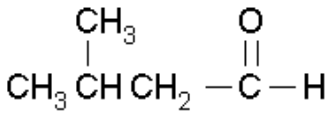
3-METHYL BUTYRALDEHYDE

SYNONYMS

3-Methyl butanal
Isoamyl aldehyde
Isopentaldehyde
Isopentanal
Isovaleraldehyde
Isovaleric aldehyde

CHEMICAL STRUCTURE



CHEMICAL FORMULA

$C_5H_{10}O$

IDENTIFIER DETAILS

CAS Number	:	590-86-3
CoE Number	:	94
FEMA	:	2692
EINECS Number	:	209-691-5
E Number	:	-

CLP CLASSIFICATION

Ingredient CLP Classification: Yes

Endpoint	Classification	Category
Acute Oral Toxicity	conclusive but not sufficient for classification	-
Acute Dermal Toxicity	conclusive but not sufficient for classification	-
Acute Inhalation Toxicity	conclusive but not sufficient for classification	-
Skin Corrosive/irritant	conclusive but not sufficient for classification	-
Eye Damage/Irritation	H319: Causes serious eye irritation	2
Respiratory Sensitisation	data lacking	-
Skin Sensitisation	H317: May cause an allergic skin reaction	1
Mutagenicity/Genotoxicity	conclusive but not sufficient for classification	-
Carcinogenicity	conclusive but not sufficient for classification	-
Reproductive Toxicity	data lacking	-
Specific Target Organ Toxicity	Single Exp. H335: May cause respiratory irritation	3
Aspiration Toxicity	conclusive but not sufficient for classification	-

REACH Statement

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/.

SPECIFICATIONS

Melting Point: -60°C [Chemfinder, 2002].

Boiling point: 90°C [Chemfinder, 2002].

PURPOSE

Flavouring substance.

STATUS IN FOOD AND DRUG LAWS

CoE limits:

Beverages (mg/kg)	Food (mg/kg)	Exceptions (mg/kg)
0.6	3	-

Acceptable Daily Intake:

ADI (mg/kg)	ADI Set by	Date Set	Comments
Acceptable	JECFA	1997	No safety concern at current levels

			of intake when used as a flavouring agent.

FDA Status: [CFR21]

Section Number	Comments
C172.515	Synthetic flavouring substances and adjuvants permitted for the direct addition to food for human consumption.

HUMAN EXPOSURE

Natural occurrence: Reported to be found in over 180 natural sources including apple, banana, berries, grape, ginger, spearmint, peppermint and other *Mentha* oils, clary sage, coffee and tea (Fenaroli, 2005).

Reported Uses: 3-Methyl butyraldehyde is reportedly used in baked goods at 28.99 ppm, frozen dairy at 11.61 ppm, meat products at 0.5 ppm, soft candy at 16.81 ppm, gelatin, pudding at 11.26 ppm, non-alcoholic beverages at 3.68 ppm, and hard candy at 2.5 ppm. Individual consumption has been reported as 0.002217mg/kg/day [Fenaroli, 2005].

Sources other than foods: Isovaleraldehyde is an intermediate in the production of alkyd resins for paints and coatings [Safronkina *et al.,* 1983].

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 3-methyl butyraldehyde at levels up to 57 ppm, “did not increase the overall toxicity of cigarette smoke” [Carmines, 2002]

Renne *et al.,* (2006) evaluated the effects of tobacco flavouring and casing ingredients on both mutagenicity, and a number of physiological parameters in Sprague-Dawley (SD) rats. Test cigarettes containing a mixture of either 165 low-uses or eight high-use flavouring ingredients which included 3-methyl butraldehyde at 0.13ppm, were compared to a typical commercial tobacco blend without flavouring ingredients. The Ames assay (TA 98, 100,102, 1535 and 1537 ± S9) did not show any increase in Mutagenicity from “low” or “high”

cigarette smoke condensate compared to the control. SD rats were exposed by nose-only inhalation for 1 h/day, 5 days/wk for 13 weeks to smoke at concentrations of 0.06, 0.2 or 0.8 mg/L from the test or reference cigarettes, or to air only. Plasma nicotine, COHb and respiratory parameters were measured periodically. Rats were necropsied after 13wk of exposure or following 13 wk of recovery from smoke exposure. Biological endpoints assessed included; clinical appearance, body weight, organ weights, and lesions (both gross and microscopic). The results of these studies did not indicate any consistent differences in toxicological effects between smoke from cigarettes containing the flavouring or casing ingredients and reference cigarettes [Renne, 2006].

In Vivo Toxicity Status

Acute Data:

Oral–Rat	LD_{50}	5600 mg/kg
Inhalation-Rat	LC_{50}	42700 mg/m^3/4H
Inhalation Rat	LC_{50}	91 mg/l (no exposure time)*
Inhalation Rat	LC_{50}	57 mg/l (4hours)*
Intraperitoneal-Rat	LDL_0	800 mg/kg
Oral–Mouse	LD_{50}	4750 mg/kg
Inhalation-Mouse	LC_{50}	50770 mg/m^3
Inhalation mouse	LC_{50}	6.2 mg/l (10 hour exposure)*
Intraperitoneal mouse	LD_{50}	237 mg/kg*
Subcutaneous-Mouse	LDL_0	2 g/kg
Skin-Rabbit	LD_{50}	3180 μL/kg
Oral-Guinea-pig	LD_{50}	2950 mg/kg
Skin-Guinea-pig	LD_{50}	>8 g/kg

[All Information obtained from RTECS, 2002 unless otherwise stated]. *ESIS [2004].

3-Methylbutanal metabolism is a normal component of human plasma and is elevated in human patients with hepatic encephalopathy. Methyl butanal is also a natural constituent of rat plasma, with the levels increased by 2-5 times in the adult rat by feeding 5% leucine in the diet. When injected at a dose of 120 mg/kg the electroencephalogram [EEG] was altered from a resting to a sleeping pattern, and sleep like behaviour occurred. Smaller doses such as 30 mg/kg lead to increased brain serotonin concentrations [Kubow *et al.,* 1981].

In humans, oral feeding with leucine lead to significant increases in the plasma levels of 3-methylbutanal concentrations in both control patients and those with cirrhosis, with peak leucine and 3-methylbutanal concentrations were occurring at approximately the same time. There was reported to be no changes in the clinical condition or psychometric performance of patients with cirrhosis fed leucine, with plasma 3-methylbutanal concentrations 700% over the basal levels [Marshall *et al.,* 1985]. The finding of no significant increase in the plasma levels of 3-methylbutanal in comma patients both with and without encephalopathy, did not support the role of 3-methylbutanal in the pathogenesis of hepatic encephalopathy [Mardini *et al.,* 1987].

The intravenous injection of 3-methylbutyraldehyde 3.2-24 mg/kg into the dog produced a rise in blood pressure, which was postulated to be possibly due to a vasoconstrictor action [Ford, 1988].

The intraperitoneal injection of 3-methyl butyraldehyde into normal and cirrhotic rats at doses of 4-120 mg/kg revealed that at 120 mg/kg the Electroencephalogram [EEG] was altered from a resting to a sleeping pattern within a period of 2-10 min. Drowsiness was observed at all dose levels, [Ford, 1988].

A study in which the relationship between elevated plasma levels of 3-methylbutyraldehyde and hepatic brain disease [encephalopathy] was investigated in rats, revealed that the single i.p injection of 30, 60 or 120 mg/kg bw increased plasma tryptophan / neutral amino acid ratios. Brain 5-hydroxyindole-acetic acid levels were also increased, but this was only observed in rats administered 30 mg/kg/bw [Ford, 1988].

Three mice, twenty guinea-pigs and five rabbits exposed to aerosols of 3-methyl butyraldehyde for up to 10 hours at a mean concentration of 6176 mg/m^3 resulted in 3/50 deaths in mice, 5/20 guinea-pigs deaths and no deaths in five rabbits, [Ford, 1988]. In rats exposed to a saturated vapour of 3 methyl butyraldehyde, the maximum time of exposure without deaths was 15 minutes, inhalation at 16000 ppm for 4 hours caused deaths in 5/6 rats [Ford 1988].

Dermal toxicity

Application of 500 mg 3-methylbutyraldehyde [24H] on rabbit skin [not stated if covered patch] was reported to be mildly irritating. Similarly 100mg [24 hour application] to adult rabbit eyes was considered a moderate irritant [RTECS, 2002]. However, application of 3-methylbutyraldehyde to the mucus membranes of rabbits eyes produced symptoms of blepharospasm, lacrimation, hyperaemia and oedema of the skin and adjacent tissues and purulent discharge [Ford, 1988].

The application of 5g/kg undiluted 3-methylbutyraldehyde on rabbit skin [24 hour occluded patch] produced irritation and scaly ulcerated skin at autopsy, [Ford, 1988]. Similarly application of 3-methylbutyraldehyde to the skin of mice and rats was reported to produce necrotisation of the rodent tail ends [Ford, 1988].

Ford, (1988), reported a study in which 29 volunteers involved in a 24 hour patch test [application to the back] with 3-methyl butyraldehyde at a concentration of 1 % produced no signs or irritation. Similarly a maximisation test carried out at the same concentration in 29 volunteers failed to produce sensitisation reactions [Ford, 1988].

Isovaleraldehyde (> 98.5%) was tested in rabbits for 4 hours under semi-occlusive conditions and evaluated with a primary irritation index of 2.83. The

material was found to be irritating to eyes according to Draize, scoring (4/10) in rabbits [OECD SIDS 2002].

Reproductive and Developmental toxicity

No data was available for isovaleraldehyde. Data were available for related substances propionaldehyde and isobutyraldehyde:

Propionaldehyde did not have specific adverse effects on the reproductive capabilities of either male or female rats and did not produce specific adverse effects on the developing offspring of laboratory animals. A NOAEL in a OECD/SIDS combined study of 1,500 ppm was found [OECD SIDS 2002].

A prenatal toxicity study with isobutyraldehyde in Wistar rats was carried out after inhalation exposures at 1,000, 2,500 and 4,000 ppm (3, 7.6 and 12 mg/l; 6 h/day; day 6 – 15 p.c., 25 pregnant animals per group). In the two higher exposure groups there were some signs of maternal toxicity, i.e. reduced body weights. No other findings were recorded during the course of this study. The fetal NOAEL was evaluated as 4,000 ppm; the NOAEL for maternal toxicity was 1,000 ppm [OECD SIDS 2002].

A prenatal toxicity study of a metabolic precursor of isovaleraldehyde, **3-**methylbutanol-1, was used as a surrogate as 3-methylbutanol-1 is biotransformed in an oxidative pathway to isovaleraldehyde and readily further oxidized to isovaleric acid. Though there are no quantitative kinetic data reported, the mechanism of the oxidative metabolism from alcohols to aldehydes was reported to be well known. Isovaleraldehyde and 3-methylbutanol-1 have the same common ultimate metabolite which is in herently assessed in the 3-methylbutanol-1 study. For the intermediary isovaleraldehyde no high bioavailability was assumed. 3- methylbutanol-1 caused no prenatal toxicity in rats and rabbits and no other form of unexpected systemic toxicity at inhalation exposure levels up to 10 mg/l. This implied that 3-methylbutanol-1 was devoid of selective fetal toxicity. Furthermore, isovaleric acid was also physiologically formed during the catabolism of leucine and occurs naturally in many edible plants [OECD SIDS 2002].

From the data of the structurally related propionaldehyde and isobutyraldehyde it was concluded that isovaleraldehyde was highly unlikely to cause selective fetal toxicity. The metabolic product of isovaleraldehyde, isovaleric acid, was also assumed to be devoid of prenatal toxicity. This conclusion was based on studies with 3- methylbutanol-1 in rats and rabbits. No additional investigations were considered necessary. The NOAEL > 1,500 ppm (propionaldehyde) in OECD/SIDS combined inhalation study and the fetal NOAEL as 4,000 ppm and maternal NOAEL as 1,000 ppm for isobutyraldehyde w2as considered to be sufficient [OECD SIDS 2002].

No data on the **e**ffects on fertility were available for isovaleraldehyde. For propionaldehyde a NOAEL of 1,500 ppm was established [OECD SIDS 2002].

Subchronic and chronic investigations with isobutyraldehyde did not indicate any evidence of testicular or ovarian toxicity. In one study epididymal weights were adversely affected at high dose levels, but this was suggested to have been a secondary, stress-related phenomenon. Furthermore, the studies available on n-butyraldehyde and repeated dose toxicity did not record toxic effects on the sex organs [OECD SIDS 2002].

Inhalation Toxicity

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. These ingredients included 3-methylbutyraldehyde at 57 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 3-methyl butyraldehyde at 47 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 3-methyl butyraldehyde 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 3 ppm in cigarettes, in a 13-week inhalation study, the presence of 3-methyl butyraldehyde "…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].

Steinhagen and Barrow (1984); reported a study in which the sensory irritation potential of aldehydes was investigated in B6C3F1 mice and Swiss-Webstar-mice. In this investigation sensory irritation was quantified by measuring respiratory rate depression before during and after exposure to five concentrations of 3-methyl butyraldehyde and a concentration effect curve constructed. The mean concentration giving a 50 % reduction in respiratory rate RD_{50} was found to be 757 ppm for B6C3F1 mice and 1008 ppm for Swiss-Webster mice. There was no statistical difference in values obtained from either mouse, with RD_{50} values ranging from 3.2-4167 ppm [Steinhagen and Barrow, 1984].

Male and female Sprague -Dawley rats were exposed 6 h/day, 5 d/week for 13 weeks to n butyraldehyde vapor at concentrations of 125, 500 and 2,000

ppm (approximating to 0.34, 1.36, 5.44 mg/l). Rats at all concentrations had a significant increased incidence of squamous metaplasia of mucosal epithelium, rhinitis and initial globlet cell atrophy followed by globlet cell hyperplasia. These alterations were more severe in rats sacrificed after 6 weeks of exposure than in those sacrificed after 13 weeks of exposure. The changes were considered indicative of a response to repeated upper respiratory tract irritation. In none of the other parameters investigated or organs any significant differences were found between test and control groups which could be related to inhalation of n-butyraldehyde vapor concentrations, i.e. no systemic toxicity was found. Thus, the NOAEL with respect to systemic toxicity was > 2,000 ppm, with respect to irritative effects < 125 ppm [OECD SIDS 2002]..

In a follow -up study with 15 male and female rats, dosed at 51.3, 10.3 and 1.1 ppm (151, 30, 3.2 mg/m³) was performed in order to find a NOAEL for effects on the upper respiratory tract. Histopathological findings indicated that no specific adverse effects could be attributed to n-butyraldehyde. A NOAEL of 51 ppm was therefore be derived from this study for irritation. In a similar 14 week study in dogs (dosed at 125, 500 and 2,000 ppm; n-butyraldehyde) besides analogous responses to the upper respiratory tract no systemic effects were found in any of the dose groups [OECD SIDS 2002].

Isobutyraldehyde (structurally closely related to isovaleradehyde) was administered by inhalation (whole-body exposure) to rats and mice up to 13 weeks or 2 years at concentrations of 0; 500; 1,000; 2,000; 4,000 or 8,000 ppm. At 2,000 ppm the rats showed lesions in the nose (olfactory epithelium degeneration), at 4,000 ppm in addition hyperplasia (epithelium), squamous metaplasia, osteodystrophy of turbinate bone and inflammation and severe necrosis at 8,000 ppm in respiratory tract. No other effects in organs/tissues were detected. Data on clinical chemistry/ hematology were not reported. In mice increased incidences of nonneoplastic lesions of the nasal mucosa and nasal turbinate bone were observed at 500 ppm (only minor effects) and at the higher concentrations. Other singular effects (organ weights of liver, kidney, thymus) were not regarded as substance-related due to a lack of dose response and the absence of a histopathological correlate. In the course of a prenatal study in rats 1,000 ppm did not show any adverse effects. Thus, a NOAEL of 1,000 ppm was derived for rats and a LOAEL of 500 ppm, with only weak effects seen in female mice [OECD SIDS 2002].

The Occupational short-term exposure limit [STEL] for Russia is 10 mg/m^3 [HSDB, 2002].

Roemer (2014) and Schramke (2014) reported on a testing program designed to evaluate the potential effects of 350 ingredients added to an experimental kretek cigarette on selected biological and chemical endpoints. The studies performed included a bacterial mutagenicity screen [Ames assay] a mammalian cell cytotoxicity assay [neutral red uptake], Mouse Lymphoma assay, determination of smoke chemical constituents, a 4-day in vivo micronucleus assay and a 90-day rat inhalation study. Based on the results of these studies, the authors concluded that the addition of ingredients

commonly used in the manufacture of kretek cigarettes, including 3-Methyl-butyraldehyde at levels up to 3 ppm, did not change the overall in vivo/vitro toxicity profile of the mainstream smoke.

Carcinogenicity and mutagenicity

Similarly, a recent mouse skin painting study investigated the carcinogenicity of condensate prepared from cigarettes containing a number of additives in combination, including 3-methyl butyraldehyde at 0.5 ppm. The authors concluded that the study "did not indicate any substantive effect of these ingredients on the tumorigenicity of cigarette smoke condensate" [Gaworski *et al.*, 1999]. [It should be noted that the cigarettes 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].

In a mouse micronucleus test after single intraperitoneal administration according to OECD protocol No. 474 isovaleraldehyde did not have any chromosome- damaging (clastogenic) effect, and there were no indications of any impairment of chromosome distribution in the course of mitosis. The test substance was administered once to male animals at dose levels of 25 mg/kg bw, 50 mg/kg bw and 100 mg/kg bw. The administration of the test substance led to evident signs of toxicity (piloerection, squatting posture, poor general state) [OECD SIDS 2002].

Behavioural data

No data identified.

Other relevant studies

In the 1990 (SIAM) it was decided that aldehydes were considered as a class in which one (or several closely related) chemical(s) may serve as a surrogate. In 1991 OECD decided that concerning isobutyraldehyde and isovaleraldehyde a developmental toxicity by inhalation route on isobutyraldehyde was sufficient.

Aldehydes have a consistent profile of the aldehyde function due to electronegativity, decreasing with increasing chain length. Protein binding is due to this electronegativity and the resulting cytotoxicty. Water solubility and Log Pow are of similar orders of magnitude and with regard to similar molecular size, the conclusion that toxicity data can be cross-read was supported, especially for butyraldehydes (n- and iso-) Propionaldehyde showed a NOAEL for systemic toxicity of 150 ppm in a combined OECD/SIDS study (No. 422); at 750 ppm, only decreased food consumption in females was seen (SIAR). For irritation (nasal damage) the LOAEL was set at 150 ppm [OECD SIDS 2002].

There are two subchronic gavage studies on n-butyraldehyde available which were peer-reviewed (NTP, 1987). The test material was administered to rats

and mice at dose levels of 75, 150, 300, 600 and 1,200 mg/kg bw/d over 13 weeks. Increased lethalities were observed at all dose groups (in rats 100% at 1,200 mg/kg bw/d), yet the deaths were regarded as gavage-related. Gastric ulceration was a frequent finding, and inflammatory lesions in the nasal cavity, both lesions resulted from the direct effect of the (irritating) compound on the mucosal epithelium of both tissues. An increased of alanine aminotransferase (male animals) and decreased alkaline phosphatise levels (female animals) were found at 600 mg/kg/day. An increased reticulocyte count, absolute reticulocytes and alkaline phosphatase levels were recorded only at 75 mg/kg bw/day dosage. In male mice; these findings, however, were regarded as incidental findings since at high doses no respective effect could be detected. With respect to systemic and local irritative effects, the NOAEL was 300 mg/kg bw/day for both male and female rats. In mice the NOAEL with respect to systemic effects was 600 mg/kg bw/d, taking into account also local irritative effects the NOAEL was 300 mg/kg bw/day [OECD SIDS 2002].

3-methylbutanol-1 is a metabolic precursor of isovaleraldehyde and is biotransformed via an oxidative pathway to isovaleraldehyde and readily further oxidized to isovaleric acid. This means that isovaleraldehyde and 3-methylbutanol-1 have the same common metabolite which was assessed in the 3-methylbutanol-1 study. For the intermediary isovaleraldehyde no high bioavailability was assumed. For an estimation of the systemic toxicity of metabolic sequel products of isovaleraldehyde, a 3-month drinking water study with the precursor 3 -methylbutanol-1 in rats was also available. Though there were no quantitative kinetic data in this case, the mechanism of the oxidative metabolism from alcohols to aldehydes was reported to be well known [OECD SIDS 2002]..

Aldehydes are reported to be readily oxidised to organic acids, which can then serve as substrates for fatty acid oxidation pathways and the Krebs cycle. The oxidation of aldehydes is catalysed by aldehyde dehydrogenase, which is reported to be found in the brain, liver and placenta, [HSDB, 2002]. Elevated levels of 3-methylbutyraldehyde were also identified in blood plasma and cerebrospinal fluids of patients with hepatic encephalopathy [Ford, 1988].

Isovaleraldehyde has also been shown to inhibit acetaldehyde oxidation in rat liver and has been shown to be the most potent inhibitor of oxidation of various mitochondrial substrates, [HSDB, 2002].

Several chemists involved in the distillation of isovaleraldehyde were reported to develop signs of nausea, vomiting and chest discomfort. These symptoms were reported to disappear within a few days, [HSDB, 2002]. Exposure to 2-methyl butyraldehyde has also been reported to produce bronchial constriction, choking coughing and a burning sensation on the skin of the face [HSDB, 2002].

Marshall, (1985) reported a study in which the plasma 3-methylbutanal concentration in man was investigated for possible correlation to hepatic encephalopathy. It was concluded in this study [involving 55 test patients and 23 controls] that ‘in man the 3-methylbutanal concentration is partly derived

from ingested leucine and is independent of the action of colonic bacteria, [3-methylbutanal was thought to primarily be obtained from the colonic breakdown of leucine]'. The need to further investigate the role of 3-methylbutanal in hepatic encephalopathy was highlighted [Marshall *et al*., 1985].

Isovaleraldehyde is soluble in water at 20,000 mg/l (20°C) and has a vapour pressure of 6100 Pa at 20°C. Based on these data a Henry-constant of 26.3 Pa.m3.mol-1 was calculated. Its measured log Pow of 1.31 indicated that there was no considerable potential for bio- or geoaccumulation. Based on these physico-chemical properties, the preferred environmental compartment is the atmosphere (Mackay I: 90.06 %) The compound does not tend to adsorb on to sediment/soil or accumulate in biota [OECD SIDS 2002]

In Vitro Toxicity Status

Carcinogenicity and mutagenicity

ASSAY TYPE	SPECIES CELL TYPE	RESULTS
Ames + / - MA [CCRIS, 2002]	*S.* t*yphimurium* TA 98, 100 & 102	Negative 0.00001-1μmol/plate
Ames + / - MA [Florin, 1980]	*S.typhimurium* TA98, 100, 1535 & 1537	Negative 3μmol/plate
Sister-chromatid Exchange [Gene-Tox, 2002]	Human [no further details]	Positive [No dose given]
Sister-chromatid Exchange [Tucker *et al*., 1993]	Human lymphocytes [no further details]	Positive [$2x10^{-3}$ Doubling of SCE frequency]

MA – metabolic activation.

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 3-methyl butyraldehyde at levels up to 57 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 3-methyl butyraldehyde at 47 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 3-methyl butyraldehyde at levels up to 70 ppm.

Roemer (2014) and Schramke (2014) reported on a testing program designed to evaluate the potential effects of 350 ingredients added to an experimental kretek cigarette on selected biological and chemical endpoints. The studies performed included a bacterial mutagenicity screen [Ames assay] a mammalian cell cytotoxicity assay [neutral red uptake], Mouse Lymphoma assay, determination of smoke chemical constituents, a 4-day in vivo micronucleus assay and a 90-day rat inhalation study. Based on the results of these studies, the authors concluded that the addition of ingredients commonly used in the manufacture of kretek cigarettes, including 3-Methyl-butyraldehyde at levels up to 3 ppm, did not change the overall in vivo/vitro toxicity profile of the mainstream smoke.

Other relevant studies

3-methyl butyraldehyde was reported to cause 13 % inhibition of cell growth in Ascites sarcoma BP8 cells at 0.1 mM. At a concentration of 1 mM, growth was inhibited by 100 % [Ford, 1988].

Oxidative metabolism, [noradrenalin-induced respiration] in hamster brown fat cells was reported to be inhibited by 45 % at a concentration of 1mM 3-methyl butyraldehyde [Ford, 1988].

Human lung fibroblast permeability was increased by 25 % after a 30-minute incubation with 3-methylbutyraldehyde. 5mM 3-methylbutyraldehyde did not

inhibit ciliary activity after a 60-minute incubation with chicken embryo tracheal organ cultures [Ford, 1988].

PYROLYSIS AND TRANSFER STUDIES

Information relating to the pyrolysis and/or transfer of 3-methyl butyraldehyde 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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