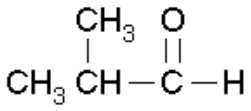
ISOBUTYRALDEHYDE

SYNONYMS

2-Methyl propanal
Isobutanal
Isobutyl aldehyde
Isobutyric aldehyde
2-Methylpropionaldehyde

CHEMICAL STRUCTURE



CHEMICAL FORMULA

C_4H_8O

IDENTIFIER DETAILS

CAS Number	:	78-84-2
CoE Number	:	92
FEMA	:	2220
EINECS Number	:	201-149-6
E Number	:	-

CLP CLASSIFICATION

Ingredient CLP Classification: No

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

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: -65°C

Boiling point: 63.5°C

PURPOSE

Flavouring substance.

STATUS IN FOOD AND DRUG LAWS

CoE limits:

Beverages (mg/kg)	Food (mg/kg)	Exceptions (mg/kg)
0.3	1	-

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.

HUMAN EXPOSURE

Natural Occurrence: Isobutyraldehyde is reported found in apple and currant aromas and in the essential oils from tobacco leaves and tea leaves; also in the essential oils of: *Pinus jeffreyi* Murr. Leaves, *Citrus aurantium* leaves and *Datura stramonium.* Also found naturally in food such as apples, carrots, currents, peas, potatoes, tomatoes, butter, cheese, eggs, yogurt, coffee, tea, beer and honey [Fenaroli, 2005].

Reported Uses: Isobutyraldehyde is reportedly used in baked goods at 5.74ppm, frozen dairy at 4.91ppm, meat products at 0.10ppm, soft candy at 5.8ppm, confection frosting at 4ppm, gelatin pudding at 11ppm, non-alcoholic beverages at 1.23ppm, and alcoholic beverages at 4.17ppm. Individual consumption reported as 0.001624 mg/kg/day [Fenaroli, 2005].

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 isobutyraldehyde at levels up to 2 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 isobutyraldehyde 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 1h/day, 5 days/wk for 13 weeks to smoke at concentrations of 0.06, 0.2 or 0.8mg/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.

In Vivo Toxicity Status

Route	Species	Test Type	Reported Dosage
Oral	Rat	LD_{50}	960 mg/kg[2]
Oral	Rat	LD_{50}	2810 mg/kg[2]
Oral	Rat	LD_{50}	960 mg/kg[1]
I.P.	Rat	LD_{50}	1600-3200 mg/kg[3]
Skin	Rabbit	LD_{50}	7130 mg /kg[1]
Inhalation	Mouse	LC_{50}	39500 mg/m^3/2H[1]
Inhalation	Rat	LCL_0	8000 ppm/4H[1]
Inhalation	Rat	TCL_0	500ppm / 6 hrs a day / 2 yrs, changes in sense organs[1].
Inhalation	Rat	TCL_0	4000ppm/6 hrs a day/13 wks, changes in the sense organs & decreased bw gain[1]
Inhalation	Mouse	TCL_0	1000 ppm /6 hrs a day / 2yrs,

			changes in the senses and decreased bw gain[1]
Inhalation	Mouse	TCL_0	1000 ppm /6 hrs a day /13 wks, changes in senses and decreased bw gain[1]
Inhalation	Rat	TCL_0	7500mg/m^3/6hrs a day / between days 6-15 of pregnancy, maternal effects[1].

[1] RTECS (2002), [2] HSDB (2002), [3] ECB (2008)

Isobutyraldehyde is reported to raise blood pressure on i.v injection in dog [4.8-41.7 mg/kg] [Legacy, 2002].

The i.p injection or direct application into the stomach of isobutyraldehyde was reported to increase the 'weight coefficient' of lungs, liver and kidney by 68, 36 and 52 % respectively [Legacy, 2002].

Carcinogenicity and Mutagenicity

In vivo isobutyraldehyde was reported to induce chromosomal aberrations in bone marrow cells of male mice, but no increases in micronuclei were reported after exposure up to the limits of toxicity [NTP, 1999].

Similarly, a recent mouse skin painting study investigated the carcinogenicity of condensate prepared from cigarettes containing a number of additives in combination, including isobutyraldehyde at 12 ppm. The authors concluded that the study "did not indicate any substantive effect of these ingredients on the tumourigenicity 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)].

Dermal Toxicity

The short-term exposure limit for skin [Russia] is reported to be 5mg/m^3 [RTECS, 2002].

Isobutyraldehyde was reported to be a severe eye irritant at 20 mg and 100 mg in rabbits [Legacy, 2002].

The application of undiluted isobutyraldehyde to the backs of two male rabbits for 20 hours with scoring carried out at 24,48 hours and eight days, isobutyraldehyde was described as corrosive [ECB, 2008].

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 isobutyraldehyde at 2 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 maximum allowable concentration which was defined as the average concentration during a working period of 8-hour shifts and a 42 hour week which does not show any adverse health effects in a worker or any of their descendents for isobutyraldehyde was reported to be 100 mg/ m^3 [Czerczak and Kupczewska, 2002].

The RD_{50} value, [the concentration required to elicit 50 % decrease in respiratory rate on exposure to isobutyraldehyde] in mice was 4167 ppm [Steinhagen, 1984].

Mice, guinea-pigs and rabbits exposed to high concentrations [mean concentration 6176 mg/m^3] of Isobutyraldehyde were reported to develop pulmonary oedema as a cause of death, [Legacy, 2002]. Similarly the chronic exposure, [4 months] of rats [no strain stated] to 50 mg/$m^3$for 4 hrs a day was observed to cause a decrease in haemoglobin content, a reduction in the number of leucocytes in the blood as well as an increase in cholinesterase activity accompanied with a decrease in gas exchange [Legacy, 2002].

When tested at 12 ppm in cigarettes, in a 13-week inhalation study, the presence of isobutyraldehyde "…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].

The addition of isobutyraldehyde at 12 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 isobutyraldehyde 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].

A study in which 4 male and 4 female rats were exposed [via inhalation] to 1000 ppm isobutyraldehyde [12 x 6 hours exposures] for a period of 3 wks. All 8 rats were reported to develop slight nose irritation and at autopsy all organs were reported to be normal [Legacy, 2002].

A 13 week study in which 20 rats F344/N, or B6C3F1 mice [10 of each sex] were exposed to concentrations of 0,500, 1000, 2000, 4000 or 8000 ppm (approximating to 1.5, 3,6,12 and 24 mg/l^{-1}) isobutyraldehyde [6 hours a day 5

days a week for 13 weeks] revealed that at a dose of 4000 and 8000 ppm a treatment related reduction in body weight decrease was seen with mortality, all rats exposed to 8000 ppm died before the end of the study. Laryngeal necrosis and inflammation of the trachea was reported. Rats exposed to 4000 ppm resulted in metaplasia of nasal respiratory epithelium and osteodysrophy of the nasal turbinate bone. With the mice at the two highest doses showing signs of necrosis of the epithelium lining of the nasal turbinate's at 13 weeks exposure. In male rats treated at 500 and 1000 ppm had significantly reduced spermatozoal activity, with females rats exposed to 4000 ppm differing significantly in the time spent in the oestrous stage compared to control rats [Abdo *et al*., 1998].

Female mice exposed to 4000 ppm and males and females exposed to 8000 ppm all died before the end of the study. The absolute and relative kidney weights of the males in the 1000 and 2000 ppm groups were significantly increased. There were no gross lesions that could be associated with isobutyraldehyde exposure. The nasal cavities and lymphopoietic tissues were considered to be the target organs with similar changes to those reported for the rats. The LOAEL was reported to be 1.5 mg/l^{-1} [Abdo *et al*., 1998].

In the 2 year study where rats and mice [50 per group] were exposed to up to 2000 ppm isobutyraldehyde [6 hours a day 5 days a week] reported no differences in survival rates between treated and control groups accompanied with no increase in neoplasm incidence related to isobutyraldehyde exposure. Non-neoplastic chemical related lesions were observed in the nasal regions of both mice and rats. These lesions included squamous metaplasia of the respiratory epithelium [rats], suppurative inflammation [rats], olfactory epithelial degeneration [rats and mice] at both 1000 and 2000 ppm. Therefore in conclusion a 105 week study in both male and female F344 rats and B6C3F1 mice exposed to up to 2000 ppm isobutyraldehyde no neoplasms, were reported that could be related to treatment [Abdo *et al*., 1998].

Fifteen men were exposed to 620 mg/m^3 (207ppm) of isobutyraldehyde for 30 minutes. No irritation was noted but there was some nausea and one man vomited [ECB, 2008].

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 isobutyraldehyde at levels up to 3 ppm, did not change the overall in vivo/vitro toxicity profile of the mainstream smoke.

Reproductive and Developmental toxicity

Morrissey *et al*., (1988) reported results from a NTP study which screened for potential reproductive toxicants. It was noted that in a 13 wk inhalation study where F344 mice were exposed to 500, 1000, and 2000 ppm no effects were observed on testis, epididymis, and cauda epididymis weights as well as sperm motility or count however, in a study in which 25 F344 rats were exposed to 500, 1000, 2000 and 4000 ppm a reduction in terminal body weight absolute cauda epididymis weights and relative and absolute epididymis weights was noted however, no effect was observed on sperm motility or count [Morrissey *et al*., 1988].

Groups of 25 pregnant Wistar rats were exposed to isobutyraldehyde at 1000, 2000 and 4000 ppm (3,7.6 and 12 mg/l-1), for 6 hours a day from days 6-15 post coitum. A drop fo bodyweight was seen in the 7.5 and 12 mg/l-1 groups in the first three days of treatment, with a statistically significant reduction in bodyweight gain was seen from day 9 at 12 mg/l-1. No substance related effects or on gestational parameters or on foetuses were observed. The NOAEL for teratogenicity was set at 12 mg/l-1 and for maternal toxicity at 3 mg/l-1 [ECB, 2008].

Behavioural data

No data identified.

Other Relevant Studies

The estimated intake of isobutylraldehyde in USA and Europe is 100 and 130 μg/person per day respectively and is reported to be of no safety concern based on current levels of intake [WHO, 1998].

It has been reported that branched-chain aliphatic acyclic alcohols, aldehydes and acids are rapidly absorbed from the gastrointestinal tract [WHO, 1998].

In human isoenzyme mixtures the oxidation of aldehydes to carboxylic acids is catalysed by NAD^+-dependent aldehyde dehydrogenase [ALD]. Isobutyraldehyde has been reported to be a good substrate for ALD and the ALD-catalysed oxidation of isobutyraldehyde has been reported to require glutathione, which the authors suggest implies the substrate for oxidation may be the thiohemiacetal formed by the rapid *in vivo* conjugation of aldehydes with glutathione [WHO, 1998].

The poisoning of animals with isobutyraldehyde has been reported to result in edema, hyperema of the eye mucosa and narcotising effects, along with histological changes in organs [no further details given [Legacy, 2002].

Isobutyraldehyde when tested in rat liver slices and heptoma was observed to depress protein synthesis in both preparations [HSDB, 2002].

Isobutyraldehyde has been detected in human urine and faeces [HSDB, 2002].

In vitro Toxicity Status

Carcinogenicity and Mutagenicity

ASSAY TYPE	SPECIES CELL TYPE	RESULTS
AMES Gradient plate	*S. typhimurium* G46, TA100	No dose stated Positive
AMES Preincubation (+/-S-9)	*S. typhimurium* TA98, 100 & 102	0.0011-110 μm/plate Negative
AMES Preincubation (-S9)	*S. typhimurium* TA100, 1535, 1537	10-10000 μg/plate Negative
AMES Preincubation Hamster & Rat S-9	*S. typhimurium* TA98, 100, 1535, 1537	33-10000 μg/plate Negative
AMES Preincubation Hamster / rat S-9	*S. typhimurium* TA100, 98, 1535, & 97	100-6666 μg/plate Negative
AMES Preincubation (-S9 rat)	*S. typhimurium* TA100 & 98.	100-10000 μg/plate Negative
AMES Preincubation Hamster/Rat S-9	*S. typhimurium* TA1535, 1537,102, 104 97,1537,102 & 104	33-3333 μg/plate Negative
AMES Preincubation	*S. typhimurium* TA98	3-10000 μg/plate Negative
AMES Preincubation	*S. typhimurium* TA102(-S9 (rat)) TA104 (+ S9 (rat))	10-1000 μg/plate Negative
AMES Preincubation	*S. typhimurium* TA97	10-1666 μg/plate Negative
AMES Preincubation (+rat S9)	*S. typhimurium* TA97	33-6666 μg/plate Negative
AMES Preincubation (+ hamster S-9)	*S. typhimurium* TA97	100-3333 μg/plate Negative

AMES Preincubation (+ hamster S-9)	*S. typhimurium* TA97	100-3333 μg/plate Negative
AMES (+ / - Rat/ Mouse S-9)	*S. typhimurium* TA102, 104,100, 100 &102	50-5000 μg/plate Negative
Mouse Lymphoma (-S9)	L5178Y (TK+/TK-) Suspension plate	62.5-1500 μg/ml Positive
Sex-linked recessive [injection] Lethal Mutations [WHO, 1998].	*Drosophila melanogaster* Canton-S wild type males	50,000ppm 80,000 ppm Negative

Data obtained from CCRIS 15/07/02 [Chemical Carcinogenisis Research Information System, 2002- CIS Record ID: CC-00001101] unless stated otherwise.

A mammalian *in vitro* assay in which evidence for genotoxicity was based on the ability of cells to increase the levels of p53 [tumour suppressing protein] in response to DNA damage revealed that isobutyraldehyde at doses of 1, 10, 20 50 and 100 μg/ml did not display any signs of cytotoxicity and did not induce p53 at any of these concentrations and therefore the result was classified as negative [Duerksen *et al*., 1999].

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, 100, 102, 1535 and 1537 (+/- S9). 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 isobutyraldehyde at levels up to 2 ppm [a multiple of its typical use in a US cigarette] [Roemer *et al.,* 2000].

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 isobutyraldehyde at 12 ppm was determined not to have affected the mutagenicity of the total particulate matter (TPM) of the smoke in either the Ames, *in vitro* micronucleus assay or the neutral red assay when compared with that of the control cigarettes [Baker *et al.,* 2004].

The 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 isobutyraldehyde at levels up to 70 ppm.

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".

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 isobutyraldehyde at levels up to 3 ppm, did not change the overall in vivo/vitro toxicity profile of the mainstream smoke.

Other Relevant Studies

Isobutyraldehyde is reported to be oxidised by rat liver mitochondria *in vitro* and found to compete with acetaldehyde for ALD [WHO, 1998].

Formaldehyde, acetaldehyde, propionaldehyde, butyraldehyde, isobutyraldehyde, and acrolein, all of which are constituents of tobacco smoke, were reacted in 5 mM concentration with the purified major fraction of normal adult human hemoglobin (hemoglobin Ao) in 1 mM concentration. A cigarette smoke condensate, diluted to contain 5 mM total aldehydes, was also reacted with 1 mM hemoglobin Ao. Cationic exchange high-performance liquid chromatography (HPLC) showed that the products formed from simple aliphatic aldehydes, with the exception of formaldehyde, were analogues of those formed from acetaldehyde, earlier shown by us to be imidazolidinone derivatives, that is, cyclic addition products of the N-terminal aminoamide function of alpha and beta chains. Formaldehyde and acrolein produced a heterogeneous mixture of derivatives including cross-linked hemoglobin dimers. The greater proportion of modified hemoglobins produced by condensate aldehydes resembled those formed from acetaldehyde, the most abundant aldehyde in the condensate. A smaller fraction consisted of cross-linked hemoglobin dimers, presumably due to the action of formaldehyde. Mass spectrometric and HPLC analyses of the 2,4-dinitrophenylhydrazones precipitated from the condensate documented the butyraldehyde,. The toxicity

of butyraldehyde is briefly discussed in the context of the findings of this study.

PYROLYSIS AND TRANSFER STUDIES

Information relating to the pyrolysis and/or transfer of isobutyraldehyde 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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