

Iishi et al., (1989). Promotion by ethanol of gastric carcinogenesis induced by N-methyl N-nitro-N-nitrosoguanidine in Wistar rats. Br J Cancer 59(5): 719-721.

Tweedie et al., (1981). Surg Forum 32: 222-224.

Capel et al., (1978). The effect of chronic alcohol intake upon the hepatic microsomal carcinogen-activation system. Oncology 35(4): 168-70.

Tanaka et al., (1989). Enhancing effect of ethanol on Aflatoxin B1-induced hepatocarcinogenesis in male ACI/N rats. Jpn J Cancer Res 80(6): 526-530.

Soffritti et al., (2002). Results of long-term experimental studies on the carcinogenicity of methyl alcohol and ethyl alcohol in rats. Ann NY Acad Sci. 982: 46-69.

Perez-Holanda S, Rodrigo L, Vinas-Salas J, Pinol-Felis C.
(2005). Effect of ethanol consumption on colon cancer in an experimental model. Rev Esp Enferm Dig. 97(2): 87-96.

Krebs, C. (1928). Ztsch. Immun. exp. Therap , 59, 203 [As cited in JECFA 1970].

Dermal Toxicity

A drop of full-strength ethanol on rabbit eyes caused reversible injury graded only 3 on a scale of 10 after 24 hr. The application of 70% alcohol to rabbit corneas injured and temporarily loosened the corneal epithelium, but the recovery is complete. The repeated application (7 drops) of 40 to 80% alcohol to rabbit eyes over an unspecified but presumably longer time is reported to cause a loss of some corneal epithelium and endothelium, followed by haemorrhages in the conjunctiva, with the infiltration and vascularisation of the corneal stroma [Grant, 1986].

Alcohol vapour exposure to humans at sufficiently high concentrations may cause prompt stinging and watering of the eyes, but there appear to be no reports of eye injury from industrial exposure to alcohol vapours [Grant, 1986]. Human volunteers exposed to alcohol vapour concentration of 0.25 % had no notable effect on the eyes. However, at concentration of 0.7 to 1% vapour in air the smell of alcohol was at first almost unbearable, although unpleasant later, and that the eyes began to burn with increasing intensity after several minutes duration [Grant, 1986].

Briefly applied to the skin, 70 % alcohol reportedly does no damage, but it is irritating if left on for long periods of time. As the result of removal of the cutaneous lipids, the frequent use of alcohol causes dry skin and scaliness. If applied to wounds or abraded skin surf, ethanol increases the injury and forms a coagulum under which bacteria may subsequently thrive. [Goodman et al., 1996].

Ethanol-based topical antiseptic hand rubs, commonly referred to as alcohol-based hand sanitizers (ABHS), are routinely used as the standard of care to reduce the presence of viable bacteria on the skin and are an important element of infection control procedures in the healthcare industry. There are no reported indications of safety concerns associated with the use of these products in the workplace. However, the prevalence of such alcohol-based products in healthcare facilities and safety questions raised by the U.S. FDA led us to assess the potential for developmental toxicity under relevant product-use scenarios. Estimates from a physiologically based

pharmacokinetic modeling approach suggest that occupational use of alcohol-based topical antiseptics in the healthcare industry can generate low, detectable concentrations of ethanol in blood. This unintended systemic dose probably reflects contributions from both dermal absorption and inhalation of volatilized product. The resulting internal dose is low, even under hypothetical, worst case intensive use assumptions. A significant margin of exposure (MOE) exists compared to demonstrated effect levels for developmental toxicity under worst case use scenarios, and the MOE is even more significant for typical anticipated occupational use patterns. The estimated internal doses of ethanol from topical application of alcohol-based hand sanitizers are also in the range of those associated with consumption of non-alcoholic beverages (i.e., non-alcoholic beer, flavored water, and orange juice), which are considered safe for consumers. Additionally, the estimated internal doses associated with expected exposure scenarios are below or in the range of the expected internal doses associated with the current occupational exposure limit for ethanol set by the Occupational Safety and Health Administration. These results support the conclusion that there is no significant risk of developmental or reproductive toxicity from repeated occupational exposures and high frequency use of ABHSs or surgical scrubs. Overall, the data support the conclusion that alcohol-based hand sanitizer products are safe for their intended use in hand hygiene as a critical infection prevention strategy in healthcare settings [Maier et al., 2015]

References - Dermal Toxicity

Grant, W.M. Toxicology of the Eye. 3rd ed. Springfield, IL: Charles C. Thomas Publisher, 1986. 54.

Goodman, et al., (1996). The Pharmacological Basis of Therapeutics. 9th Edition. McGraw Hill, New York.

Maier, A., et al. (2015). Safety assessment for ethanol-based topical antiseptic use by health care workers: evaluation of developmental toxicity potential. Regulatory Toxicology and Pharmacology, 73(1), 248-264.

Reproductive/ Developmental Toxicity

Treatment of CD-1 mice with 7 g/kg of ethanol i.p. on one of gestational days 7, 8, 9, 10, or 11 significantly increased the percentage of malformed foetuses and decreased foetal birth weight [Blakley et al., 1984].

Pregnant Wistar rats [15/group] were exposed in inhalation chambers to 0, 10,000, or 16,000 ppm ethanol for 7 hours a day on gestation days 1-20 of gestation. In a second experiment groups of male Wistar rats [18 per group] were exposed ethanol at 0, 10, 000 or 16,000 ppm for 7 hours a day for six weeks. Exposed males were then mated individually with unexposed virgin females for 5 days after completion of exposures. After parturition, pups from both maternally- and paternally-exposed groups were fostered to untreated females. Neither female nor male rats exposed to 10,000 or 16,000 ppm ethanol showed any adverse effects, or any of their offspring. Behavioural testing revealed no differences between any of the offspring of exposed parents to the controls after maternal exposure to ethanol [Nelson et al., 1985].

Groups of female mice were admin 5.8 g/kg [95% alcohol in saline intragastrically in a single intubation of 0.3 mg/10 g body wt] on day 10 of pregnancy, producing a blood ethanol concentration of approximately 450 mg/l-1 at 60 minutes after injection. Control group animals were intubated with saline made isocalorific to the ethanol dose by addition of sucrose. On day 19 of gestation, the fetal urogenital systems were examined by injection of indigo carmine into the bladders of all mice. A total of 53 control and 116 ethanol treated foetuses were examined. The administration of ethanol resulted in a significant decrease in fetal wt. Malformed limbs mostly in the form of fused digits [25 in all] were also seen in the treated animals but not in the controls. There was a significant increased in the incidence of hydronephrosis and hydroureter and a significant increase in reflux in the ethanol treated foetuses [Boggan et al., 1989].

Foetal alcohol syndrome, in children born to alcoholic mothers consists of decreased CNS dysfunction [such as low IQ and microcephaly], a slow growth rate, a characteristic range of facial abnormalities [such as a short nose short, palpebral fissures, and a hypoplastic upper lip]. A variable set of major and minor malformations have also been reported. These characteristic features features have been attributed in part, to the direct action of ethanol to

inhibit embryonic cellular proliferation early on in gestation. Those children with foetal alcohol syndrome generally have a greatly increased susceptibility to both life threatening and minor infectious diseases, due to extensive impairment of their immune system [Goodman et al., 1996].

In a study by Day et al., (1989) a cohort of 650 women were interviewed at each trimester of pregnancy about their level of ethanol intake. The mothers were then classified on their alcohol intake rates as heavy alcohol users if they had an average of greater or equal than 1 drink/day; moderate users for an average consumption of 3 to 6 drinks per week, and light users for an average of < 2.9 drinks per week. A relationship was demonstrated between prenatal maternal alcohol use and growth and morphologic abnormalities in the offspring. A Low birth weight, decreased head length and circumference, and an increased rate of foetal alcohol effects were all found to be significantly correlated with exposure to alcohol during the first 2 months of the first trimester of pregnancy [Day et al., 1989].

It has generally been accepted that long-term chronic ethanol consumption by young rats will lead to significant losses in or cerebellar granule neurones [GN]. Young and adult rats F344 and Wistar-Kyoto [WKY] rats were exposed to chronic ethanol consumption for either 40 weeks as young rats or 40 weeks as adults, during the second half of their life span. There was found to be no effect of ethanol consumption on total numbers of cerebellar GN volumes in young or aged F344 or WKY rats [Petney et al., 2002].

Cebral et al., (2011) conducted a study in which ethanol 10% was administered to CF-1 adult male (treated males, TM) and female (treated females, TF) mice for 27 days, whereas water was given to controls from both sexes too (CM and CF). Post-treatment micronucleus frequency (MN-PCE/1,000/mouse) and gamete morphology were evaluated. To test whether change of female reproductive status results in maternal genotoxicity, CF-1 females received ethanol 10% (exposed group, periconceptionally treated females (PTF)) or water (control group, pregnant control females (PCF)) in drinking water for 17 days previous and up to 10 days of gestation. TM had a high percentage of abnormal spermatozoa vs CM ($p < 0.001$) and elevated parthenogenetic activated oocyte frequency appeared in TF vs CF ($p < 0.001$). Sub-chronic ethanol ingestion induced increased MN frequency in TM and TF ($p < 0.01$). In PTF, where blood alcohol concentrations were between 19-28 mg/dl, very significantly increased MN frequency was found vs PCF ($p < 0.01$), whereas MN values were similar to TF. These results show that sub-chronic alcohol ingestion in CF-1 mice produces sperm head dysmorphogenesis and oocyte nuclear anomalies, suggesting that morphological abnormalities in germ cells are probably related to parental genotoxicity after ethanol consumption. [Cerbal et al., 2011]

References - Reproductive/ Developmental Toxicity

Blakley et al., (1984). Determination of the proximate teratogen of the mouse fetal alcohol syndrome. 2. Pharmacokinetics of the placental transfer of ethanol and acetaldehyde. Toxicol Appl Pharmacol 72 (2): 355-63.

Nelson et al., (1985). Comparison of behavioral teratogenic effects of ethanol and n-propanol administered by inhalation to rats. Neurobehav Toxicol Teratol 7:779-783.

Boggan et al.,(1989). Effect of prenatal ethanol administratrion on the urogenital system of mice. Alcohol Clin Exp Res 13(2): 206-208.

Goodman, et al., (1996). The Pharmacological Basis of Therapeutics. 9th Edition. McGraw Hill, New York.

Day et al., (1989). Prenatal exposure to alcohol: effect on infant growth and morphologic characteristics. Pediatrics 84(3): 536-541.

Petney et al., (2002). The total numbers of cerebellar granule neurones in young and aged Fischer 344 and Wistar-Kyoto rats do not change as a result of lengthy ethanol treatment. Cerebellum (1); 79-89.

Cebral et al., (2011) Male and female reproductive toxicity induced by sub-chronic ethanol exposure in CF-1 mice. Cell Biol Toxicol (4):237-48

Inhalation Toxicity

A study investigating the effect of exposure to inhaled ethanol vapours on the rat pulmonary inflammatory response (from an allergic asthmatic reaction) found that ethanol vapour did not significantly modify pulmonary inflammation. The study was conducted using Brown Norway rats that were sensitized and challenged (15 min inhalation, 14 days later) with chicken egg ovalbumin (OVA). Leukocytes were counted in bronchoalveolar lavages (BAL) performed at 6, 24, 36, 48 and 72 h following the challenge after ethanol exposure (3000 ppm, 6 h/day, daily). Exposure to ethanol did not significantly affect BAL leukocytes after OVA challenge; the authors concluded that allergic pulmonary inflammation is not up-regulated by the inhalation of ethanol [Scarino et al. 2012].

Inhalation concentrations up to 3500 ppm are reported in man to have caused no irritation nor any subjective symptoms, or any rise in blood alcohol levels [Treon, 1958].

Uncertainty exists regarding the validity of a previously developed physiologically-based pharmacokinetic model (PBPK) for inhaled ethanol in humans to predict the blood levels of ethanol (BLE) at low level exposures (<1000 ppm). The objective of this study was to document the BLE resulting from low levels exposures in order to refine/validate this PBPK model. Human volunteers were exposed to ethanol vapors during 4 h at 5 different concentrations (125-1000 ppm), at rest, in an inhalation chamber. Blood and exhaled air were sampled. Also, the impact of light exercise (50 W) on the BLE was investigated. There is a linear relationship between the ethanol concentrations in inhaled air and (i) BLE (women: r^2= 0.98/men: r^2= 0.99), as well as (ii) ethanol concentrations in the exhaled air at end of exposure period (men: r^2= 0.99/women: r^2= 0.99). Furthermore, the exercise resulted in a net and significant increase of BLE (2-3 fold). Overall, the original model predictions overestimated the BLE for all low exposures performed in this study. To properly simulate the toxicokinetic data, the model was refined by adding a description of an extra-hepatic biotransformation of high affinity and low capacity in the richly perfused tissues compartment. This is based on the observation that total clearance observed at low exposure levels was much greater than liver blood flow. The results of this study will facilitate the refinement of the risk assessment associated with chronic inhalation of low levels of ethanol in the general population and especially among workers [Dumas-Campagna et al., 2014]

In a study by Mouton et al. (2014), adult male Sprague-Dawley rats were exposed to daily CIEV for a period of 8 weeks (14HR ON/10HR OFF), producing blood alcohol levels of ~200 mg/dl. Controls were exposed to room air. After 8 weeks, echocardiography was performed to assess cardiac function. Indices of liver injury (alanine and aspartate aminotransferases [ALT and AST]; cytochrome p450 2E1 [CYP2E1]; alcohol dehydrogenase [ADH]; Oil Red O and triglyceride content; lipid peroxidation; inflammatory cytokine expression; and macrophage infiltration), and lung inflammatory cell count, proinflammatory cytokine expression, and lipid peroxidation were measured. Left ventricular posterior wall thickness was significantly decreased, and systolic blood pressure was significantly elevated by CIEV compared with air controls. CIEV led to a significant increase in plasma ALT and triglycerides compared with room air controls. CIEV did not affect liver triglyceride content, lipid staining or peroxidation, but increased CYP2E1 and chemokine (C-C motif) ligand 2 (CCL2) protein expression, while decreasing ADH expression. CIEV significantly increased numbers of both polymorphonuclear neutrophils and lymphocytes in the bronchoalveolar lavage fluid, indicative of pulmonary inflammation. However, CIEV did not produce significant changes in lung mass, pulmonary lipid peroxidation, inflammatory cytokine expression, or edema.

References - Inhalation Toxicity

Scarino, A., A. Noel, et al. (2012). "Impact of emerging pollutants on pulmonary inflammation in asthmatic rats: ethanol vapors and agglomerated TiO2 nanoparticles." Inhalation Toxicol. 24(Copyright (C) 2014 American Chemical Society (ACS). All Rights Reserved.): 528-538.

Treon, JF, (1958) in F. A. Patty, Industrial Hygiene & Toxicology Vol. II,

Dumas-Campagna, J., Tardif, R., Charest-Tardif, G., & Haddad, S. (2014). Ethanol toxicokinetics resulting from inhalation exposure in human volunteers and toxicokinetic modeling. Inhalation toxicology, 26(2), 59-69.

Mouton et al. (2016) Alcohol Vapor Inhalation as a Model of Alcohol-Induced Organ Disease. Alcohol Clin Exp Res.40(8):1671-8.

Cardiac Toxicity

No data identified

References - Cardiac Toxicity

No data identified

Addictive Data

No data identified

References - Addictive Data

No data identified

Behavioral data

A recent study by Philpot and Kirstein [2004] revealed that adolescent animals are unique in their responses to ethanol since brain reinforcement pathways (e.g., the mesolimbic dopamine [DA] pathway) are undergoing developmental transition. Ethanol was administered to preadolescent, adolescent and young adult animals. The resulting data indicated an age-dependant difference in the homeostatic alterations of mesolimbic systems in response to repeated ethanol treatment, an effect that may manifest itself as differences in behavioural responsivity and conditionability to the drug and the drug's effects [Philpot and Kirstein, 2004].

In another study the neurobehavioral effects of alcohol or nicotine exposure were investigated in adolescent animal models. Rats were either exposed to ethanol for 10-14 days using an intermittent vapour inhalation paradigm, or to nicotine continuously for 5 days using Nicoderm CQ transdermal patches. Alcohol-induced changes included enhanced anxiety-like behaviour and enhanced depressive-like behaviour, whilst nicotine-induced changes included increased anxiety-like behaviour. The authors concluded that these findings supported the hypothesis that adolescents are uniquely susceptible to the effects of chronic alcohol and nicotine exposure [Slawecki et al., 2004].

Nicotine's counteraction of adverse effects of ethanol on cognitive function and motor coordination may play a major role in the observed high incidence of smoking among alcoholics. Previously, Tizabi et al., (2005) observed protective effects of nicotine against ethanol-induced neurotoxicity in cultured cortical and cerebellar granule cells as determined by lactate dehydrogenase assay. Tizabi et al., (2005) determined whether ethanol induced formation of caspase 3 (reflective of apoptosis) in these cells and whether these effects may be blocked by nicotine pretreatment. Primary cultures of cerebral cortical and cerebellar granule cells were prepared from the brains of 20 day old Sprague-Dawley foetuses. Exposure of cells to ethanol (10-100 mM) for 3 days resulted in a dose-dependent increase in caspase 3 activity and cytotoxicity. Pretreatment with nicotine (5-20 microM) dose dependently attenuated these effects of ethanol. Complete block of ethanol effects was achieved by the highest dose of nicotine (20 microM). The results suggested that at least some of the neurotoxic effects of ethanol may be mediated by apoptosis and that pretreatment with nicotine can prevent these effects of ethanol. Anti-apoptotic

effects of nicotine in this model may be suggestive of potential use of nicotinic agonists in neurotoxic insults and/or neurodegenerative disorders [Tizabi et al., 2005].

Quertemont et al., (2005) stated that acetaldehyde has long been suggested to be involved in a number of ethanol's pharmacological and behavioural effects, such as its reinforcing, aversive, sedative, amnesic and stimulant properties. However, the role of acetaldehyde in ethanol's effects has been an extremely controversial topic during the past two decades. Opinions ranged from those virtually denying any role for acetaldehyde in ethanol's effects to those who claimed that alcoholism is in fact "acetaldehydism". Considering the possible key role of acetaldehyde in alcohol addiction, it is critical to clarify the respective functions of acetaldehyde and ethanol molecules in the pharmacological and behavioural effects of alcohol consumption. Quertemont et al., (2005) reviewed the animal studies reporting evidence that acetaldehyde was involved in the pharmacological and behavioural effects of ethanol. A number of studies demonstrated that acetaldehyde administration induced a range of behavioural effects. Other pharmacological studies indicated that acetaldehyde might be critically involved in several effects of ethanol consumption, including its reinforcing consequences. However, conflicting evidence has also been published. Furthermore, it remains to be shown whether pharmacologically relevant concentrations of acetaldehyde are achieved in the brain after alcohol consumption in order to induce significant effects. Finally, the authors review current evidence about the central mechanisms of action of acetaldehyde [Quertemont et al., 2005].

Oshiro et al. (2014) evaluated the potential cognitive consequences of ethanol inhalation by exposing pregnant Long Evans rats to clean air or ethanol vapor from gestational day 9-20, a critical period of neuronal development. Concentrations of inhaled ethanol (5,000, 10,000, or 21,000 ppm for 6.5 h/day) produced modeled peak blood ethanol concentrations (BECs) in exposed dams of 2.3, 6.8, and 192 mg/dL, respectively. In offspring, no dose-related impairments were observed on spatial learning or working memory in the Morris water maze or in operant delayed match-to-position tests. Two measures showed significant effects in female offspring at all ethanol doses: 1) impaired cue learning after trace fear conditioning, and 2) an absence of bias for the correct quadrant after place training during a reference memory probe in the Morris water maze. In choice reaction time tests, male offspring (females were not tested) from the 5,000 and 10,000 ppm groups showed a transient increase in decision times. Also, male offspring from the 21,000 ppm group made more anticipatory responses during a preparatory hold period, suggesting a deficit in response inhibition. The increase in anticipatory responding during the choice reaction time test shows that inhaled ethanol yielding a peak BEC of ~ 200 mg/dL can produce lasting effects in the offspring. The lack of a dose-related decrement in the effects observed in females on cue learning and a reference memory probe may reflect confounding influences in the exposed offspring possibly related to maternal care or altered anxiety levels in females. The surprising lack of more pervasive cognitive deficits, as reported by others at BECs in the 200 mg/dL range, may reflect route-dependent differences in the kinetics of ethanol. Overall, these data suggest that ethanol inhalation selectively impairs cognitive function at relatively high concentrations. However, there is low likelihood of cognitive deficits in children of mothers who inhale ethanol vapors at the levels predicted at the gasoline pump (rarely exceeding 100 ppb).

References - Behavioral data

Philpot R, and Kirstein C (2004). Developmental differences in the accumbal dopaminergic response to repeated ethanol exposure. Ann N Y Acad Sci. 1021, 422-6.

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In Vivo - Other Relevant Studies

A case control study involved interviews with the next of kin or close friends of 120 black males who recently died of oesophageal cancer and compared to 250 similarly aged black males who died of other causes. This was undertaken to discover reasons for the exceptionally high mortality from this form of cancer in Washington, DC. The major factor found to be responsible for the high cancer rate, was alcoholic beverage consumption, with an estimated 81% of the oesophageal cancers attributed to the use; or high use of alcoholic beverages, which was also found among the controls. The relative risk increased with the amount of ethanol consumed and was highest among drinkers of hard liquor, although the risk was also elevated among consumers of wine and/or beer only [Pottern et al., 1981].

In a review by the IARC (1998), of fifteen cohort studies looking at the effects of ethanol and lung cancer risk in those humans with higher than normal alcohol consumption, smoking was taken in to consideration in only five of the cases. On examining data from five case control studies they found that there was no correlation between ethanol consumption and lung cancer [IARC, 1998].

Groups of 10 mice were fed for 5 weeks on a control diet with ethanol added to the drinking water at the following concentrations 0, 0.8, 4, and 20 %. Mortality increased with the increasing dose but there was little effect on the mean weight of survivors [College Pharmaceutical Society, 1962].

Nine groups of rats containing 5-25 animals were reported to have received 20 % alcohol in their drinking water and additional cystine with or without choline in their diet. The observation period extended from 8 to 24 weeks. No tumours were observed in any of the treatment groups [Wanscher, 1953].

Groups of 15 rats received 15 % alcohol in water for up to 14 weeks. No tumours were observed. Groups of male rats received 15 % alcohol in their drinking water. After 177 days there were no tumours reported [JECFA, 1970]. A total of 24 rats were given 15 % alcohol in their drinking water. After 120 days there were no tumours reported [Klatskin et al., 1951].

A total of 64 rabbits were given 20 % ethanol in water by stomach tube in quantities from 20-100 ml daily for 304 days. Thirteen of the 64 rabbits died of an unspecified infection, no tumours were observed in the animals that survived to the end of the study [JECFA, 1970].
Twenty-three dogs received 40 % aqueous solutions at a rate of 10 ml/kg body-weight daily for a total of 6 to 26 months without any signs of tumour development [JECFA, 1970].

Ethanol acts principally on the brain whether ingested or inhaled, first as an inhibitor of the higher functions and then as an anaesthetic. The lethal dose for man is reported to be 8-10 ml per kg body-weight or a blood level of 0.5 % or more [Haag et al., 1951]. Death occurs from severe and probably irreversible injury to CNS. The acute intoxication affects visual acuity, fields of vision, eye hand co-ordination and distance judgement [Haag et al., 1951].

At 100-199 mg ethanol per 100 ml/dl/ blood has been reported to produce the following symptoms: a staggering gait; marked impairment on mental tests; marked impairment of driving ability and lengthened reaction time. At 200 299 mg/100 ml/dl symptoms included nausea and vomiting; diplopia; marked ataxia and extreme clumsiness. At 300-399 mg/100 ml/dl hypothermia with cold, clammy skin; a loss of ability to speak; amnesia; anesthesia and heavy breathing are reported. Blood levels greater than 400 mg/100 ml/dl/ are reported to produce deep coma and possibly death. If the coma persists for 8 to 10 hr, shock and circulatory collapse may develop [International Encyclopaedia of Pharmacology and Therapeutics, 1970].

There is currently considerable controversy surrounding the possible activity of ethanol in the dominant lethal assay [Phillips et al., 2001]. In mice no dominant lethal effect was found when ethanol was administered as 20 or 30% of the calories of a liquid diet for four weeks [Randall et al., 1982].

When rats were given 5 % ethanol in the drinking water for 8 months at 4.3 g/kg/day, there was found to be no increase in DNA adducts in the liver, lung, heart or oesophagus as assayed using 32P-postlabelling technique [Izzotti et al., 1998]. DNA adducts induced in the heart and lungs by cigarette smoke were increased by ethanol consumption and were in increased in the oesophagus only after the combined administration of both cigarette smoke and ethanol consumption [Izzotti et al., 1998].

Groups of six adult male Sprague Dawley rats were administered saline or ethanol at 0, 0.5, 1.0 and 3 .0 mg/ml and in saline via the intraperitoneal [i.p.] route daily for 7 days and killed 24 hr after the last dose. Other groups of 6 rats were administered saline or ethanol (3 ml/kg i.p.) for 7 days. Carbon tetrachloride in corn oil [1.0 ml carbon tetrachloride/kg as a 50 % solution in corn oil, i.p.] was admin on day 8, and measurements of butanol oxidase were made 24 hr later. 2-Butanol was incubated with microsomal lung and liver preparations from rats, and methyl ethyl ketone production was measured by gas chromatography. The rate of production of methyl ethyl ketone was 6 to 8 times lower in lung than in liver. Administration of both the two low doses of ethanol did not alter activity in the liver but was found to be inhibitory in the lung. The high dose of ethanol at 3.0 ml/kg caused a 41 % inhibition in the liver and a 51 % inhibition in the lung. The effects of ethanol and carbon tetrachloride were not additive [Carlson, 1989].

Groups of 36 adult rats [18 males and 18 females] belonging to either UChA or UChB strains were exposed to either air [control group] or ethanol vapor [6 to 7 mg/l for 46 hours] in an inhalation chamber. Each group was further divided into 4 subgroups according sex and strain [9 rats per each subgroup]. Ethanol blood levels were measured during day 2 of exposure. At 6 hours after exposure, rats from both groups received 60 mM/kg ethanol i.p. The blood alcohol level data showed no significant difference by strain, but a marked difference according to sex. The mean blood alcohol level of females was 196 + or - 27 mg/dl and that of males was 114 + or - 22 mg/dl [$p < 0.05$] [Alvarado et al., 1989].

Ethanol is rapidly absorbed from the stomach and small intestine and colon. Many factors influence the rate of absorption of ethanol including the presence of food. Absorption from the small intestine is almost complete and extremely rapid and it is largely independent of the presence of food in the stomach or intestine. After absorption, ethanol is fairly well distributed throughout all the tissue fluids of the body. The placenta is freely permeable to ethanol and hence it rapidly enters the foetal circulation [Goodman et al., 1996].

Between 90-98 % of the ethanol that enters the body is completely oxidised, using zero order kinetics (the rate of oxidation being relatively constant and independent of blood concentrations). The amount of ethanol oxidised per unit time depends upon the bodyweight and liver weight. Ethanol is metabolised predominantly in the liver, principally by alcohol dehydrogenase, the product acetaldehyde is converted to acetyl CoA, which is then oxidised through the citric acid cycle or used in the synthesis of cholesterol, fatty acids and other tissue constituents. Ethanol can also be metabolised to acetaldehyde by the mixed function oxidase [MFO]. The extent of this process is thought to be small but increases with increasing consumption of alcohol. There is also known to be genetic variation/polymorphism in the activities of both alcohol dehydrogenase and aldehyde dehydrogenase both in different human races throughout the world and between males and females [Goodman et al., 1996; Baraona et al., 2001] with both enzymes requiring NAD as a cofactor [Westerfeld, 1961]. Alcohol dehydrogenase activity has been demonstrated in several other organs and tissues, including the stomach and intestine [Spencer et al., 1964; Mistilis et al., 1969], kidney [Buttner, 1965], lung [Moser et al., 1968], and brain [Sergy, 1997], but their contribution to the overall metabolism of ethanol is reported to be slight in comparison to that made by the liver [Bartlett et al., 1949].

Acetaldehyde dehydrogenase controls the further oxidation of acetaldehyde to acetic acid [Camps, 1968]. Using

14C labelled alcohol it has been shown that rats exhale approximately 75 % of the ethanol as CO2 within 5 hours and 90 % within 10 hours. Two percent of the ethanol was eliminated unchanged in the urine and expired air, whilst a further 0.5-2.0 % was conjugated and excreted in the urine as ethylglucuronide [Bartlett et al., 1949, Kamil et al.,. 1953]. In an adult, the average rate at which alcohol can be metabolised is reported to be approximately 30 ml in 3 hr with the maximal daily metabolism of alcohol being about 450 ml in man [Goodman et al., 1996].
Ethanol is present as an endogenous substance in the blood of man, being produced probably in the intestinal tract, at an average level of 1.5 mg/l. Resting human subjects developed blood concentrations of less than 100 mg/l when exposed to ethanol vapour concentrations of 7500-8500 ppm for 3 hours, while an exercising subject developed a blood level of 450 mg/l under the same conditions. A single oral administration of 0.5 ml/kg [35 ml for a 70 kg male] of pure ethanol given to four fasting men produced an average maximal blood concentration of about 400 mg/l at 2 hours after dose administration. A dose of 1.4 mg/l [98 ml/70 kg] produced a level of 1200 mg/l at 1 hour; and 2.0 ml/kg [140 ml/70 kg], a level of 2000 mg/l at one hour after dose administration. The levels declined at a mean rate for the 21 subjects of 189 mg/l/hr [Baselt, 1988].

The metabolic effects of ethanol are due to a direct action of ethanol or its metabolites. Ethanol causes hyperglycaemia or hypoglycaemia depending on whether glycogen stores in the body are adequate. Ethanol inhibits protein synthesis and results in a fatty liver with elevations in serum triglyceride levels. The increase seen in high density lipoprotein cholesterol following ethanol ingestion may help to explain the lower risk of myocardial infarction and death from coronary disease after moderate drinking. Increases in serum lactate, resulting from the increased NADH/NAD+ ratio, and hyperuricemia, are most likely to be the result of an increased turnover of adenine nucleotides, are common transient effects of ethanol ingestion [Goodman et al., 1996].

The causes of vitamin deficiencies associated with alcoholism include a decreased dietary intake of vegetables, decreased intestinal absorption, and alterations in vitamin metabolism. Ethanol decreases thiamine absorption and decreases the enterohepatic circulation of folate. Acetaldehyde increases the degradation of pyridoxal 5'-phosphate by displacing it from its binding protein and making it susceptible to hydrolysis by membrane bound alkaline phosphatase. Ethanol decreases hepatic vitamin A concentration and its conversion to active retinal, and modifies retinal metabolism of vitamin D [Mezy, 1985].

Acute alcoholic intoxication in man is probably not believed to be associated with any great change in hepatic function. Alcohol consumption increases the rate at which isolated liver slices synthesise fat. It also causes mobilisation of fat from peripheral tissue. Fat thus accumulates in the liver of normal individuals after the ingestion of relatively small amounts of alcohol. Alcohol inhibits the secretion of protein from hepatic cells, and its prolonged use also results in the accumulation of protein. The accumulation of fat and protein is benign at first, and the associated hepatic disorders are reversible on abstinence from ethanol. However, these processes can become irreversible and may proceed eventually to the characteristic cirrhosis of the liver seen in many alcoholics. Malnutrition and vitamin deficiencies in alcoholics may also contribute to the hepatic and gastrointestinal disorders in man, particularly if alcoholic liver disease is present [Goodman et al., 1996].

Ethanol produces a marked increase in serum cholesterol in the dog, while in man a small but significant increase occurs after large consumption rates. Hyperlipademic subjects show a greater rise [Grande et al., 1960].

Alcohol ingestion impairs glottic reflexes, and alcoholics are predisposed to pneumonia’s and lung abscesses from aspiration of oropharyngeal bacteria. Alcohol intoxication also increases the incidence of sleep apnoea and may result in respiratory failure due to over sedation of the CNS [Krumpe et al., 1984].

The interaction of ethanol with drugs and xenobiotics is complex because ethanol can affect any of the following steps; absorption, plasma protein binding, hepatic blood flow, distribution, hepatic uptake of drugs, and phase I and II hepatic metabolism. The principal effect of acute ethanol ingestion is upon drug metabolism and the inhibition of microsomal drug metabolism. The synergistic effects of ethanol on central nervous system

depressants can be explained by this mechanism. In contrast, chronic ethanol consumption increases mixed function oxidation and increases drug metabolism. The cross tolerance between ethanol and sedatives in chronic alcoholics has been reported to possibly be due to this effect of alcohol. In addition, increased production of hepatotoxic products from certain drugs and xenobiotics and an increased activation of procarcinogens to carcinogens can result from this microsomal induction. The increased susceptibility to hepatotoxins and the enhanced carcinogenesis in the alcoholic may be explained by this fact. Other effects of the interaction between drugs and alcohol are the result of changes in organ susceptibility, best demonstrated for the central nervous system. Subsequently, the presence of liver disease has a great effect upon drug metabolism capability of alcoholics [Seitz, 1985].

Ethanol consumption induces the CYP2E1 isoform of cytochrome P450, being inducible in human bronchial epithelial cells after a single administration of ethanol [Runge et al., 2001]. Cigarette smoke has been shown to significantly enhance CYP2E1 activity, as measured as the total clearance of chlorzoxazone in an intra individual study in humans [Zevin et al., 1999].

Wistar rats when given either an acute dose of ethanol 5 g/kg by oral gavage or as 5 % of a liquid diet for 1 week, there was found to be a significant increase in the specific etheno DNA adducts examined [ethenodeoxyadenosine and ethenodeoxycytidine]. Single gel electrophoresis was performed of liver hepatocytes of rats exposed to ethanol and N-nitrosodimethylamine [NDMA] or NDMA on its own. There was a small but significant increase in the number of DNA strand breaks in rats exposed to both ethanol and NDMA after 1 week compared to those treated with NDMA on its own, with a difference of 1.4 fold after 4 weeks [Navasumrit et al., 2001].

Although alcohol dehydrogenase is the major enzyme that metabolises alcohol [Goodman et al., 1996], the CYP2E1 has been proposed to account for only 20 % of the ethanol metabolism at physiologically relevant blood levels [Lieber 1994]. ADH activity is not induced by ethanol [Lieber 1994] this suggests that the induction of CYP2E1 is therefore responsible for the 2-fold increase in ethanol metabolism of heavy drinkers [Lieber, 1994, 1999]. The increase in activity of CYP2E1 has also been shown to be due to pathological conditions such as diabetes and obesity [Lieber, 1999].

CYP2E1 generates ethanol and oxygen derived free radicals that can initiate oxidative stress and lipid peroxidation and so cause cell injury and DNA strand breaks [Jarvelainen et al., 2000]. There is reported to be a synergistic effect between ethanol consumption, smoking and cancer formation being approximately 50 % higher than that for either activity alone [Lieber et al., 1997]. It is the induction of CYP2E1 by both tobacco smoke and ethanol consumption that is believed to be responsible for the effect. Ethanol induced CYP2E1 is almost exclusively located in the in the centrilobular region of each liver cell and may increase the toxicity of metabolites that found in this region of the liver cell [Lieber et al., 1999; Howard et al., 2001].

Wang et al., (2006), explored the effects of ethanol on cell death pathways in the pancreas. Adult male Wistar rats were fed with ethanol diets. In the pancreas from rats fed with ethanol, the protein expression and activity of caspase-8 decreased by 48 % and 45 %, respectively, and caspase-3 activity decreased by 39 %. In contrast, cathepsin B protein expression and activity increased with ethanol feeding by 189 % and 143 %, respectively. The effect of ethanol on these pathways was largely transcriptional. Wang et al., (2006) demonstrated effects of ethanol on the expression of several signals involved in cell death in the pancreas through alteration of transcriptional regulators. The decrease in caspase expression and increase in cathepsin B expression indicated that ethanol feeding may prevent apoptosis and promote necrosis of pancreatic tissue with stresses that cause pancreatitis [Wang et al., 2006].

It has been reported that although there is a dose response relationship between alcohol consumption and liver damage, less than one-third of alcoholics develop alcoholic liver disease [ALD]. This individual susceptibility to the development of alcoholic liver disease may be explained by genetic and environmental factors. Of the genetic factors, female sex is clearly a significant risk factor, HLA status is probably important factor but it is not clear at the present time. Of the environmental factors, no consistent evidence explains the correlation between hepatitis B

viral infection and the subsequent susceptibility to developing alcoholic liver disease [Johnson, 1985].

Rats exposed to 5 % ethanol via drinking water increased the mutagenicity of NMBA nitrosamine N-nitrosomethylbenzylamine a specific rat esophagus mutagen (no further information provided) [De Boer et al., 2004].

References - In Vivo - Other Relevant Studies

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In Vitro Data

In Vitro Carcinogenicity/Mutagenicity

Recently in a review of the mutagenicity of ethanol, it was concluded by the Committee on Mutagenicity [COM, 2000], that there was no evidence to suggest that ethanol had any mutagenic potential. With regards to acetyl aldehyde the committee concluded that 'acetaldehyde had a direct acting mutagenic potential in vitro but would only be expected to have the potential of in vivo activity at sites where it is not rapidly metabolised to acetic acid' [COM, 2000].

Using a pre-incubation assay Zeiger et al., (1992) found ethanol to be negative in the Ames assay in the following Salmonella typhimurium strains TA97, TA98, TA100, TA104 and TA1535 at concentrations up to 10 mg/plate both with or without metabolic activation. Similar results have been found by several other authors including De flora et al., (1984) and Hellmer et al., (1992).

Most of the reported chromosome aberration assays with ethanol have been negative [Phillips et al., 2001]. Ethanol administered at 0.8 mg/ml for 9 days to Hela cells failed to induce and chromosome aberrations or micronuclei [Obe et al., 1979].

Ethanol is sometimes used as a vehicle in chromosome aberration tests. Data from Safepharm Laboratories consistently demonstrated no effect in either human lymphocytes, Chinese hamster ovary or Chinese hamster lung cells at concentrations approximating to 8 mg/ml both with and without metabolic activation [Phillips et al., 2001].

In the mouse L5178Y lymphoma assay, ethanol was found to be negative at concentrations up to 35.9 mg/ml (780 mM) [Amacher et al., 1980]. Background data from Safepharm Laboratories, ethanol solvent controls at 8 mg/ml failed to show any mutagenic effect, both with or without metabolic activation [Phillips et al., 2001].

References - In Vitro Carcinogenicity/Mutagenicity

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In Vitro - Other Relevant Studies

Ethanol extract (EE) from cigarette smoke (CS) dose-dependently stimulated gastric cancer cell proliferation (no figures provided). Activation of ornithine decarboxylase (ODC) activity, COX-2, and c-myc expressions was observed. Antisense c-myc and a-difluoromethylornithine (DFMO, specific ODC inhibitor) inhibited cell proliferation without affecting COX-2 expression in response to CS extracts. A selective COX-2 inhibitor (SC-236) blocked proliferation, ODC activity and c-myc protein expression induced by CS in gastric cancer cells. SC-236 inhibition was reversed by exogenous prostaglandin (PG) E(2). It was concluded by the author that CSE-induced gastric carcinogenesis occurs via a COX-2/c-myc/ODC and PGE(2)-dependent pathway. Indicating that selective COX-2 inhibitors may act therapeutically to treat gastric cancer in smokers [Shin et al., 2004].

Ethanol-induced breast cancer cell proliferation was investigated using the MCF-7 breast cancer cell line. A 1.3-fold increase in MCF-7 cell proliferation was observed after 6 days exposure to 0.1% ethanol. This increase was also confirmed using a clonogenic assay which demonstrated a 1.5-fold increase in clonal growth in the presence of 0.1% ethanol. Higher concentrations of ethanol (0.3%) produced no significant increase. A non-significant 1.7-fold increase in ER-alpha mRNA was detected using RT-PCR after 6 days exposure accompanied by a significant 3.3-fold increase in ER-alpha protein content (analysed by western blotting) and a 2.4-fold increase in aromatase at both the mRNA and protein level [Etique et al., 2004].

Vignesh et al., (Vignesh, 2006) aimed to determine the dose response effects of ethanol on osteoblast-like human osteosarcoma cells (SaOS-2) proliferation, differentiation, mineralization and cyto-toxicity. At high doses, there was a significant reduction in cell number, whereas at lower doses there were variable effects. Ethanol treatment caused a dose- and time-dependent increase in LDH activity and altered the quality of mineralization at 10 mM dose whereas completely inhibited mineralization at 100 mM dose, despite the presence of serum.

References - In Vitro - Other Relevant Studies

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Emissions and Associated Toxicity Data

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 ethanol at levels up to 4802 ppm, "did not increase the overall toxicity of cigarette smoke" [Carmines, 2002].

In a recent mouse skin painting study [Gaworski et al., 1999], investigated the carcinogenicity of condensate prepared from cigarettes containing a number of additives in combination, including ethanol at 1111 ppm. The authors concluded that the study "did not indicate any substantive effect of these ingredients on the tumorigenicity of cigarette smoke condensate". It should be noted that the cigarettes contained a typical American blend humectant and sugar component (i.e. glycerine approx. 20,000 ppm, propylene glycol at approx. 24,000 ppm, and brown invert sugar at approx. 24,000 ppm) [Gaworski et al., 1999].

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 ethanol at 4802 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].

The addition of ethanol at 23,400 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 ethanol 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].

When tested at 1111 ppm in cigarettes, in a 13-week inhalation study, the presence of ethanol "…had no discernible effect on the character of extent of the biologic responses normally associated with inhalation of mainstream cigarette smoke in rats."[Gaworski et al., 1998]. [However, it should be noted that the cigarettes had been spiked with a number of flavour ingredients in combination prior to smoking, and they contained a typical American blend humectant and sugar component (i.e. glycerine 20,000 ppm, propylene glycol at 24,000 ppm, and brown invert sugar at 24,000 ppm)] [Gaworski et al., 1998].

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 Ethanol at levels up to 4802 ppm (a multiple of its typical use in a US cigarette) [Roemer et al., 2002].

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 ethanol at 23,400 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].

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".

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 ethanol at levels up to 13252 ppm.

Information relating to the pyrolysis and/or transfer of ethanol 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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