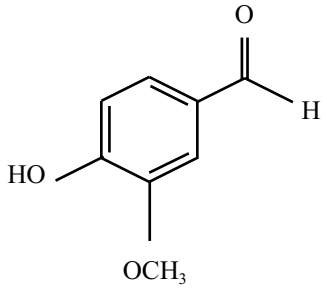
Vanillin

Document History
Published: 2009
Rebranded: 2025

I. IDENTIFICATION[1]

Chemical Name: Vanillin
Synonyms: benzaldehyde, 4-hydroxy-3-methoxy-; 4-formyl-2-methoxyphenol; lioxin; vanilla; vanillaldehyde; vanillic aldehyde
CAS Number: 121-33-5
Molecular Formula: C_8-H_8-O_3
Chemical Structure:



II. CHEMICAL AND PHYSICAL PROPERTIES[1, 2]

Physical State: white to yellow crystalline powder
Odor Description and Threshold: pleasant aromatic odor, threshold not available
Molecular Weight: 152.15
Conversion Factors: 1 ppm = 6.25 mg/m^3; 1 mg/m^3 = 0.16 ppm
Melting Point: 80°C (176°F)
Boiling Point: 285°C (545°F)
Vapor Pressure: 1.18 X 10-4 mm Hg at 25°C (77°F)
Saturated Vapor Concentration: 0.29 ppm @ 25°C (77°F)
Vapor Density: 5.2 (Air = 1)
Flammability Limits: LEL: not available UEL: not available
Flash Point: 153°C (307°F) closed cup
Autoignition Temperature: >400°C (>752°F)
Specific Gravity/Density: 1.056 g/mL at 20°C (68°F)
Solubility in Water: 10 g/L at 20°C (68°F)
Stability: Not available
Reactivity: Not available
Log K_{ow}: 1.37

III. USES[2]

Used as a flavoring agent, reagent in analytical chemistry, pharmaceutic aid (flavor), and in perfumery. Vanillin is included on the US FDA's food additive status list as 'Generally Recognized as Safe' (GRAS). The FAO/WHO Joint Expert Committee on Food Additives acceptable daily intake level for vanillin is 3 mg/kg/day.

IV. ANIMAL TOXICITY DATA

A. Acute Toxicity and Irritancy

1. *Oral Toxicity*

LD_{50} = 3925 mg/kg, rat[3]

LD_{50} = 4730 mg/kg, rat[4]

LD_{50} = 4333 mg/kg, mouse[4]

LD_{50} = 1400 mg/kg, guinea pig[5]

2. *Eye Irritation*

Vanillin as a powder caused irritation in the rabbit eye, but when applied as a saturated aqueous solution produced only lacrimation. The ocular irritation scores are not available.[4]

In a second rabbit eye irritation study, 55 mg of vanillin was applied as a finely ground powder to the eyes of six rabbits. A Draize irritation score of 18.8/110 (mild) was observed 24 hours after instillation of the test material. All eyes showed gradual improvement over the following 120 hours, with Draize scores of zero at 168 hours after application of the test material.[6]

3. *Skin Absorption*

A dermal LD_{50} of >2000 mg/kg was determined in a GLP study performed in accordance with OECD test guideline 402, in which the shaved skin of five rats/sex was exposed to a paste of 70% vanillin in purified

water for 24 hours under a semi-occlusive patch. No mortality or clinical signs, cutaneous lesions, or gross pathologic effects were observed.[7]

4. Skin Irritation

The dermal irritancy of vanillin was tested in six rabbits with 24 hours of exposure to 0.5 g of finely ground powder moistened with water. Under the conditions of this study no irritation was observed. The precise dermal irritation scores were not available.[6]

5. Skin Sensitization

Vanillin was tested in a Guinea Pig maximization test (GPMT) performed in accordance with OECD test guideline 406 under GLP conditions.[8] Ten albino Dunkin-Hartley guinea pigs of each sex were allocated to a control or a test group. Controls were exposed to vehicle during induction and to vanillin during challenge, while test animals were exposed to vanillin during both the induction and challenge phase. Induction exposure of test group animals consisted of two intradermal injections with either Freund's Complete Adjuvant (FCA) plus 35% vanillin in ethanol or FCA plus 17.5% vanillin in ethanol followed by topical application of 73% vanillin paste in absolute ethanol for 48 hours under an occlusive bandage. All animals were subjected to an 11-day rest period followed by topical challenge with 73% vanillin paste in absolute ethanol for 24 hours under an occlusive bandage. None of the animals in the test group responded to the challenge dose. Under the conditions of this study vanillin was considered negative for dermal sensitization.

Vanillin was also tested in a modified Buehler test similar to OECD 406.[9] Albino guinea pigs of both sexes 6–8 weeks old were used in the study. Ten guinea pigs were used for the vanillin group and a control group of 10 animals was treated simultaneously with pure petrolatum. For induction, about 0.l g of vanillin (10% in petrolatum) was applied to the shaved napes of the test animals and covered with an occlusive bandage. The test material was removed 24 hours after application. The procedure was repeated three times a week for 2 weeks. Two weeks after the final induction, pure vanillin was applied to the shaved left flank of the animals for the challenge tests. Pure petrolatum served as a control. The test material was removed 48 hours after application and readings were performed at 1, 24, and 48 hours thereafter. No dermal sensitization reactions were observed in any of the animals challenged with vanillin.

In another study experiments were performed with guinea pigs using the modified FCA (Freund's Complete Adjuvant) method.[10] Ten guinea pigs were used for the vanillin and control groups. The results obtained were presented as the mean response (mr), which is the quotient of the sum of all dermal reactions obtained, divided by the total number of animals treated. The challenge concentration was 10% vanillin in acetone. The mr reported for vanillin was 1.17, which the authors considered to be an indication of weak dermal sensitization activity.

Vanillin was also studied in a mouse ear swelling test (MEST).[11] In this study, groups of 10-15 female CF-1 mice were induced on four consecutive days with 100 µl of 50% vanillin (in 70% ethanol) by topical application of shaved and tape-stripped abdominal skin. A group of 5–10 control mice were exposed in an identical manner to 100 µl 70% ethanol. One week after the last induction dose, all test and control group mice were challenged with 20 µl of 50% vanillin (in 70% ethanol) topically applied to one ear, and the same volume of ethanol to the other ear. None of the mice showed signs of dermal sensitization under the conditions of this study, and vanillin was judged negative in the MEST assay.

6. Inhalation Toxicity

No mortality was observed in rats or mice following 4- or 2-hour exposures, respectively, to a saturated atmosphere at 20°C (0.29 ppm).[4]

B. Subacute Toxicity

No data found for vanillin.

C. Subchronic Toxicity

Rats (group size, sex/strain and controls unspecified) were exposed for 4 hours/day, 5 days/week, for 30 days to the saturated vapor concentration of vanillin at 20°C.[4] No animals died on test, but body weights were depressed and animals were lethargic following exposure. A drop in haemoglobin and white and red blood cells was reported, reduced pain sensitivity. Gross necropsy revealed hypermia of the lungs, spleen and liver. Histopathology indicated (unspecified) damage to the lungs, liver and kidney.

Ten rats/sex/group were fed diets containing 0.3, 1.0, or 5.0% vanillin (3000, 10,000, or 50,000 ppm) for 13 weeks.[1,12] No adverse effects were seen at the 0.3% dietary exposure level. Growth depression and enlargement of the liver, kidney and spleen were seen in the 5.0% exposure level while undefined mild changes were reported at the 1.0% dose level. The authors set the no observed adverse effect level (NOAEL) at 3000 ppm.

D. Chronic Toxicity/Carcinogenicity

In a 2-year feeding study, 12 rats/sex/dose were fed diets containing ethyl vanillin or vanillin at 5000, 10,000 or 20,000 ppm (250, 500, or 1000 mg/kg/day).[1,12,13] A control group comprised of 12 rats/sex was fed standard rodent diet. Both control and test diets also contained 3% polypropylene glycol as a binder. Commercially-available vanillin or ethyl vanillin was used in this study. The body weight, food intake, and general condition were recorded every week. Hematological examinations were made at 3, 6, 12, and 22 months. These examinations included white cell counts, red cell counts, hemoglobins, and hematocrits. At the end of the treatment regiment the tissues of all the rats were examined macroscopically and the liver, kidneys, spleen, heart, and testes were weighed. These organs, the remaining abdominal and thoracic viscera, and one hind leg, fore bone, bone marrow, and muscle, were histopathologically evaluated. Two years of dietary exposure had no effect on growth or hematology and produced no macroscopic or microscopic changes in the tissues. The authors set the NOAEL for this study at >20,000 ppm. (1000 mg/kg/day)

Vanillin was also studied in a Strain A mouse lung tumor bioassay.[1,14] For the bioassays, male and female Strain A mice, 6–8 weeks old, weighing an average of 18–22 g, were randomly distributed among experimental and control groups. Animals were housed in groups of four in plastic boxes. Groups of 15 Strain A mice/sex/dose were injected intraperitoneally (i.p.) 3 times/week, for a total of 8 weeks with vanillin. Mice were injected with the maximum tolerated dose (750 mg/kg) or 1/5 the MTD (150 mg/kg). The experimental group was compared to the untreated control, vehicle control (tricaprylin) and positive control (urethane) groups. The study was terminated 16 weeks after the last i.p. injection (24 week duration). The tumors on each lobe of the lung were counted and recorded while examining the lungs with a dissecting microscope. The lungs were also examined grossly and microscopically for the presence of other abnormalities (e.g., inflammatory reactions and adenomatosis). Liver, kidney, spleen, thymus, intestine, and salivary and endocrine glands were examined at necropsy for the presence of abnormalities. A chemical was considered to be a carcinogen in Strain A mice if it induced a significant increase in the average number of lung tumors per mouse when compared to that in the appropriate vehicle control. Under the conditions of this study vanillin was judged negative.

E. Developmental/Reproductive Toxicity

Although it has not been tested in traditional teratology or reproductive toxicity studies, vanillin was investigated *in vivo* in the mouse spot test using pregnant female C57BL mice.[1,15] Female mice were injected i.p. with 0, 12.5, 25 or 50 mg/kg ethylnitrosourea (ENU) on the 10th day of pregnancy and received three successive oral administrations of 500 mg/kg vanillin 0, 4, and 24 hours after the ENU injection. The number of pups born per female and the appearance of recessive color spots (RS) and white midventral spots (WMVS) was examined in pups aged about 30 days. Three successive oral administrations of vanillin at 500 mg/kg decreased by approximately 50% the number of mice with RS induced by ENU at 50 mg/kg. Vanillin did not affect the number of mice with WMVS and pups per female, indicating that oral exposure to 1500 mg/kg vanillin on Gestation Day 10 did not produce adverse effects on the fetal development or survival.

In another study with mice, the ability of vanillin to modify the teratogenic effects of a direct-acting monofunctional alkylating agent, N-methyl-N'-nitro-N-nitrosoguanidine (MNNG), was examined.[1,16] To determine the effect of vanillin on fetal development, mice were separately given a single i.p. dose of 50 mg/kg of vanillin on Day 11 of gestation. In another experiment, two groups of mice were given i.p. doses of 40 or 60 mg/kg of MNNG, respectively on Day 11 of gestation. A single 50 mg/kg i.p. dose of vanillin was administered one hour after MNNG treatment. On Gestation Day 18, the dams were humanely killed and the number of implants, resorptions, dead fetuses and live fetuses were counted. Live fetuses were weighed and examined for external malformations, and then cleared and stained for skeletal examination. Vanillin was comparable to the saline control with respect to the number of live fetuses, fetal body weight, fetal mortality and incidence of external and skeletal abnormalities. The incidence of syndactyly in the fore- and hind-limbs and cleft palate was significantly decreased when MNNG was co-administered with Vanillin, with a decrease in oligodactyly also being noted. In conclusion, the

incidence of MNNG-induced syndactyly in the fore- and hind-limbs was markedly decreased by administration of Vanillin, and decreases in other malformations, such as microcephaly, cleft palate and oligodactyly were also noted.

F. Genotoxicity/Mutagenicity

Vanillin was negative in several Ames tests performed with *Salmonella typhimurium* and *E. Coli* WP2, with or without metabolic activation.[1,17–19] Vanillin produced negative[18] and equivocal[17] results in *in vitro* chromosome aberration assays with Chinese Hamster Ovary (CHO) cells. Vanillin was also negative in a mitotic recombination assay with *Saccharomyces cerevisiae*.[20] The antimutagenic effect of vanillin was investigated in cultured Chinese hamster V79 cells *in vitro*.[15] The frequencies of 6-TG-resistant mutations induced by UV or X-rays were decreased by treatment with vanillin during the expression time. The decreases were not due to cytotoxic effects on cellular growth or killing effects on damaged cells.

The effects of vanillin on the induction of sister-chromatid exchanges (SCEs) and structural chromosome aberrations by mitomycin C (MMC) were investigated in cultured CHO cells.[21] Vanillin induced neither SCEs nor chromosome aberrations by itself. However, an obvious increase in the frequency of SCEs was observed when MMC-treated cells were cultured in the presence of vanillin. The effect of vanillin was S-phase-dependent. In contrast, the frequency of cells with chromosome aberrations was significantly decreased by post-treatment with vanillin at the G2 phase.

Vanillin was also studied in a mouse micronucleus assay alone and in combination with mitomycin C (MMC).[22] A single MMC dose dissolved in physiological saline was administered by i.p. injection to male BDF1 mice at 2 mg/kg in a volume of 5 ml/kg. Vanillin suspended in 2% Tween-80 solution was given to mice by gastric intubation at a volume of 10 ml/kg. Mice received MMC at 2 mg/kg and were post-treated by gavage with vanillin at 125-500 mg/kg. In order to determine the most sensitive period for vanillin treatment, vanillin was administered every 3 hours (3, 6, 9, 12, 15, 18, and 21 hours) after MMC injection. In all cases, except for the time-course study, the bone marrow cells were sampled 24 hours after the injection of MMC. In the time course study, vanillin at 500 mg/kg was given 7.5 or 9 hours after MMC injection, and the bone marrow cells were sampled at 12, 16, 20, 24, 30, 36, 48, and 72 hours after administration of MMC. The micronucleus assay was performed according to the method described by Schmid. Vanillin itself did not induce any micronucleated polychromatic erythrocytes (MN-PCEs). No reduction in the frequency of induced MN-PCEs was observed by simultaneous administration with vanillin and MMC, indicating that it is unlikely that vanillin or its metabolites inactivate MMC by chemical interaction. When vanillin was given to mice from 6 to 9 hours after the injection of MMC, a significant reduction in the frequencies of MN-PCEs by 38–50% was observed. The suppression by vanillin gradually decreased from the 12- to 18-hour interval and disappeared at the 21-hour interval. The effect of vanillin administration on the time-course of formation of MN-PCEs indicated that the suppressing effect was not due to a delay in the formation of MN-PCEs by the cytotoxic action of vanillin. The authors concluded that vanillin acts as an anticlastogenic factor *in vivo*.

G. Metabolism and Pharmacokinetics

Rats dosed orally with 100 mg/kg vanillin excreted most metabolites in the urine within 24 hours. Seven percent of the administered dose was unchanged, 47% was metabolized to vanillic acid, 19% to vanillyl alcohol, 10% to vanilloglycine, 8% to catechol, and 2% to 4-methylcatechol.[2]

In a human liver cytosol preparation, 1.3 micromoles vanillin produced 50% non-competitive inhibition of the sulphotransferase-mediated sulphation of the xenobiotic steroid 17 alpha-ethinyloestradiol (EE2).[23]

H. Other Toxicity Data

In an immunotoxicity screening assay with mouse peritoneal macrophages (PM) vanillin was tested for its ability to alter nonspecific phagocytosis and killing of *S. cerevisiae*.[24] Under the conditions of this assay, vanillin decreased the percentage of PM capable of ingesting yeast, but not below 50% of the control, and was classified as a weak modulator of PM function.

V. HUMAN USE AND EXPERIENCE

Human dermal exposure to vanillin was assessed in two closed patch tests.[9,25] In the more recent study[9], 30 occupationally exposed workers and 15 persons with no prior history of exposure were included in the study. All subjects were exposed on the back to 2% vanillin for 48 hours, with skin reactions read 1 hour after removal of the patch. No dermal irritation reactions were observed in any of the subjects. In the earlier study[25], no irritation reactions were observed when 29 normal subjects were patch tested for 48 hours on the small of the back with 20% vanillin in vaseline.

'Vanillism', commonly seen among workers handling vanilla pods, is characterized by skin reactions including papulous itching eruptions, swelling of the hands and face, and swelling of the eyelids, headache, gastric trouble, vertigo, and drowsiness.[1]

In a standard (24/48-hour covered) patch test of cosmetic ingredients, six of 585 subjects with skin complaints had local reactions to 5% vanillin; a concentration that produced no reactions in 94 healthy subjects.[1] Other investigations have reported cross sensitization between balsam of Peru and vanillin.[1]

VI. RATIONALE

Vanillin has a low level of acute toxicity via the oral and dermal routes. It is not a skin irritant, and produced only mild eye irritation in the rabbit. No reliable long term studies with vanillin are available via the inhalation route. The available data are not sufficient to support a DSEN notation.

Vanillin was negative in several Ames tests, with and without metabolic activation. It was also negative or equivocal in chromosomal aberration assays with CHO cells, and negative in a mitotic recombination assay with *Saccharomyces cerevisiae*. Vanillin did not induced SCEs in CHO cells and was negative in an in vivo mouse micronucleus assay suggesting that it is not clastogenic.

In the rat, two years of dietary exposure to vanillin at up 20,000 ppm (1000 mg/kg/day) had no effect on growth or hematology and produced no macroscopic or microscopic changes in the tissues or organs. Vanillin showed no evidence of carcinogenicity in a strain A mouse lung tumor bisoasay.

Although it has not been tested in traditional teratology or reproductive toxicity studies, oral exposure of pregnant C57BL mice to 1500 mg/kg vanillin on Gestation Day 10 did not produce adverse effects on fetal development or survival. In another mouse study, a single i.p. dose of 50 mg/kg vanillin on Gestation Day 11 had no effect on the number of live fetuses, fetal body weight, fetal mortality or the incidence of external and skeletal abnormalities.

The study of longest duration with vanillin established a dietary NOAEL of 20,000 ppm (1000 mg/kg/day) in the rat. An OEL of 10 mg/m^3, based on good industrial hygiene practice, is expected to provide an acceptable level of protection for all known health effects of vanillin. The application of appropriate uncertainty factors to the 1000 mg/kg/day NOAEL would have resulted in a higher OEL value.

VII. RECOMMENDED OEL

10 mg/m^3 8-hour time-weighted average.

VIII. REFERENCES

Databases consulted during this review include: IUCLID; TSCATS; RTECS

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