(b) (4)

Ethyl vanillin

Toxicity monograph

October 2016

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Toxicity monograph

INTRODUCTION

(b) (4) was asked to produce a toxicity monograph of ethyl vanillin (CAS RN[1] 121-32-4), focussing on the inhalation route of exposure. Data on the inhalation of tobacco smoke containing the ingredient (if available) have not been included in this monograph.

EXPERTISE

(b) (4) was founded[2] in 1961 to provide independent, high-quality research, information and advice on chemical toxicology to industry and governmental departments. Its risk assessors have been working together for many years (more than 40 years in some instances) and have a record of objectivity and scientific excellence. All senior and principal scientists in the current team are accredited and listed in the European (Eurotox) and UK Royal Society of Biology/British Toxicology Society Registers of Toxicologists and are thus bound by their specific codes of conduct.

TOXICITY DATA SEARCH CRITERIA

Searches of the (b) (4) database (see Appendix for details) identified the EFSA (2012) review, on which this monograph is based on. JECFA (2002) was also consulted for additional information on the studies cited in EFSA (2012). Due to time constraints, limited additional searches were conducted in an attempt to identify critical data not included in the EFSA (2012) review. A subsequent search of the primary literature was restricted to (b) (4) and Toxline (the toxicity subset of Medline, via TOXNET) in an attempt to identify more recent data since the 2012 review. The remainder of the TOXNET system (which includes HSDB, GENETOX, DART, CCRIS, IRIS, ITER and CPDB) was also consulted. Since the key review focussed on the use of ethyl vanillin in food and, as such, could not necessarily be relied upon to identify all critical local and systemic inhalation data, no date restriction was placed on searches in PubMed tailored to identify such information (and also cardiopulmonary data).

All searches were conducted in October 2016 using the CAS RN(s) and (in PubMed only) name identified below, as appropriate.

The data summarised in this report refers to the unheated form unless otherwise stated.

[1] Chemical Abstracts Service Registry Number.

[2] (b) (4)

IDENTIFICATION, REACH STATUS AND EU CLASSIFICATION

Identifier / status	
Name	Ethyl vanillin
Synonym(s)	3-Ethoxy-4-hydroxybenzaldehyde
CAS RN	121-32-4
REACH registration number[3]	01-2119958961-24-xxxx
Classification, according to EU CLP (EC 1272/2008)	**Harmonised classification:** None available

TOXICOLOGY

LOCAL EFFECTS

Respiratory tract irritation

No substance-specific data were identified.

Skin irritation

Human

Mild skin irritation was seen in 25 subjects following a 48-hour covered application of 2% ethyl vanillin in petrolatum (Kligman, 1970). [See also Skin sensitisation section.]

No skin irritation was observed in a 48-hour closed patch test in which 100 consecutive dermatitis patients were tested with ethyl vanillin at 5% (Frosch *et al*., 1995). [See also Skin sensitisation section.]

Non-human

No skin irritation was observed when undiluted ethyl vanillin was applied to the skin of rabbits for 24 hours (Monsanto, 1991).

Eye irritation

Human

No substance-specific data were identified.

Non-human

Ethyl vanillin was considered to be non-irritating when instilled undiluted (100 mg of substance, finely ground) in one eye of each of six rabbits (Monsanto, 1991).

Other local effects

No substance-specific data were identified.

[3] REACH registration numbers are substance and company specific. Therefore, the substance-specific part of the registration number is included here, from data disseminated on the ECHA 'registered substance' website. The REACH dossier has not been consulted for toxicity data.

SENSITISATION AND INTOLERANCE

Respiratory tract sensitisation

No substance-specific data were identified.

Skin sensitisation

Expert-group opinion

Ethyl vanillin has been categorised as a "likely contact allergen" by the SCCS based on limited human evidence and Structure Activity Relationship (SAR) considerations (SCCS, 2012).

Human

In a maximisation test[4], no skin sensitisation reactions were produced when 25 subjects were tested with ethyl vanillin at 2% in petrolatum (Kligman, 1970). [See also Skin irritation section.]

No skin sensitisation reactions were observed in a 48-hour closed patch test in which 100 consecutive dermatitis patients were tested with ethyl vanillin at 5% (Frosch *et al.*, 1995). [See also Skin irritation section.]

Non-human

In a high quality local lymph node assay (LLNA), groups of 4 female CBA/Ca mice were topically treated on each ear with 25 µl ethyl vanillin at concentrations of 0, 2.5, 5, 10, 25 or 50% for three consecutive days. An EC3[5] value of >50% was calculated, indicating that ethyl vanillin was a weak sensitiser (Basketter *et al.*, 2001).

Oral allergy/intolerance

No substance-specific data were identified.

INHALATION TOXICITY STUDIES

No substance-specific data were identified.

TOXICITY STUDIES – OTHER EXPOSURE ROUTES

Single dose toxicity

Human

No substance-specific data were identified.

Non-human

Oral LD_{50} values[6] of >2000-4470 mg/kg bw were reported for rats and 2000 mg/kg bw for rabbits (cited in EFSA, 2012).

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[4] The test procedure typically involves an initial induction phase of five 48-hour covered patch tests, followed 10-14 days later by a 48-hour covered challenge patch.

[5] OECD test guideline 429 defines stimulation index (SI) as the ratio of lymphocyte proliferation in a treated group to that in the vehicle control group. An SI of ≥3 is considered positive and the estimated concentration three (EC3) is the estimated concentration of a test substance needed to produce an SI of 3. ECETOC definitions of potency are: extreme sensitiser (EC3 value <0.1); strong sensitiser (EC3 >0.1 - ≤1); moderate sensitiser (EC3 value >1 - <10; and weak sensitiser (EC3 value >10).

[6] Lethal Dose 50, i.e. the dose that is lethal to 50% of the exposed group.

Repeated dose toxicity

Human

No substance-specific data were identified.

Non-human

Groups of Sprague-Dawley rats were given ethyl vanillin in the diet for 13 weeks at 0, 500, 1000 or 2000 mg/kg bw/day. Endpoints assessed included food consumption, body weight, eye examination, haematology, clinical chemical examinations, necropsy and complete histological examination. A NOAEL[7] of 500 mg/kg bw/day was established based on enlarged cervical lymph nodes in males at 1000 mg/kg bw/day and in both sexes at 2000 mg/kg bw/day. At the top two doses relative liver weights and the incidence of hepatic peribiliary inflammatory changes [around the bile duct] were increased. Minor bile-duct hyperplasia was also observed in a few males at 1000 mg/kg bw/day and above (Hooks *et al*., 1992). JECFA selected this study as the basis for setting its ADI[8] (JECFA, 2002). [See also Cardiopulmonary effects section.]

Dietary administration of ethyl vanillin (dissolved in corn oil) to groups of five male rats for 1 year, providing 1000 or 2500 mg/kg bw/day, was reported to be without adverse effect on body weight, food intake, general condition or haematological endpoints compared with vehicle-fed controls. The NOAEL was therefore 2500 mg/kg bw/day. Similarly a NOAEL of 1000 mg/kg bw/day (the highest dose tested) was established when rats (12/sex/group) were fed ethyl vanillin in the diet for 2 years (Hagan *et al*., 1967). Several other repeated dose studies were cited by EFSA, albeit rather old and often rather limited by modern standards (e.g. no control group) (EFSA, 2012).

GENOTOXICITY

Expert-group opinions

In its evaluation of benzyl alcohols, benzaldehydes, a related acetal, benzoic acids and related esters, in which ethyl vanillin was included as a supporting substance, the EFSA CEF Panel[9] concluded that there was no safety concern with respect to genotoxicity for the substances in the group (EFSA, 2012).

Mammals (*in vivo*)

Ethyl vanillin did not induce micronuclei when tested in two micronucleus assays. In the first test, male BDF_1 mice received an intraperitoneal injection [dose unspecified] and in the second NMRI mice received a dose of 1000 mg/kg bw [route unspecified] (cited in EFSA, 2012).

[7] No-observed-adverse-effect level.
[8] Acceptable Daily Intake.
[9] EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids.

Mammalian cells (*in vitro*)

In a chromosome aberration assay, ethyl vanillin induced polyploidy, but not chromosome aberrations, in Chinese hamster lung cells when tested at 250 µg/mL without S9[10] (Ishidate *et al*., 1984).

In a mouse lymphoma assay L5178Y cells were exposed to ethyl vanillin at 125-800 µg/mL. No mutations were detected when tested without S9, however in the presence of S9 a 2.1-3-fold increase in mutant frequency was reported (Heck *et al*., 1989).

Ethyl vanillin did not induce sister chromatid exchanges in human lymphocytes when tested without S9 at up to 332 µg/mL, nor in Chinese hamster ovary K1 cells at 17 µg/mL [metabolic activation not specified] (cited in EFSA, 2012].

An *in vitro* unscheduled DNA synthesis assay in rat hepatocytes was negative for DNA damage (Heck *et al*., 1989).

Micro-organisms

Five bacterial reverse mutation (Ames) assays were cited by EFSA, all showing an absence of mutagenicity for ethyl vanillin (EFSA, 2012). One of the most reliable of these involved the treatment of *Salmonella typhimurium* strains TA97, TA98, TA100, TA1535 and TA1538 at up to 8 mg/plate with and without S9 (Mortelmans *et al*., 1986).

A rec assay for DNA damage[11] in *Bacillus subtilis* [strains M45 and H17; metabolic activation not specified] conducted with ethyl vanillin at 0.021 mg/disc did not show any genotoxic effects (Oda *et al*., 1978).

Other

No mutagenicity was observed in *Drosophila melanogaster* in a sex-linked recessive lethal assay with ethyl vanillin (Wild *et al*., 1983).

CARCINOGENICITY

No substance-specific data were identified.

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

Human

No substance-specific data were identified.

Non-human

No standard substance-specific fertility data were identified.

[10] Induced mammalian liver post-mitochondrial fraction used for metabolic activation.

[11] An indicative test, based on DNA repair.

No developmental toxicity was seen in a rat study in which females were orally dosed with ethyl vanillin at 0, 200, 1000 or 2000 mg/kg bw/day by gavage from 7 days prior to cohabitation and throughout the 7-day mating period, gestation and delivery, until 4 days post-parturition (Vollmuth *et al.*, 1990).

CARDIOPULMONARY EFFECTS[12]

No treatment-related effects on the heart or lungs were reported following the complete histological examination of rats fed ethyl vanillin at up to 2000 mg/kg bw/day for 13 weeks (Hooks *et al.*, 1992) [See also Repeated dose toxicity section.]

No gross effects were seen in an unspecified range of organs [presumably including the heart and/or lungs] from rats fed ethyl vanillin in the diet for 1 and 2 years at up to 2500 mg/kg bw/day and 1000 mg/kg bw/day respectively (Hagan *et al.*, 1967). [See also Repeated dose toxicity section.]

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(b) (4) includes information from peer-reviewed toxicology and nutrition journals as well as secondary sources and websites. In addition to primary literature on the health effects of chemicals, (b) (4) covers official publications and evaluations issued by authoritative groups including:

- WHO/IPCS reports and evaluations (including CICADs and EHCs, and IARC, JECFA and JMPR monographs), and the WHO Air Quality and Drinking-Water Quality Guidelines
- OECD SIDS dossiers/SIARS
- IUCLID data sets
- EU Risk Assessment Reports
- EU expert committee opinions (including EU scientific committees, and EFSA scientific panels) and other reports from EU agencies and institutes etc (including ECHA, ECVAM, EMA and CPS&Q)
- ECETOC, HERA, Council of Europe and other pan-European programmes
- UK government agency (including Defra, EA, FSA, DoH, HSE, HPA, PSD and VMD) and advisory committee (e.g. COT, COM, COC, ACNFP, SACN, ACP, ACAF, VPC, VRC and ACRE) reports and evaluations
- Opinions from other UK organisations such as the Royal Society
- US agency reports and evaluations (EPA, ATSDR, FDA, NTP, OSHA, NCEA, CFSAN, CERHR, NIEHS, CDC, OEHHA and ACGIH)
- Health Canada evaluations
- BUA, DFG, BG Chemie and BfR reports and monographs
- Gezondheidsraad opinions, including those from its various committees such as DECOS
- RIVM reports
- Danish EPA reviews
- Reports and other information provided by Swedish governmental organisations, including the National Food Administration and the Swedish Chemicals Agency
- Nordic Expert Group for Criteria Documentation of Health Risks from Chemicals
- Australian agency reviews including NICNAS Priority Existing Chemical Assessments, APMVA reports and (jointly with New Zealand) FSANZ assessments
- Japanese Chemical Industry Ecology-Toxicology & Information Center reports
- CIR, RIFM and other specialist industry groups
- (b) **Toxicity Profiles**