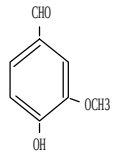
FOREWORD INTRODUCTION

VANILIN

CAS N°: 121-33-5

SIDS INITIAL ASSESSMENT PROFILE

CAS No.	121-33-5
CHEMICAL NAME	Vanillin
STRUCTURAL FORMULA	

CONCLUSIONS

Only minor quantities of vanillin are released from the vanillin production sites. Some occupational exposure to vanillin by inhalation of dust has been identified. The consumers will be directly exposed to vanillin as most of the produced material is ingested as flavour additive to food and beverages.

Environment:

Vanillin occurs widely in plants in the nature, usually as a glycoside bound to sugar or as a percusor to vanillin bound to the large lignin molecule abundant in wood. Free vanillin in the environment will be distributed to the aqueous compartment, and there is no tendency to bioaccumulation. The emission of vanillin from the vanillin production and from consumer products to the environment, is not considered to represent any biohazard.

Human Health:

During the present reviewing of the available toxicity data for vanillin, no particular risk has been identified which should give reason to concern or additional toxicity testing in animals.

The use of vanillin as a food additive is approved by authorities world wide, and FDA has granted GRAS status to its use. This is in agreement with the experience with vanillin in consumer products during many years without any confirmed report of adverse events.

RECOMMENDATIONS

It is recommended to make efforts to obtain more data on the occupational exposure through monitoring of inhalation of vanillin dust in contaminated working areas. Such monitoring data should also include particles larger than those normally considered respirable.

SIDS SUMMARY

DATE: 20.08.96

CAS NO: 121-33-5		Information available	OECD Study	GLP	Other Study	Estimation Methods	Acceptable	SIDS Testing Required
STUDY		**Y/N**	**Y/N**	**Y/N**	**Y/N**	**Y/N**	**Y/N**	**Y/N**
PHYSICAL-CHEMICAL								
2.1	Melting Point	Y	N	N/?	Y	N	Y	N
2.2	Boiling Point	Y	N	N/?	Y	N	Y	N
2.3	Density	Y	N	N/?	Y	N	Y	N
2.4	Vapour Pressure	Y	N	?	Y	N	Y	N
2.5	Partition Coefficient	Y	N	N/?	Y	Y/N	Y	N
2.6	Water Solubility - pH and pKa values	Y	N	N/?	Y	Y/N	Y	N
2.12	Oxidation: Reduction Potential	n.a.						
OTHER P/C STUDIES RECEIVED		Flash point, Autoflammability, Flammability, Explosive properties, Oxidizing properties, Additional data						
ENVIRONMENTAL FATE and PATHWAY								
3.1.1	Photodegradation	Y	N	N	Y	Y	Y	N
3.1.2	Stability in water	Y	Y	Y	N	N	Y	N
3.2	Monitoring data	Y	N	n.a.	Y	N	Y	N
3.3	Transport and Distribution	Y	Y	n.a.	N	Y	Y	N
3.5	Biodegradation	Y	N	N/?	Y	N	Y	N
OTHER ENV. FATE STUDIES RECEIVED		Stability in soil, Additional remarks						
ECOTOXICOLOGY								
4.1	Acute toxicity to Fish	Y	N	N	Y	N	Y	N
4.2	Acute toxicity to Daphnia	Y	N	N	Y	N	Y	N
4.3	Toxicity to Algae	Y	N	N	Y	N	Y	N
4.5.2	Chronic toxicity to Daphnia	Y	Y	Y	N	N	Y	N
4.6.1	Toxicity to Soil dwelling organisms	Y	N	?	Y	N	Y	N
4.6.2	Toxicity to Terrestrial plants	Y	N	N/?	Y	N	Y	N
4.6.3	Toxicity to Birds	N					Y	N
OTHER ECOTOX. STUDIES RECEIVED		Toxicity to bacteria						
TOXICOLOGY								
5.1.1	Acute Oral	Y	Y	Y/N/?	Y	N	Y	N
5.1.2	Acute Inhalation	N	N	N	N	N	Y	N
5.1.3	Acute Dermal	Y	Y	Y/N	Y	N	Y	N
5.4	Repeated Dose	Y	N	N/?	Y	N	Y	N
5.5	Genetic Toxicity in vitro							
	• Gene mutation	Y	Y	Y/N/?	Y	N	Y	N
	• Chromosomal aberration	Y	N	?	Y	N	Y	N
5.6	Genetic Toxicity in vivo	Y	Y	N/?	Y	N	Y	N
5.8	Reproductive Toxicity	N					Y	N
5.9	Development/Teratogenicity	Y	N	N/?	Y	N	Y	N
5.11	Human experience	Y	N	N	Y	N	Y	N
OTHER TOXICOLOGY STUDIES RECEIVED		Acute tox. - other routes of administration, Skin irritation/ corrosion, Eye irritation/corrosion, Skin sensitisation, Carcinogenicity, Immunotoxicity, Cytotoxicity, Metabolism						

SIDS INITIAL ASSESSMENT REPORT

1. IDENTITY

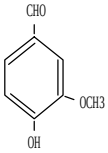
Name (OECD): Vanillin

CAS number: 121-33-5

Molecular formula: $C_8H_8O_3$

Molecular Weight: 152,14

Structural formula:



Other names: 4-hydroxy-3-methoxybenzaldehyde
para-vanillin
vanillic aldehyde
4-hydroxy-m-anisaldehyde
methylprotocatechuic aldehyde
3-methoxy-4-hydroxybenzaldehyde
hydroxy-4-methoxy-3-benzaldehyde

Vanillin is a white crystalline material melting at about 81 ^{o}C. The purity is generally above 99.0% w/w on dried basis. In vanillin obtained from guaiacol, ethyl vanillin can be an impurity comprising up to 250 ppm. Calcium stearate is occasionally added (0.5%) to improve flow-ability. Vanillin has a characteristic pleasant smell and taste of vanilla which is reason for its widespread use.

Vanillin has a vapour presssure of 0.33 Pa and saturated air has a concentration of 0.00029 % at 25 ^{o}C, corresponding to 18.0 mg/m^3. Vanillin is soluble in water and solubility increases with increasing temperature. At 25 ^{o}C the solubility is 10 g/l. Log P_{ow} value ranges from 1.21 to 1.35 (calculated and measured) indicating that vanillin is unlikely to bioaccumulate. The pH of a 5 % solution of vanillin in water is 4.3. The phenol group of vanillin has a pK_a value of 7.38. With increasing pH the molecule will loose a proton, become negatively charged and more soluble in water.

2. GENERAL INFORMATION ON EXPOSURE

For the years 1993-95 the estimated world wide production of vanillin was 10,000 tonnes per year, and an annual increase of 2 % is estimated.

Vanillin is mainly used as an additive to food and beverages (60 %), but considerable amounts are used for flavour and fragrances (20-25 %), while 5-10 % is used for intermediates for pharmaceuticals. Vanillin is added to a whole range of food and beverage products in concentrations, depending on the product category, from 0.00002 % up 0.1 %. As fragrance ingredient for perfumes etc. vanillin is added at concentrations ranging from 0.005 % to 0.8 %.

Vanillin occurs widely in nature both as free vanillin and as constituent of larger molecules. It has been reported to be present in several essential oils and vanilla pods. Vanillin is also present in plants bound to sugar as a glycoside. A precursor of vanillin is also part of the macromolecule lignin, one of the major constituents of wood.

Vanillin has been identified in smoke from burning wood, and also in cigarette smoke.

The majority of vanillin is produced from guaiacol, but significant amounts are also made from lignin, a by-product from the wood-pulp industry. Both production processes are closed except from packaging, where some dust might be formed. Maximum occupational level of organic dust in countries where such limits have been set is 6 mg/m^3 (Germany) or 5 mg/m^3 (Norway). There is no specific occupational limit for vanillin. From the production site there should be minimal waste to water, but minor dry waste to air and deposit will result. The resulting vanillin product is traded and distributed to the food and perfume industry in closed containers. Leakage and exposure during transport can happen accidentally, but this is not considered a problem.

It can thus be concluded that man is exposed to vanillin by ingestion of food and beverages with added vanillin. Through the use of perfumes and skin care products some vanillin will also be applied topically. Occupational exposure to the industrial product is limited to rather few individuals.

3. ENVIRONMENT

3.1 Environmental Exposure

3.1.1 General Discussion

Level I fugacity calculation, using a six compartment global reference model shows that vanillin will be distributed mainly to water (98.5%), minor quantities will be distributed to soil solids (1.41%), while negligible quantities will be distributed to other compartments and there are no trends to bioaccumulation. This is in agreement with the Log P_{ow} value of 1.23. Compounds with a Log $P_{ow} < 3$ will not tend to bioaccumulate.

Abiotic degradation of vanillin has been studied as photodegradation and hydrolytic degradation in water. Vanillin has been estimated to be degraded by sunlight in air with a half-life of 4.7 hours. Meaurement of hydrolysis in water at different levels of pH, indicated slow rates and the hydrolyses did not reach 10 % in any of the pH systems investigated. The compound is thus considered stable in sterile water.

Biotic degradation of vanillin has been studied in soil, and in water after inoculation with *Aspergillus terrus*, anaerobic sludge or benthic microorganisms from an eutrophic lake.
In unamended garden soil, biodegradation was slow, with about 10 % degraded after 4 weeks, however after amending the soil with "active garden soil" a 41 %degradation was achieved after 21 days. Under aerobic conditions in water after inoculation with *Aspergillus terrus*, 62.5% was degraded after 6 days. Under anaerobic conditions in water after inoculation with anaerobic sludge, 72 % was degraded after 28 days. Benthic microorganisms, dependent on a carbon source in the growth medium, were able to grow on vanillin as the only carbon source after 6 days of incubation. It is concluded from these studies that vanillin is readily biodegradable.

The biochemical oxygen demand (BOD_5) and chemical oxygen demand (COD_{Cr}) of vanillin has been determined. Values of 1.26 mg/mg and 1.76 mg/mg respectively gives an aerobic degradation during 5 days incubation at 20 ^oC of BOD/COD = 72 %. This qualifies vanillin as a compound which is readily biodegradable.

The degradation of vanillin by soil invertebrates has been studied by injecting ^{14}C-labelled vanillin into 20 specimens of each of an isopod (*Osniscus asellus*), millipede (*Pseudopolydesmus serratus*), slug (*Deroceras teticulatum*), snail (*Oxychilus draparnaldi*) and earthworm (*Eisenia foetida*). Of the injected vanillin; 9-14 % was oxidized to ^{14}C-CO_2 after 6 days. At this sample point 2-10 % of non-metabolised and 13 - 48 % of metabolised vanillin was present in animal tissue, while 1-4 % of non-metabolised and 22-66 % of metabolised materials were found in egesta (sand and faeces). Mortality for the different species ranged from 0 to 20 %. It can be concluded from the study that vanillin is readily metabolised in the tested species. Significant amounts are emitted as CO_2, large amounts are excreted as vanillin metabolites, while only a minor fraction of unmetabolised vanillin is recovered from excretion products or from the animals.

Vanillin ingested by man and other mammals is metabolised prior to excretion.

It can be concluded from the degradation experiments that vanillin is susceptible to photodegradation in air, is rather stable to hydrolysis in water, but is readily biodegradable under aerobic conditions. The the study of biodegradability under anaerobic conditions shows that vanillin also degrades rapidly under these conditions. A study in soil invertebrates shows that vanillin is metabolised by all five species tested.

3.1.2 Predicted Environmental Concentration

Monitoring data on vanillin in the environment is limited, but measurements have been done in fog-water in an area polluted by smog from firewood burning. Content in fog-water was measured during a two months period during the winter season in a residential area, 8 measurements ranged between 2 µg/l and 51 µg/l (mean 27µg/l). No vanillin was detected in an area with normal activity (no burning). Air samples taken 50 m from burning of fruit trees had a concentration of between 7 and 21 µg/l.

In a monitoring well close to an industrial site a concentration of 54 mg/l was found.

It is assumed that emission of vanillin is highest close to production plants. Emission data has been gathered from a plant with an annual production of about 2000 ton/y. This is thought to represent a large production unit category. Shown in the Table below are the estimated and measured emissions for this production plant.

Emission table for a large vanillin production plant. Emissions are either measured or calculated. Production is continuously throughout the year.

Recipient system	**Secondary treatment**	**Emission based on:**	**Emission kg/day**
-Soil (deposition of dust)	none	estimates	0.15
-Sewage water	sewage treatment	measurement/estimated	1.15
-Surface water	none	intermittent release concentration in river <100 ppm, estimated	2.3
-by-product	burnt at 600-700 ^oC	measurement/estimated	150

As the frequency and magnitude of intermittent release directly to surface water is not known, it is assumed that this release is continuos. A dilution factor of 10 is assumed from STP to river. The STP is assumed to receive water from 10000 people equivalents each with an output of 200 l/day. It is reported that 65 % of vanillin going into STP is degraded. Applying these emission rates as input to the EUSES

model, gave the following local PECs. $PEC_{aquatic}$ = 0.0219 mg/l, $PEC_{sediment}$ = 0.0234 mg/kg wwt, PEC_{soil} = 0.00197 mg/kg wwt. $PEC_{sewage\ plant}$ = 0.219 mg/l.

3.2 Effects on the Environment

3.2.1 Aquatic effects

Fish

The acute toxicity of vanillin to fish has been tested in Fathead Minnow (*Pimephales promelas*). The following LC_{50} values were observed for the following observation periods: 1 hr; 173 - 370 mg/l, 24 hr; 100 - 131 mg/l, 48 hr; 68.3 - 123 mg/l, 72 hr; 57 - 123 mg/l and 96 hr; 57 - 123 mg/l. It was observed that fish stopped schooling, became hypoactive, swam at the surface and lost equilibrium prior to death. There are no data available on the chronic toxicity to fish.

Daphnia

The acute toxicity to aquatic invertebrates has been tested in daphnia. After 24 hours exposure, the EC_{50} value was reported to be 180 mg/l.

A 21 day reproduction study has been performed on daphnia, according to OECD 202 and GLP. After an exposure for 13 days to 100 mg/l, immobilisation occurred in all animals. The EC_{50} for immobilisation in the period 13 to 21 days exposure was estimated to 75 mg/l. The reproductive function was however more sensitive to vanillin, and after 21 days exposure the EC_{50} (reproduction) was 16 mg/l (24 mg/l), NOEC 5.9 mg/l (10 mg/l) and LOEC 10 mg/l (18 mg/l) (nominal concentrations given in parentheses).

Algae

The toxicity of vanillin to algae was tested in a screening study in a variety of species. Only one concentration level (2 mg/l) was used. The green algae *Scenedesmus obliquus* and *Chlorella variegata* showed reduced growth after 3 days but no effect was observed after 7, 14 and 21 days exposure. When the diatoms *Gomphonema parvulum* and *Nitzschia palea* were tested for 2 mg/l of vanillin, the former showed no growth after 3 days exposure and reduced growth after 7, 14 and 21 days. The latter was unaffected after 3 days, but showed reduced growth after 7 days, however growth was normalised after 14 and 21 days. No effect was seen with the blue-green algae *Cylinderospermum licheniforme* and *Microcystis aeruginosa.* These results could indicate that green algae and diatoms are the most sensitive algae, but since this was a screening test using only one dose level, one should not attach too much importance to this finding.

In another report, 50 % growth inhibition of *Chlorella vulgaris* was observed after 80 hr exposure to 152 mg/l. After 160 hr exposure to this concentration the growth inhibition was 30 %. No effects were observed at 15 mg/l and 1.5 mg/l.

Conclusion

To summarise the effects of vanillin to the aquatic environment, fish, bacteria and most algae seems moderately sensitive to vanillin. There are indications that some algae species are more sensitive to vanillin, and the reproductive function of daphnia was shown to be sensitive. A $PNEC_{aquatic}$ based on the NOEC value (5.9 mg/l) found in the Daphnia reproduction test would give a value of 0.059 mg/l (only one long term NOEC, application factor=100). A PNEC value based on most sensitive EC_{50}/LC_{50} value (LC_{50} fish = 57 mg/l) and an application factor of 1000 give a $PNEC_{aquatic}$ of 0.057 mg/l.

Microorganisms

Another study tested the effect of vanillin on bacteria, yeast and blue-green algae in water. The effect of vanillin on anaerobic methane formation from sludge was measured, and 49 hr incubation with a concentration of 1,800 mg/l reduced the methane production with 50 %. *Photobacterium phosphoreum*

was however seen to be more sensitive to vanillin, and after 5 minutes incubation, an EC_{50} value of 58 mg/l was observed.

Yeast (*Saccharomyces cerevisiae*) showed an EC_{50} value of 179 mg/l after 210 minutes incubation with vanillin.

Conclusion microorganisms
Photobacterium phosphorum being the most sensitive organism and using an application factor of 10 gives a $PNEC_{STP}$ of 5.8 mg/l.

3.2.2 Terrestrial effects

Plants
Toxicity of vanillin to plants has been tested with lettuce, wheat and cotton. Germination testing with lettuce using water with 650 ±30 mg/l produced a 50 % reduction in germination compared to controls. Using a Petri dish bioassay, the elongation of cotton radicels was visually observable inhibited (11 %) after addition of 30 mg vanillin/dish, while no effect was observed on the wheat.

Earthworm
The effect of vanillin has been tested on growth and survival of the earthworm *Eisenia foetida*. The tested concentrations in soil, were 0, 0.1, 1.0, 4 and 8 %, the exposure period was 42 days. Of these, 4% was the lowest concentration which significantly reduced growth rate and caused death (in 80 % of the worms), the NOEC value was 10 g/kg drw.

Conclusion
As plants seem more sensitive than eartworm the EC_{50} value is multiplied with an application factor of 1000, giving a $PNEC_{terr}$ of 0.647mg/kg wwt.

3.2.3 Other effects

The white root fungi, which are responsible for breakdown of most of the lignin in nature, are rather tolerant to vanillin, showing 0-33 % inhibition at 152 mg/l, 76-100% at 760 mg/l and no growth at 1520 mg/l.

3.3 Initial Assessment for the Environment

With only a few production sites in Europe a regional or contintal exposure assessement does not seem relevant for this fairly nontoxic compound. A local scenario with a production plant in the large scale category should ensure that any possible environmental risks are covered. In the Table below based on such a local scenario no PEC/PNEC are found with values above 1. One may therefore conclude that risks associated from industrial production of vanillin and from consumer products is very limited. Waste from packaging of the raw material, waste of food and use of detergents, soaps and perfumes with added vanillin account for an added environmental exposure, however this gives a very disperse emission. Seen in relation to observed effect levels in the range of environmental species tested, no hazard to the environment can be identified.

Summary table of PEC/PNEC founds for a local scenario for a large vanillin production plant.

Scenario	**PEC/PNEC**
Surface water	0.385
Agricultural soil	0.003
Sediment	0.474

Local STP	0.038

Most monitoring values are for the air compartment, the origin of this vanillin have all been connected to burning of wood. As vanillin is quite soluble in water, exposure in air is not thought to be of importance. A rather high value (54 mg/l) found in water in a monetoring well close to an industrial plant is clearly above the PNEC established here for aquatic organisms. In conclusion open air burning of wood may represent a more important source of vanillin exposure in the environment than industrial production.

4. HUMAN HEALTH

4.1 Human Exposure

4.1.1 Occupational exposure

The industrial production of vanillin is a closed process. However, the milling of the dried crystals and the filling of the product into containers generate some dust to which a limited number of workers are exposed. Also some dust formation is expected in the food and beverage industry during addition of vanillin.

A study was carried out in the packaging section at Borregaard EuroVanillin, Norway, to assess the inhalation exposure of the operators to vanillin dust. The work in the packaging section is divided among 12-18 persons, meaning 2-3 persons per shift. On average, these workers are exposed to vanillin for 1 to 1.5 hours per day. For the rest of the day they conduct other operations where they are not exposed to vanillin. The exposure was measured by a constant flow sampler (2 l air/min) carried by the operator during the exposure period. The total amount of dust collected corresponded to air concentrations of 4.2, 4.3 and 6.0 mg/m^3 for the three consecutive days. From microscopic examination of the dust particles, only 10 % of the dust particles were considered to be vanillin.

Occupational exposure to vanillin other than from dust, is of little relevance. For the risk assessment, it is anticipated that occupational exposure is maximum 5 mg/m^3. Assuming a person has a respiratory minute volume (RMV) of 20 l and an exposure for 6 hours per day at the limit concentration, will give a daily exposure by inhalation of 36 mg. For a 70 kg person this makes 0.5 mg/kg/day.

Absorption of vanillin by inhalation of dust will also depend on particle size. Sifting analysis of crystalline vanillin revealed that less than 1 % of the particles were smaller than 53 microns. Since only particles smaller than 5 microns are respirable, much less than 1 % of inhaled vanillin can be absorbed. This is valid for vanillin produced from both lignin and guaiacol.

4.1.2 Consumer exposure

The majority of industrially produced vanillin is ingested in the form of food and beverages. Minor amounts are applied topically as skin care products, perfumes etc. The global use of vanillin in food and beverages imply that almost every human globally is exposed to minute amounts of vanillin by ingestion. The individual doses and exposure can vary due to eating habits and preferences. An Acceptable Daily Intake (ADI) of 10 mg/kg has been agreed between FAO/WHO and EU. For a 70 kg person the ADI is 700 mg vanillin which as an example corresponds to minimum 700 g chocolate, or 7000 g of ice cream. For the risk assessment it is assumed that even persons with a high intake of vanillin containing food and beverages do not have a vanillin intake above the ADI.

4.1.3 Indirect exposure via the environment

Human exposure from the environment is negligible.

4.2 Effects on Human Health

a) Mode of action of the chemical, toxicokinetics and metabolism

Metabolism studies in rats have shown that vanillin is metabolised to a number of urinary products, primarily vanillic acid, in both free and conjugated forms. Only minor amounts of unmetabolised vanillin is excreted. One person who ingested 100 mg vanillin excreted 96 mg as vanillic acid (94% of the dose) in the next 24 hour period.

b) Acute toxicity

The acute toxicity studies conducted with vanillin are summarised in the following table.

Acute toxicity studies with vanillin				
Species, strain	**No**	**Administration**	**Endpoint**	**Value (mg/kg)**
Rat, Sprague Dawley (GLP)	40	Oral, gavage	LD_{50}	3925-3976
Rat, Sprague Dawley	40	Oral, gavage	LD_{50}	4200
Rat, Sprague Dawley	15	Oral, gavage	LD_{50}	3300
Rat, Osborn Mendel	10	Oral	LD_{50}	1580
Rat	-	Oral	-	2000
Rat, albino	25	Oral	LD_{50}	3830
Guinea Pig	10	Oral	LD_{50}	1400
Rat, Sprague Dawley (GLP)	10	Dermal, paste	LD_0	[3]2000
Rabbit	3	Dermal	LD_{50}	[3]5010
Rat, Sprague Dawley	-	Intraperitonal	LD_{50}	1160
Mouse	-	Intraperitonal	LD_{50}	780
Mouse	-	Intraperitonal	LD_{50}	475
Guinea Pig	-	Intraperitonal	LD_{50}	1190
Rat, albino	50	Subcutaneous	LD_{50}	2600
Dog	-	Intravenously	LDL_0	1320

The acute toxicity (LD_{50}) of orally administered vanillin to rats seems, when taking into account the more recent and properly conducted studies, to be in the range of 3500 - 4000 mg/kg. When administered intraperitonally, intravenously or subcutaneously, the toxicity seems somewhat higher, while little toxicity has been seen after dermal application. No data are available on the acute toxicity of inhaled vanillin.

c) Repeated dose toxicity

Many repeated dose toxicity studies have been carried out with vanillin in several animal species. None of the studies have been carried out recently, and none have been carried out according to GLP, but some of them are well conducted and hold a high scientific standard. The different studies are summarised in the following table.

Repeated dose toxicity studies conducted with vanillin.

Species, strain	No	Dur-ation	Adm. strat.	Doses	End-point	Value (unit)
Rat, O-M	20	27 weeks	Oral, feed	1000 ppm (50 mg/kg/day)	NOEL	³1000 ppm (50 mg/kg/day)
Rat, O-M	20	16 weeks	Oral, feed	10,000 ppm (500 mg/kg/day)	NOEL	³10,000 ppm (500 mg/kg/day)
Rat, O-M (males)	15	1 year	Oral, feed	20,000/50,000 ppm (1000/2500 mg/kg/day)	NOEL	³50,000 ppm (2500 mg/kg/day)
Rat	80	91 days	Oral feed	3000/10,000/50,000 ppm (150/500/2500 mg/kg/day)	NOEL LOEL	³3000 ppm (150 mg/kg/day) ³10,000 ppm (500 mg/kg/day)
Rat, males	40	26 weeks	Oral, feed	1000/5000/10,000 ppm (50/250/500 mg/kg/day)	NOEL	³10,000 ppm (500 mg/kg/day)
Dog	8	26-27 weeks	Oral, caps	0, 25, 100 mg/kg/day	NOEL	³100 mg/kg/day

(O-M: Osborn-Mendel)

The repeated oral administration studies in rat suggest that the NOEL can be as high as 50,000 ppm (2500 mg/kg/day). One oral feed study for 91 days did, however report a NOEL ³3000 ppm (150 mg/kg/day) and a LOEL ³10,000 ppm (500 mg/kg/day). This study is unpublished, but the information has been taken from a citation in 1963. The design and the details of the study are unknown, thus, the other studies seem more reliable. For the assessment, the NOEL of ³50,000 ppm in the 1 year study is used. No particular toxicity has been observed in dogs after repeated oral administration.

No information has been obtained on toxicity after repeated administration through other routes of exposure.

d) Reproduction developmental toxicity

There are no studies conducted according to the OECD-guidelines, with the aim of assessing the potential toxicity of vanillin on the reproductive system of mammals. Most of the repeated dose toxicity studies conducted in different animal species have, however, included both macroscopic and microscopic histopathological evaluation of the reproductive organs with no reported effect of the test substance. Also, throughout the many years of wide use of vanillin, there are no indications that vanillin is toxic to the reproductive system.

In a mouse spot test designed to measure the antimutagenic effect of vanillin, pregnant mice were given 3 successive oral administrations of vanillin at 125-500 mg/kg at 0, 4 and 24 hours after the injection of the mutagen ethylnitrosourea. Vanillin was also given to females in the control group (not given the mutagen). Vanillin was shown to have an antimutagenic effect. Even if this study was designed for another purpose, it indicates that administration of vanillin has no toxic effect on the mouse embryos.

In another study to test the antimutagenic activity of vanillin, the substance was injected intraperitonally as a single dose of 50 mg/kg to mice at the 11th day of gestation, either with or without the mutagen. On day 18 of gestation, dams were killed and examined with their foetuses. It was concluded that the effect of vanillin was comparable to that of saline control with respect to the number of live foetuses, foetal body weight, foetal mortality and incidence of external and skeletal abnormalities.

Vanillin turned out to be non-teratogenic when tested in the developing chicken embryo test. Four exposure conditions were used; injection via the air cell and via the yolk and at two different time points (0 and 96 hr). For each condition and at each of five dose levels 100 embryos were treated. All embryos and hatched chicks were examined grossly for any structural or functional abnormality. The highest dose tested was 10 mg vanillin/egg. When injecting the test substance into the air-cell at 0 hr, the observed LD_{50} value for the embryo was 0.82 mg/egg.

e) Genetic toxicity

Testing of the potential genotoxicity of vanillin has been conducted in a whole range of tests in bacteria (*in vitro*), in mammalian cells (*in vitro*) and in different *in vivo* test. The results of these testing are summarised in the following 3 tables.

Testing of mutagenicity in tests utilising bacteria.						
Test/ species strain	**No. of strains**	**Concentration range (mg/plate)**	**Cytotoxicity (mg/plate)**		**Genotoxicity**	
			+ S9	**- S9**	**+ S9**	**- S9**
Ames test/ S. typhimurium (GLP)	5	100 - 5000	> 5000	> 5000	-	-
Ames test/ S. typhimurium (GLP)	5	50 - 5000	> 5000	> 5000		-
Ames test/ S. typhimurium	4	100 - 10.000	> 10,000	> 10,000	-	-
Ames test/ S. typhimurium	5	0.5 - 5000	5000	5000	-	-
Ames test/ S. typhimurium	4	456	> 456	> 456	-	-
Ames test/ S. typhimurium	6	£10,000	> 10,000	> 10, 000	-	-
Ames test/ S. typhimurium	2	0.05 - 1000	> 1000	> 1000	-	-
Ames test/ S. typhimurium	2	0 - 1520		> 1520		-
Rev. mutation assay/ E. coli S. typhimurium	2	50 - 150			-	

Rev. mutation assay/ E. coli WP2	1	0 - 4560		> 4560		-
Rev. mutation assay/ E. coli PQ37	1	15 - 1500		> 1500		-
DNA-repair/ E. coli K12/PJ5	2	0 - 1520		> 1520		-
DNA-repair/ E. coli K12	1	500 (plasm.recomb.) 600 (UV-survival)		> 600		-

Testing of potential mutagenicity for vanillin in Ames test, DNA repair test and reverse mutation assays with *E. coli* are all negative both in the presence and the absence of a metabolic activation system (S9). Few signs of cytogenicity have been observed, even with doses up to 10 mg/plate.

***In vitro* mutagenicity testing in mammalian cells.**			
Test/ Cell type	**Concen-tration**	**Observed cytotoxicity**	**Observed genotoxicity**
Cytogenicity test/ Human lymphocytes	0 - 612 mg/l	Without metabolic activation, cytotoxicity observed at 612 mg/l	Increased number of aberrations including gaps at 612 mg/l
Cytogenicity test/ Mouse fibroblasts	0 - 1224 mg/l	Without metabolic activation, cytotoxicity observed at 1224 mg/l	Vanillin at concentrations 153 - 918 mg/l induced multinuclear mutations in the fibroblasts
DNA-repair assay/ Chinese Hamster ovary cells - K1	1.5 - 15 mg/l		Negative. No chromosomal aberration observed. Vanillin antimutagenic
Gene mutation assay/ CHO-Cells (GLP)	250-1500 mg/l (- S9) 183-2440 mg/l (+ S9)	-S9: Complete cytotoxicity at 1700 mg/l, Cell cycle delay at 509 mg/l. +S9: 2440 ml/l severe cytotoxicity.	Negative for chromosomal aberrations except at 2440 mg/l with S9.
Gene mutation assay/ Chinese Hamster fibroblast cells	£1000 mg/l No metabolic activation	No cytotoxicity	Negative.
Gene mutation assay/ Chinese Hamster V79 cells	0-15 mg/l	No cytotoxicity	Negative. Vanillin showed antimutagenic activity on UV and X-ray induced mutations
Gene mutation assay/ Chinese Hamster B241 cells	0.05 - 1000 mg/plate	No cytotoxicity, neither in presence or absence of S9	Negative in presence and absence of S9
Sister chromatid	0 - 50.6 mg/l	No cytotoxicity	No sister chromatid exchange or

exchange assay/ CHO cells			chromosomal aberration
Sister chromatid exchange assay/ Human lymphocytes	114 and 152 mg/l		Positive. Significant induction of SCE when first tested at 152 mg/l and confirmed positive when retested at 114 and 152 mg/l
Sister chromatid exchange assay/ Human lymphocytes	153, 306 and 612 mg/l		Positive effect of vanillin on SCE at 153 and 306 mg/l. Positive on chromosomal aberrations at 612 mg/l when gaps are included. Negative when gaps are excluded

(CHO: Chinese Hamster Ovary)

Testing of vanillin mutagenicity in mammalian cells *in vitro* have shown positive effects in some test systems. Increased number of aberrations has been seen at high vanillin concentrations in human lymphocytes only when gaps are included. The biological significance of gaps are, however, debated and is not alone any proof of genotoxicity. In mouse fibroblasts, vanillin has been shown to induce multinuclear mutations. In two independent studies in human lymphocytes, vanillin induced sister chromatid exchange. High concentrations of vanillin is cytotoxic to mammalian cells. The mutagenicity testing *in vitro* in mammalian cells indicates a genotoxic potential of vanillin. However, several of these studies were performed at high, unphysiological concentrations that could lead to false positives.

Genetic toxicity testing of vanillin *in vivo*			
Test	**No**	**Doses**	**Result**
Mouse Micronucleus Assay	10 female/ group	500 mg/kg x 2 1000 mg/kg x 2	Negative. No significant increase in micronuclei in erythrocytes.
Mouse Micronucleus Assay	3 male/ group	125 - 500 mg/kg x 8 or 500 mg/kg x 2	Vanillin did not induce micronuclei. When vanillin was administered in combination with a positive control (mitomycin C), vanillin decreased its mutagenic effect.
Mouse Micronucleus Assay	4 male/ group	250 - 500 mg/kg x 7 or 500 mg/kg x 8	Vanillin did not induce micronuclei. Vanillin reduced the micronuclei formation induced by X-rays.
Mouse spot test	about 30 pregnant females/ group	125 - 500 mg/kg x 3	Vanillin showed no mutagenic effect, but reduced the mutagenic effect induced by ENU (ethylnitrosourea)
Ring-X loss test in Drosophila melanogaster	Adult female flies	0.5 - 2 % vanillin in 5 % sucrose	Vanillin was seen to have a suppressor effect on ring-X loss that occurs spontaneously in Drosophila melanogaster.

The testing for genetic toxicity of vanillin *in vivo* is negative in all systems tested and gave no indication of any genotoxicity.

f) Carcinogenicity testing

Carcinogenicity testing conducted with vanillin				
Test Type	**No**	**Dosing**	**Duration**	**Result**
Oral feeding carcinogenicity study in the rat	12 male and 12 females/ group	Daily in feed, 0, 5000, 10,000 and 20,000 ppm (250, 500 and 1000 mg/kg/day)	2 years	Negative. Vanillin not a carcinogen. No toxicity observed
Pulmonary tumour test in mice	15 male and 15 female/ group	150 mg/kg or 750 mg/kg 3 x weekly intraperitonal injections for 8 weeks	8 weeks + 16 weeks observation	Negative. Vanillin not a carcinogen
Tumour initiating activity and cocarcinogenic effect on mouse skin	18	10 thrice weekly applications of 0.3 ml of 20 % vanillin in acetone followed by 18 weekly applications of 0.3 ml croton oil	Totally 21 weeks observa-tion period	No significant increase in local tumours. No cocarcinogenic effect
Short term cell proliferation of rat stomach	5/ group	Daily combined administration of 2 % vanillin ± 0.3 % $NaNO_2$ in drinking water.	4 weeks	No significant effect on oesophagus and on the forestomach mucousa. In glandular stomach, thickness and labelling indices significantly increased by the combination.
Anti-photocarcinogenic properties of vanillin in mice	3/ group	0.5% vanillin in diet	40 weeks	Vanillin did not reduce tumour latency but significantly reduced tumour multiplicity (- 48 %)

A full 2 years oral feeding carcinogenicity study has been conducted in rats with a negative result. There was no indication of vanillin being an experimental carcinogen. The other tests conducted confirm this finding and some tests even indicate that vanillin reduces the tumourgenicity of carcinogenic treatments.

None of the carcinogenicity studies presented have been conducted according to GLP, but the 2 years study (conducted prior to GLP regulations) seem to hold a high scientific standard and includes an appropriate number of animals and groups. Less emphasis should be put on the other studies mentioned, but they support the conclusions from the 2 years study.

g) Other data

Skin irritation testing with vanillin			
Test type	**No**	**Dosing**	**Result**

Rabbit skin irritation test	6	Application of grounded sample for 24 hr	No irritation
Closed patch test in Guinea Pigs	5	1, 2, 5 or 10 % vanillin applied for 48 hours	All observations were negative, there were no signs of irritation

The two skin irritation studies were both negative.

Skin sensitisation testing with vanillin			
Test type	**No**	**Dosing**	**Result**
Open Epicutaneous test (OET) in Guinea Pigs	6 - 8	0.03 - 30 % and undiluted vanillin applied on 2 cm^2 of clipped flank skin. Read after 14 hr. Repeated administration for 21 days	Minimum irritation concentration (30 %) after 1 application, but 3 % after 21 applications
Closed patch test in Guinea Pigs	10	10 % Vanillin applied for 24 hours 3 times a week for 2 weeks and then read after 1, 24 and 48 hours	All observations were negative, there were no signs of sensitisation or allergy in any of the animals.
Draize test in Guinea Pigs	6 - 8	Day 0: 0.05 ml of 0.1 % solution in saline. Day 1-9: 0.1 ml of 0.1%. Challenged again on days 35 and 49 with 0.05 ml	No allergic effects were seen in Guinea Pigs with vanillin.
Maximisation Test in Guinea Pigs	6-8 /group	Intradermal injection of 0.1 ml 5% vanillin ±Freunds complete adjuvance	Vanillin showed positive allergic effects in Guinea Pigs
Freunds complete adjuvance test in Guinea Pigs	6-8	0.05 ml of undiluted vanillin mixed with 0.05 ml Freunds complete adjuvance and injected intradermally into the neck on days 0, 2, 4, 7, and 9	Vanillin showed positive allergenic effects in Guinea Pigs
Freunds complete adjuvance test in Guinea Pigs	10	10 % in acetone	Weak sensitive
Maximisation Test in Guinea Pigs (GLP)	40	17.5 - 35 % in ethanol, 73 % in paste	The test article did not provoke any reaction or cutaneous sensitisation in any of the animals examined
Maximisation Test in Guinea Pigs	21	50 % vanillin	Positive result in 60 % of animals
Maximisation Test in Guinea Pigs	-	10 - 50 % vanillin	Positive
Mouse ear swelling	15 -	50 % vanillin in ethanol	No sensitisation

test	25		

In the skin sensitisation testing with vanillin, 5 out of 10 tests showed positive results indicating that vanillin has an allergenic potential. The other tests were negative, including the only test conducted according to GLP.

Eye irritation testing			
Test type	**No**	**Dosing**	**Result**
Rabbit eye irritation test	6	55 mg sample of finely grounded powder, 24 hours exposure	Slightly irritating. Gradually improving from 48 - 120 hr. After 168 hr, all scored zero.

Only vanillin powder was irritating to the eye due to mechanical stress from crystals.

Immunotoxicity testing of vanillin			
Test type	**No**	**Dosing**	**Result**
Immunotoxicity in mice	30/ group	250, 500 and 1000 mg/kg intragastrically daily for 5 days	No signs of immunotoxicity
Test for immunosuppression *in vitro*		50 mg/culture. Test for plaque-forming cell response	Vanillin stimulated plaque forming cell response up to 300 %, indicating an immunostimulatory effect
Immunotoxicity in an *in vitro* antibody producing assay		Vanillin, 200 mg/culture. Anti sheep red blood cells	Vanillin suppressed the *in vitro* anti sheep red blood cell antibody response indicting an immune suppressing effect.
Immunomodulatory effect *in vitro*		1, 10, 100, 300, 1000 mg/ml. Microbicidal activity of mouse macrophages tested	Vanillin is a weak modulator of macrophage function

Testing of possible immunotoxicity indicated that vanillin is not immunotoxic, but one study indicated an immunostimulating effect, while another indicated an immunosuppressive effect.

h) Human data

Vanillin was tested in the closed patch test on healthy normals and on subjects with dermatoses. In the test with the healthy volunteers, test substance was applied on the back (20% concentration) of 29 subjects for 48 hr or at the upper inside of the arm (2 % concentration) of 30 subjects for 24-72 hr. Vanillin (0.4 % concentration) was applied for 24 -48 hr on the upper inside of the arm of 35 subjects with dermatoses. Both in the healthy persons and in those with dermatoses vanillin was negative in all tests. No sign of irritation or erythema was observed.

A patch test was carried out on 30 workers in a vanillin factory and compared with 15 controls. About half of the workers had dermatitis. Vanillin was applied undiluted and removed after 48 hours. No signs of irritation or sensitisation was observed with vanillin.

Vanillin (2 %) was tested in the Maximisation test, where the skin area is made permeable by applying sodium lauryl sulphate for 24 hr prior to 48 hr repeated application of the test material. Vanillin was negative for all 25 subjects tested and concluded to be non-sensitising in humans.

In two different studies, positive reactions to vanillin have been reported in patients who already were sensitised to Balsam of Peru. From these studies vanillin was considered to be a secondary allergen.

In a double blind challenge test, an asthmatic patient was given vanillin by inhalation at 1.5 hr intervals, two or three times, providing no reaction occurred within 15 minutes of challenge. There was some evidence that vanillin reduced lung function at oral doses of 0.24 and 1 mg. Itching of the ears and throat was also described.

4.3 Initial Assessment for Human Health

In the human health risk assessment the consumer exposure (by oral intake) is taken as the ADI value (10.0 mg/kg/day).

Occupational exposure has been identified as inhalation of vanillin dust by operators in the packaging area of the factory. This exposure has been quantified to a maximum of 0.5 mg/kg/day. This level is likely to never be reached since the amount of vanillin in the total dust is estimated to only about 10 %. Additionally, much less than 1 % of the product has a particle size small enough to reach the lungs.

Skin exposure to vanillin has not been quantified, but both consumers and workers will be subject to a minor extent of such exposure.

Assessment from acute and repeated dose toxicity

From the acute toxicity studies, the LD_{50} value for rats (3500 - 4000 mg/kg) gives a safety margin of 350 - 400 for consumers, and this is considered satisfactory.

From the oral repeated dose toxicity studies a NOEL of 2500 mg/kg/day was observed after oral feeding to rats. This gives a safety margin of 250 for consumers use, and this is considered acceptable.

Assessment from reproduction and development studies

The potential toxicity of vanillin to the reproductive system has been studied in several connections. Microscopic and macroscopic histopathological evaluation of reproductive organs were performed in connection with repeated dose studies. In the mouse spot test and in a study to test the possible antimutagenic effect of vanillin, it was given to pregnant mice. The potential teratogenicity of vanillin has also been tested in the developing chicken embryo test. There has been no sign of vanillin being toxic to the reproduction system, or to the developing embryo in any of these tests. Even though a reproduction/development study carried out according to OECD-guidelines is lacking, the present use and production of vanillin indicate no such risk.

Assessment from genotoxicity and cancer studies

The testing of the potential genotoxicity of vanillin is comprehensive. Mutagenicity testing in bacteria was negative. Testing *in vitro* in mammalian cells gave positive results in tests for sister chromatid exchange and chromosomal aberration in human lymphocytes. These results indicate that vanillin under certain testing conditions might be genotoxic. However, *in vivo* genotoxicity tests were negative. An overall evaluation of the test results indicate that vanillin is not likely to pose a genetic risk to humans.

The carcinogenicity studies, including a 2 years study in rats, did all give negative results. It is concluded that the present use and production of vanillin represent little risk for genotoxicity and there are no signs of carcinogenicity.

Assessment from skin irritation and sensitisation testing
Skin irritation and sensitisation testing have shown that in some tests vanillin turned out to be a sensitiser and thus a potential allergen. These results are, however, not conclusive and the negative human data support the opinion that vanillin is not a human allergen.

5. CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

Only minute quantities of vanillin are released from the vanillin production sites. Some occupational exposure to vanillin as inhalation of dust has been identified. The consumers will be directly exposed to vanillin as most of the produced material is ingested as flavour additive to food and beverages.

From a distribution model, it seems that vanillin in the environment will be distributed to the aqueous compartment, and there is no trend to bioaccumulation. The emissions of vanillin from the vanillin production and from the consumer products to the environment, are undetectable and represent no hazard.

During the present reviewing of the environmental and human safety data for vanillin, no particular risk has been identified which should give reason to concern or additional toxicity testing in animals.

The use of vanillin as a food additive is approved by authorities world wide, and FDA has granted GRAS status to its use. This is in agreement with the experience with vanillin in consumer products during many years without any confirmed report of adverse events.

5.2 Recommendations

It is recommended to make efforts to obtain more data on the occupational exposure through monitoring of inhalation of vanillin dust in contaminated working areas. Such monitoring data should also include particles larger than those normally considered respirable.

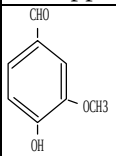
6. REFERENCES

References are not given in the SIDS initial assessment report. It is referred to those given in the Full SIDS Dossier.

SIDS PROFILE

SIDS Dossier

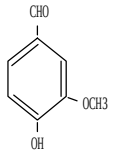
DATE: 20.08.96

1.01 A.	**CAS No.**	121-33-5
1.01 C.	**CHEMICAL NAME (OECD Name)**	Vanillin
1.01 D.	**CAS DESCRIPTOR**	Not applicable
1.01 G.	**STRUCTURAL FORMULA**	
	OTHER CHEMICAL IDENTITY INFORMATION	
1.5	**QUANTITY**	Estimated global production in 1993 was 10,000 tons.
1.7	**USE PATTERN**	(a) Wide dispersive use as foodstuff additive to food and food essences industry (0.005-0.1%). (Vanilla sugar 2%). (b) Non dispersive use as intermediate for industrial synthesis of pharmaceuticals. (c) Wide dispersive use as odour agent in cosmetics for personal and domestic use (usual: 0.005-0.01%, max: 0.03-0.1%). (d) Wide dispersive use as odour agent in perfumes for personal and domestic use (usual: 0.2%, max: 0.8%).
1.9	**SOURCES AND LEVELS OF EXPOSURE**	1. Amount released from production site (EuroVanillin, Norway) to soil: < 100 kg/year. 2. Amount released from production site (Borregaard EuroVanillin, Norway) to water: < 1500 kg/year. 3. Exposure through food products: 0.005-0.1% in foods containing Vanillin. 4. Exposure through cosmetic products (perfumes, creams, lotions, soap, detergents): 0.001-0.8% in cosmetics containing Vanillin.
ISSUES FOR DISCUSSION (IDENTIFY, IF ANY)		

1. GENERAL INFORMATION

1.01 SUBSTANCE INFORMATION

A. CAS-Number: 121-33-5
B. Name (IUPAC): 4-hydroxy-3-methoxybenzaldehyde
C. Name (OECD): Vanillin
D. CAS Descriptor: Not applicable
E. EINECS-Number: 204-465-2
F. Molecular Formula: $C_8H_8O_3$
G. Structural Formula:



H. Substance Group: Not applicable
I. Substance Remark:
J. Molecular Weight: 152.14

1.02 OECD INFORMATION

A. Sponsor Country: Norway

B. Lead Organisation: Norwegian Pollution Control Authority
Contact Person: Marit Kopangen
Address: P.O.Box 8100 Dep.
N-0032 OSLO
NORWAY
Tel: +47 22 57 34 00
Fax: +47 22 67 67 06

C. Name of Responder: Borregaard EuroVanillin
Address: P.O.Box 162
N-1701 SARPSBORG
NORWAY
Tel: +47 69 11 80 00
Fax: +47 69 11 86 40

1.1 GENERAL SUBSTANCE INFORMATION

A. Type of Substance: Element ( ); Inorganic ( ); Natural substance ( ); Organic (x); Organometallic ( ); Petroleum product ( )

B. Physical State
(at 20°C and 1.013 hPa): Gaseous ( ); Liquid ( ); Solid (x)
Crystalline

C. Purity: > 99.0% w/w on dried basis

1.2 SYNONYMS

para-Vanillin
Vanillic aldehyde
Vanillaldehyde
4-hydroxy-m-anisaldehyde
methylprotocatechuic aldehyde
Protocatechualdehyde-3-methyl ether
3-methoxy-4-hydroxybenzaldehyde
hydroxy-4-methoxy-3-benzaldehyde

1.3 IMPURITIES

CAS No: 121-32-4
EINECS No: 204-464-7
Name: Ethyl Vanillin (3-ethoxy-4-hydroxybenzaldehyde)
Value: < 250 ppm
Remarks: In vanillin obtained from guaiacol only, and not in vanillin from lignin raw material.
Valid for EuroVanillin KS, Norway.

1.4 ADDITIVES

CAS No: 1592-23-0
EINECS No: 216-472-8
Name: Calcium stearate
Value: Approx. 0.5%
Remarks: Is added only occasionally (one single product) to improve flowability.
Valid for EuroVanillin KS.

1.5 QUANTITY

Remarks: Estimated global production:
1993: 10,000 metric tonnes.
(Divided on 2 big (>2000 metric tonnes/year)
and 5-8 smaller producers.) [1]
1994: 10,000 metric tonnes [2]
1995: 10,000 metric tonnes [2]

Reference:
1) Project Euromarketing, 1993.
2) Estimated 2% increase, by EuroVanillin KS, Norway.

1.6 LABELLING AND CLASSIFICATION

A. Labelling

(a)
Type: Directive 67/548/EEC
Specific limits:
Symbols:
Nota:
R-phrases:
S-phrases:
Text of S-phrases:
Remarks: No labelling required (no dangerous properties)

(b)
Type: Other: Directive 88/388/EEC.
Remarks: Requirements:
- Name of producer
- "Aroma" or "Vanillin"
- For food
- Nature identical flavouring
- Identification of the lot
- Weight/Volume

This is valid for Vanillin sold for manufacturing of food products.

B. Classification

Type: Directive 67/548/EEC
Category of danger:
R-phrases:
Remarks: No classification required (no dangerous properties)

1.7 USE PATTERN

A. General

(a)
Main category: Wide dispersive use
Industrial category: Other: Food and food essences industry
Use category: Food/foodstuff additives
Remarks: Vanillin was given GRAS (Generally Recognized As Safe) status by FEMA (Flavor and Extract Manufacturers' Association) in 1965 and is approved by the FDA for food use (GRAS). The Joint FAO/WHO Expert Comittee on Food Additives (1967) has published a monograph and specifications for vanillin, giving an unconditional ADI (Acceptable Daily Intake) of 0-10 mg/kg. The Council of Europe (1974) listed vanillin, giving it an ADI of 10 mg/kg (Opdyke, 1977).

(b)
Main category: Non dispersive use
Industrial category: Chemical industry: synthesis
Use category: Intermediate for pharmaceuticals

(c)
Main category: Wide dispersive use

Industrial category: Personal and domestic use
Use category: Odour agent for cosmetics
Remarks: Added to cosmetics by industry

(d)
Main category: Wide dispersive use
Industrial category: Personal and domestic use
Use category: Odour agent for perfumes
Remarks: Added to perfumes by industry

(e)
Remarks: Vanillin is used as pharmaceutic aid (flavour), as flavouring agent in confectionary, beverages, foods, as a odour agent in perfumery and as reagent in analytical chemistry (The Merck Index, 1989). Besides being a very popular flavour in the food industry, vanillin is also useful in the synthesis of drugs; 40% of the vanillin is consumed in manufacturing drugs such as Aldomet (antihypertensive drug), L-dopa (treatment of Parkinson's disease) and Trimethoprim (treatment of upper respiratory-tract infections and some strains of venereal disease). Vanillin is also used in the perfume and metal-plating industries. Other uses for vanillin include prevention of foaming in lubrication oils, as a brightener in zinc coating baths, as an activator for electroless plating of zinc, as an aid to the oxidation of linseed oil, as an attractant in insecticides, as an agent to prevent mouth roughness caused by smoking tobacco, in the preparation of syntans for tanning, as a solubilizing agent for riboflavin and as a catalyst to polymerize methyl methacrylate (Kirk-Othmer, 1983).

(f)
Remarks: Estimated global Vanillin consumption by end use application:

Food & Beverages:	60%
Flavour and Fragrances:	20-25%
Pharmaceutical intermediates:	25%

Reference: Garshol, 1996.

B. Uses in Consumer Products

(a)

Function:	**Amount present:**	**Physical state:**
Flavouring agent in foods and beverages:		Dissolved or dispersed
Non-alcoholic beverages	0.0063% [1]	
Ice cream, Ices etc.	0.0095% [1]	
Candy	0.020% [1]	
Baked goods	0.022% [1]	
Gelatines and puddings	0.012% [1]	
Chewing gum	0.027% [1]	
Syrups	0.033-2.0% [1]	
Chocolate	0.097% [1]	
Toppings	0.015% [1]	
Margarine	0.00002% [1]	

Bonbons	up to 0.050% [2]	
Chocolate, plain	up to 0.055% [2]	
Chocolate milk	up to 0.030% [2]	
Fondants	up to 0.015% [2]	
Fudge	up to 0.055% [2]	
Marshmallow	up to 0.040% [2]	
Nougat	up to 0.055% [2]	
Vanillin sugar	2% [2]	Powder
Fragrance ingredient for perfumes:	0.2% usual 0.8% max [3]	Dissolved
Fragrance ingredient for creams, lotions:	0.005% usual 0.03% max [3]	Dissolved
Fragrance ingredient for detergents:	0.001% usual 0.01% max [3]	Dissolved
Fragrance ingredient for soap:	0,01% usual 0,1% max [3]	Dissolved

Reference: 1) Furia et al, 1975, Ash, 1995.
2) ACF ChemieFarma NV.
3) Opdyke, 1977

(b)

Food category	**Level of use (weighted mean), ppm**	
	Usual	**Maximum**
Baked goods	74.5	106.0
Break cereals	353.0	353.0
Fats, oils	96.0	100.0
Sweet sauce	358.0	363.0
Gelatine pudding	47.7	117.0
Snack foods	200.0	200.0
Beverage type I	39.4	97.4
Milk products	221.0	314.0
Frozen dairy	26.7	55.2
Meat products	1.5	2.7
Soft candy	247.0	408.0
Beverage type II	30.1	47.1
Hard candy	26.4	193.0
Chewing gum	1.5	445.0
Miscellaneous	468.0	2090.0

Reference: FEMA, 1985.

1.8 OCCUPATIONAL EXPOSURE LIMIT VALUES

A. Exposure limit value

(a)

Type: Norwegian Administrative Norm
Value: 5 mg organic dust/m^3 (average through 8 hours)
No particular limit for Vanillin.
Reference: Direktoratet for Arbeidstilsynet, 1995.

(b)

Type: MAK (DE)
Value: 6 mg dust/m^3
No particular limit for Vanillin.
Reference: List of MAK and BAT Values, 1995.

(c)

Type: BAT (DE)
Value: No values for neighter Vanillin nor organic dust
Reference: List of MAK and BAT Values, 1995.

(d)

Type: TRK (DE)
Value: Not relevant. TRK is for carcinogenic and mutagenic substances only.
Reference: List of MAK and BAT Values, 1995.

(e)

Type: OEL (EEC)
Value: Neighter Vanillin nor organic dust has been treated yet.
Reference: Directive 91/322/EEC, 1991.

(f)

Type: COSHH (UK)
Value: 10 mg total inhalable dust/m^3 (8-hour TWA)
5 mg respirable dust/m^3 (8-hour TWA)
No particular limit for Vanillin.
Reference: Health and Safety Executive, 1996.

(g)

Remarks: Percent in "saturated air"at 25^{o}C, 760 mmHg: 0.00029 % = 2.9 ppm[1].
Conversion factors in air: 1 mg/l = 161 ppm; 1 ppm = 6.2 mg/m^3 [2].
Reference: 1) Clayton et al, 1993, 2) HSDB, 1994.

B. Short time exposure limit value

Value: No short time exposure limit value (Norway)
Length of exposure period:
Frequency:
Remarks:
Reference: Direktoratet for Arbeidstilsynet, 1995.

1.9 SOURCES OF EXPOSURE

(a)

Source: **Media of release:** From air in the packing area to soil.
Quantities per media: < 100 kg/year

Remarks: Valid for EuroVanillin KS site, Norway (see 1.5 QUANTITY for information regarding estimated number of producers).
The whole process is closed, except from the packing station.
Some Vanillin is lost due to raise of dust in the packing area.
When cleaning the packing area, the Vanillin dust is treated as ordinary organic waste, and is being deposited at a public landfill.

Reference: Estimation done by EuroVanillin, 1995.

(b)

Source: **Media of release:** Discharge to water.
Quantities per media: < 500 kg/year

Remarks: Valid for Borregaard EuroVanillin site, Norway (see 1.5 QUANTITY for information regarding estimated number of producers).
The raffinate from K1800 in factory 2 is transferred to the biological treatment plant, where at least 65% of the Vanillin in the raffinate is decomposed.

Reference: Estimation done by Borregaard EuroVanillin, 1996.

(c)

Source: **Media of release:** Discharge to water.
Quantities per media: < 1000 kg/year

Remarks: Valid for Borregaard EuroVanillin site, Norway (see 1.5 QUANTITY for information regarding estimated number of producers).
Although the process is closed, there are from time to time water discharged to the river that contains Vanillin, but in concentrations of less than 100 ppm.

Reference: Estimation done by Borregaard EuroVanillin, 1996.

(d)

Source: **Media of release:** From raffinate to by-product.
Quantities per media: < 150 mt/year

Remarks: Valid for Borregaard EuroVanillin site, Norway (see 1.5 QUANTITY for information regarding estimated number of producers).
The various raffinate and tar streams from both plants are collected, evaporated and sold to Scandinavian craft mills for make-up.
The make-up product are burnt at temperatures around 600-700^{o}C, and Vanillin is decomposed to CO_2 and water, so practically no Vanillin is released.

Reference: Estimation done by Borregaard EuroVanillin, 1996.

(e)

Source: **Media of release:** Food products
Quantities per media: 0.005-0.1%

Remarks:

Reference: 1.7 USE PATTERN B. Uses in Concumer Products (this SIDS)

(f)

Source: **Media of release:** Cosmetic products (perfumes, creams, lotions, soap, detergents)

Quantities per media: 0.001-0.8%

Remarks:

Reference: 1.7 USE PATTERN B. Uses in Concumer Products (this SIDS)

(g)

Remarks: *Waste from end products:* Information not available.

Occupational exposure: Dust (inhalation).

Occurence: Vanillin occurs widely in nature. It has been reported in the essential oil of Java Citronella, in benzoin, Peru balsam, and clove-bud oil, and chiefly in Vanilla pods. Vanillin is also present in the plants as glucose and vanillin. Anothersource of vanillin is the lignin from the wood-pulp industry. (BIBRA Toxicity Profile, 1990. Opdyke, 1977).

Vanillin occurs in nature as a glycoside, which hydrolyses to vanillin and sugar. It has been identified in many oils, balsams, resins and woods. The best known source of vanillin is the vanillin plant (Vanilla Planifolia), a member of the orchid family (Kirk-Othmer, 1983)

Vanillin occurs naturally in vanilla, in potato parings, and in Siam benzoin (The Merck Index, 1989).

Vanillin occurs in balsams and is a constituent of lignins (Parke, 1968).

Vanillin occurs in tissues of certain plants, crude beet sugar, asparagus, and Asafetida (Osol et al, 1975).

Vanillin occurs in essential oil of Java Citronella and in Clove bud oil (Furia et al, 1975).

Vanillin has been identified in over 40 food products, in fruits (berries, urrants up to 0.02 ppm), in asparagus (1.2 ppm), in spices (mint, nutmeg), crispbread, alcoholic beverages (at levels varying fro 0 to 9.6 ppm), coffee, tea and vanilla. The highest concentration, up to 23.000 ppm, has been found in vanilla (Feron et al, 1991).

Vanillin has been found in anise, asparagus, Balsam Peru, roasted, barley, beer, brandy, bread volatiles, rye crispbread (0.14 ppm), grape musts, maple sap, maple syrup, evaporated milk, heated milk, dried mushroom, yellow passion fruit, popcorn, smoked belly pork (23.4 ppm), rum, jamaican rum (0.25 ppm), spearmint oil, tomato, vanilla extract (200-3100 ppm, 1000-2400 ppm, 250-1880 ppm), Bourbon vanilla pods, rice vinegar, whiskey, wine (FEMA, 1985).

(h)

Value: Day 1: 4.2 mg/m^3 total dust (0.42 mg/m^3 Vanillin, estimated)
Day 2: 4.3 mg/m^3 total dust (0.43 mg/m^3 Vanillin, estimated)
Day 3: 6.0 mg/m^3 total dust (0.60 mg/m^3 Vanillin, estimated)

Length of exposure period: Day 1: 2 hours
Day 2: 1.25 hours
Day 3: 1 hour

Frequency: The above exposure periods were the total exposure to Vanillin dust for the personnel during the actual days of working. The packaging operation was divided between 2-3 operators, so it is not nessesarily the same person who was exposed all the three days (see remarks).

Remarks: The operators are exposed to Vanillin dust at the packaging section only, i.e. the filling operation. The rest of the production process is closed.
The work in the packaging section is divided between a 12-18 persons (6 shifts, 2-3 persons per shift). The packaging operation counts for approximately 3 hours per shift per day. This means that each operator is exposed to Vanillin dust 1-1.5 hour per day on average, or more likely 3 hours 2-3 days per week. The rest of the time they are also carrying out other operations of which they are not exposed to Vanillin dust.
The test was carried out during the packaging of Vanillin ex lignin at Borregaard Eurovanillin, Norway.
A pump carried by the operator was used (Du Pont constant flow sampler S-2500). 2 liters air/min.
Filters: Closed filter holders. Filters of cellulose acetate, diameter: 37 mm, mesh opening 0.8 microns. The filter was exposed in the zone of breathing.
Microscopic evaluation showed that most of the dust particles seemed to be dust from outside the location. The gates were open during the exposure periods.
The Vanillin concentration is estimated to be max. 10% of the total dust.

Reference: Ramberg, 1996.

Remarks: Sifting analysis of Vanillin samples:
Vanillin ex lignin: 1.0 % < 53 microns
Vanillin ex guaiacol: 1.1% < 53 microns
Mean value of 4 parallel samples.
Method: 3 g sample, LP3 Sonic Sifter Separator (ATM Corporation), pulse at amplitude 5, 10 minutes, US Standard Sieves (ATM Corporation).

Reference: Pettersen, 1996.

1.10 ADDITIONAL REMARKS

A. Options for disposal

Remarks: Sweep, scoop or vacuum up all spilled material, contaminated soil and other contaminated material and place in clean, dry container for disposal. The material may be incinerated in a suitable chemical incinerator.
If there are arrangements for treatment of effluents on site, wash down with water to sewer.

B. Other remarks

Remarks: *Water pollution factors*[1]*:* Reduction of amenities:

- T.O.C. in water:	0.2 ppm
	4 ppm
- Detection:	0.06 mg/l
	0.2 mg/kg
- Recognition:	4.0 mg/kg

Odour detection/recognition[2]*:*

- Recognition in water:	4.00 ppm
- Detection in water:	0.20 ppm
- Detection in air:	$1.10x10^{-8}$ ppb
- Detection in air:	$2.00x20^{-4}$ ppb

Odour detection may be different by orders of magnitude because methods for making these determinations are totally subjective and scales used in these evaluations are not standardized. Additionally, the olfactory receptors may accomodate very rapidly to odours, resulting in inconsistent human responce.

Reference: 1) Verschueren, 1983.
2) Clayton et al, 1993

2. PHYSICAL-CHEMICAL DATA

2.1 MELTING POINT

(a)

Value: 80-81 °C
Decomposition: Yes ( ) No (x) Ambigous ( )
Sublimation: Yes ( ) No ( ) Ambigous ( )
Method: No data
GLP: Yes ( ) No ( ) ? (x)
Remarks:
Reference: Lewis, 1992, HSDB, 1994.

(b)

Value: 80-81°C (81-83 °C)
Decomposition: Yes ( ) No ( ) Ambigous ( )
Sublimation: Yes ( ) No ( ) Ambigous ( )
Method: No data
GLP: Yes ( ) No ( ) ? (x)
Remarks:
Reference: The Merck Index, 1989.

(c)

Value: 80-82 °C
Decomposition: Yes ( ) No ( ) Ambigous ( )
Sublimation: Yes ( ) No ( ) Ambigous ( )
Method: No data
GLP: Yes ( ) No ( ) ? (x)
Remarks:

Reference: Numata et al, 1992.

(d)

Value: 81-82 °C
Decomposition: Yes ( ) No ( ) Ambigous ( )
Sublimation: Yes ( ) No ( ) Ambigous ( )
Method: No data
GLP: Yes ( ) No (x) ? ( )
Remarks:
Reference: Inoue et al, 1981.

(e)

Value: 81-83°C
Decomposition: Yes ( ) No (x) Ambigous ( )
Sublimation: Yes ( ) No ( ) Ambigous ( )
Method: No data
GLP: Yes ( ) No ( ) ? (x)
Remarks:
Reference: Hawley, 1971, Kirk-Othmer, 1983, Sigma-Aldrich, 1988, Food Chemicals Codex, 1994, The United States Pharmacopeia 23/The National Formulary 18, 1995.

(f)

Value: 81-84 °C
Decomposition: Yes ( ) No ( ) Ambigous ( )
Sublimation: Yes ( ) No ( ) Ambigous ( )
Method: Capillary method
GLP: Yes ( ) No ( ) ? (x)
Remarks:
Reference: British Pharmacopeia, 1993, Deutsches Arzneibuch, 1994.

(g)

Value: ca. 82 °C
Decomposition: Yes ( ) No (x) Ambigous ( )
Sublimation: Yes ( ) No ( ) Ambigous ( )
Method: No data
GLP: Yes ( ) No ( ) ? (x)
Remarks:
Reference: Rhône-Poulenc, 1994.

(h)

Value: 82-83 °C
Decomposition: Yes ( ) No ( ) Ambigous ( )
Sublimation: Yes ( ) No ( ) Ambigous ( )
Method: No data
GLP: Yes ( ) No (x) ? ( )
Remarks:
Reference: Di Biase et al, 1979.

(i)

Value: 82-83.5 °C

Decomposition: Yes ( ) No (x) Ambigous ( )
Sublimation: Yes ( ) No ( ) Ambigous ( )
Method: No data
GLP: Yes ( ) No ( ) ? (x)
Remarks:
Reference: Clayton et al, 1993.

2.2 BOILING POINT

(a)

Value: 127 ^{o}C
Pressure: 1.33 hPa
Decomposition: Yes ( ) No ( ) Ambigous ( )
Method: No data
GLP: Yes ( ) No ( ) ? (x)
Remarks:
Reference: Kirk-Othmer, 1983.

(b)

Value: 170 ^{o}C
Pressure: 20 hPa
Decomposition: Yes ( ) No ( ) Ambigous ( )
Method: No data
GLP: Yes ( ) No ( ) ? (x)
Remarks:
Reference: Kirchhof et al, 1934, Sigma-Aldrich, 1988, The Merck Index, 1989, Weast et al, 1989.

(c)

Value: 285 ^{o}C
Pressure: 1013 hPa
Decomposition: Yes (x) No ( ) Ambigous ( )
Method: The temperature at which the liquid phase is in equilibrium with the vapour at a pressure of 760 mmHg (101.325 kPa)*.
GLP: Yes ( ) No ( ) ? (x)
Remarks: When heated to decomposition vanillin emits acrid smoke and irritating fumes **.
Reference: Susuki et al, 1978, Verschueren, 1983, Weast et al, 1989, The Merck Index, 1989, Lewis, 1992 (**), Clayton et al, 1993, Lide, 1994-1995 (*).

2.3 DENSITY

(a)

Type: Bulk density ( ); Density (x); Relative density ( )
Value: 1.056 g/cm^{3}
Temperature: 20 ^{o}C
Method: No data
GLP: Yes ( ) No ( ) ? (x)

Remarks:
Reference: Kirk-Othmer, 1983, Verschueren, 1983, The Merck Index, 1989, Weast et al, 1989, Lewis, 1992, Clayton et al, 1993, Lide, 1994-1995, Rhône-Poulenc, 1994.

(b)
Type: Bulk density (x); Density ( ); Relative density ( )
Value: 400-900 kg/m^3
Temperature: Room temperature
Method: No data
GLP: Yes ( ) No (x) ? ( )
Remarks: Varying because of different crystal sizes.
Reference: EuroVanillin KS, 1995b, Rhône-Poulenc, 1994.

2.4 VAPOUR PRESSURE

(a)
Value: 0.0022 hPa
Temperature: 25°C
Method: Calculated ( ); Measured ( )
No data
GLP: Yes ( ) No ( ) ? (x)
Remarks:
Reference: Clayton et al, 1993.

(b)
Value: 0.17 hPa
Temperature: 65°C
Method: Calculated ( ); Measured ( )
No data
GLP: Yes ( ) No ( ) ? (x)
Remarks:
Reference: Rhône-Poulenc, 1994.

(c)
Value: 1.33 hPa
Temperature: 107°C
Method: Calculated ( ); Measured ( )
No data
GLP: Yes ( ) No ( ) ? (x)
Remarks:
Reference: Verschueren, 1983.

(d)
Value: 9.73 hPa
Temperature: 140°C
Method: Calculated ( ); Measured ( )
No data
GLP: Yes ( ) No ( ) ? (x)
Remarks:

Reference: Rhône-Poulenc, 1993.

(e)
Value: 13.3 hPa
Temperature: 154°C
Method: Calculated ( ); Measured ( )
No data
GLP: Yes ( ) No ( ) ? (x)
Remarks:
Reference: Verschueren, 1983.

(f)
Value: 133 hPa
Temperature: 214.5°C
Method: Calculated ( ); Measured ( )
No data
GLP: Yes ( ) No ( ) ? (x)
Remarks:
Reference: Verschueren, 1983.

2.5 PARTITION COEFFICIENT $\log_{10} P_{ow}$

(a)
Log P_{ow}: 1.21
Temperature: No data
Method: Calculated ( ); Measured ( )
No data
GLP: Yes ( ) No ( ) ? (x)
Remarks:
Reference: Holmes, et al, 1976, LOGP and Related Parameters Computerized Database, 1987, Rhône-Poulenc, 1994.

(b)
Log P_{ow}: 1.23
Temperature: 22°C
Method: Calculated ( ); Measured (x)
GC-analysis of saturated o/w in equilibrium.
GLP: Yes ( ) No (x) ? ( )
Remarks: The compound is slightly dissociative.
Reference: EuroVanillin KS, 1993.

(c)
Log P_{ow}: 1.21-1.35
Temperature: No data
Method: Calculated (x); Measured (x)
GLP: Yes ( ) No ( ) ? (x)
Remarks: Different experimental and estimation methods.
Reference: Bodor, N. et al, 1989.

(d)
Log P_{ow}: 1.29-1.33

Temperature: No data
Method: Calculated (x); Measured ( )
Other
GLP: Yes ( ) No (x) ? ( )
Remarks:
Reference: Leo, 1978.

(e)
Log P_{ow}: 1.31
Temperature: No data
Method: Calculated ( ); Measured ( )
No data
GLP: Yes ( ) No (x) ? ( )
Remarks:
Reference: Korenman et al, 1975.

2.6 WATER SOLUBILITY

A. Solubility

(a)
Value: 3 g/l
Temperature: 4.4°C
Description: Miscible ( ); Of very high solubility ( ); Of high solubility ( ); Soluble ( ); Slightly soluble ( ); Of low solubility ( ); Of very low solubility ( ); Not soluble ( )
Method: No data
GLP: Yes ( ) No (x) ? ( )
Remarks:
Reference: Mange et al, 1924.

(b)
Value: 5.2 g/l
Temperature: 15.6°C
Description: Miscible ( ); Of very high solubility ( ); Of High solubility ( ); Soluble ( ); Slightly soluble ( ); Of low solubility ( ); Of very low solubility ( ); Not soluble ( )
Method: No data
GLP: Yes ( ) No (x) ? ( )
Remarks:
Reference: Mange et al, 1924.

(c)
Value: 9 g/l
Temperature: 23.9°C
Description: Miscible ( ); Of very high solubility ( ); Of High solubility ( ); Soluble ( ); Slightly soluble ( ); Of low solubility ( ); Of very low solubility ( ); Not soluble ( )
Method: No data
GLP: Yes ( ) No (x) ? ( )

Remarks:
Reference: Mange et al, 1924.

(d)
Value: 10g/l
Temperature: 25°C
Description: Miscible ( ); Of very high solubility ( ); Of High solubility ( ); Soluble ( ); Slightly soluble (x); Of low solubility ( );Of very low solubility ( ); Not soluble ( )
Method: No data
GLP: Yes ( ) No ( ) ? (x)
Remarks:
Reference: The Merck Index, 1989, Food Chemicals Codex, 1994, The United States Pharmacopeia 23/The National Formulary 18, 1995.

(e)
Value: 50 g/l
Temperature: 75°C
Description: Miscible ( ); Of very high solubility ( ); Of High solubility ( ); Soluble ( ); Slightly soluble ( ); Of low solubility ( ); Of very low solubility ( ); Not soluble ( )
Method: No data
GLP: Yes ( ) No (x) ? ( )
Remarks:
Reference: Tieman, 1877.

(f)
Value: 50 g/l
Temperature: 80°C
Description: Miscible ( ); Of very high solubility ( ); Of High solubility ( ); Soluble ( ); Slightly soluble ( ); Of low solubility ( ); Of very low solubility ( ); Not soluble ( )
Method: No data
GLP: Yes ( ) No ( ) ? (x)
Remarks:
Reference: Food Chemicals Codex, 1994, The United States Pharmacopeia 23/The National Formulary 18, 1995.

(g)
Value: 62.5 g/l
Temperature: 80°C
Description: Miscible ( ); Of very high solubility ( ); Of High solubility ( ); Soluble ( ); Slightly soluble ( ); Of low solubility ( ); Of very low solubility ( ); Not soluble ( )
Method: No data
GLP: Yes ( ) No ( ) ? (x)
Remarks:
Reference: The Merck Index, 1989.

(g)
Value:
Temperature:

Description: Miscible ( ); Of very high solubility ( ); Of High solubility ( ); Soluble ( ); Slightly soluble (x); Of low solubility ( ); Of very low solubility ( ); Not soluble ( )
Method: No data
GLP: Yes ( ) No ( ) ? (x)
Remarks:
Reference: Lide, 1994-1995.

B. pH Value, pKa Value

(a)
pH Value: 4.3
Concentration: 5% in water, saturated solution
Temperature: 25°C
Method: Measured by electrode.
GLP: Yes ( ) No (x) ? ( )
pKa Value:
Remarks:
Reference: EuroVanillin KS, 1995a.

(b)
pH Value:
Concentration:
Temperature:
Method:
GLP: Yes ( ) No (x) ? ( )
pKa Value: 7.38 at 25°C
Remarks: pKa value: Measured; spectrophotometric, solvent: water.
Reference:. Robinson et al, 1955.

2.7 FLASH POINT

Value: 153°C
Type of test: Closed cup (x); Open cup ( ); Other ( )
Method: No data
GLP: Yes ( ) No ( ) ? (x)
Remarks:
Reference: Rhône-Poulenc, 1993, Rhône Poulenc, 1994.

2.8 AUTO FLAMMABILITY

Value: > 400°C
Pressure: No data
Method: No data
GLP: Yes ( ) No ( ) ? (x)
Remarks:
Reference: Rhône-Poulenc, 1994.

2.9 FLAMMABILITY

Results: Extremely flammable ( ); Extremely flammable - liquified gas ( ); Highly flammable ( ); Flammable ( ); Non flammable ( ); Sponaneously flammable in air ( ); Contact with water liberates highly flammable gases ( ); Other (x)
Dust explosivity hazard in air.
Method: No data
GLP: Yes ( ) No ( ) ? (x)
Remarks:
Reference: Rhône-Poulenc, 1994.

2.10 EXPLOSIVE PROPERTIES

Results: Explosive under influence of a flame ( ); More sensitive to friction than m-dinitrobenzene ( ); More sensitive to shock than m-dinitrobenzene ( ); Not explosive ( ); Other (x) Dust explosivity hazard in air under influence of ignition sources[1].
Method: No data
GLP: Yes ( ) No (x) ? ( )
Remarks: Can react violently with Br_2, $HClO_4$, potassium-tert-butoxide, tert-chlorobenzene + NaOH, formic acid + thallium nitrate[2].
Reference: (1) Rhône-Poulenc, 1993.
(2) Lewis, 1992.

2.11 OXIDIZING PROPERTIES

Results: Maximum burning rate equal or higher than reference mixture ( ); Vigorous reaction in preliminary test ( ); No oxidizing properties (x); Other ( )
Method: No data
GLP: Yes ( ) No ( ) ? (x)
Remarks:
Reference: Rhône-Poulenc, 1994.

2.12 OXIDATION: REDUCTION POTENTIAL

Not Applicable

2.13 ADDITIONAL DATA

A. Partition co-efficient between soil/sediment and water (Kd)

Value: No data
Method:
GLP: Yes ( ) No ( ) ? ( )
Remarks:
Reference:

B. Other data

(a)

Results: Freely soluble in ethanol, methanol [1,2,3,4,5], chloroform [1,2,3], carbon disulfide, glacial acetic acid and pyridine [1].
Soluble in ether [1,2,3,4,5], aceton , benzen [3] and oils [1]
Dissolves in dilute solutions of alkali hydroxides [1,4,5].

Remarks:

Reference: (1) The Merck Index, 1989.
(2) Food Chemicals Codex, 1994.
(3) Lide, 1993-1994.
(4) British Pharmacopeia, 1993.
(5) Deutsches Arzneibuch, 1994.

(b)

Results: Decomposes above 160°C; differential thermal analysis on crude Vanillin before purification showed slightly exothermic decomposition at 130-200°C and strongly exothermic decomposition at 300°C.

Remarks:

Reference: Rhône-Poulenc, 1988.

(c)

Results: Slowly oxidizes somewhat when exposed to moist air, affected by light.

Remarks:

Reference: The Merck Index, 1989, HSDB, 1994.

(d)

Results: Incompatibilities with aluminium above 100°C, with strong oxidizing and reducing agents, with strong bases or alkalis. Decomposition products: phenol (pyrolysis), CO and CO_2.

Remarks:

Reference: Sigma-Aldrich, 1988, Rhône-Polenc, 1994.

(e)

Results: Exposure to air causes Vanillin to oxidize slowly to vanillic acid. When Vanillin is exposed to light in an alcoholic solution, a slow dimerization takes place, with the formation of dehydrovanillin.

Remarks:

Reference: Kirk-Othmer, 1983.

3. ENVIRONMENTAL FATE AND PATHWAYS

3.1 STABILITY

3.1.1 PHOTODEGRADATION

Type:	Air (x); Water ( ); Soil ( ); Other ( )
Light source:	Sun light (x); Xenon lamp ( ); Other ( )
Light spectrum:	Average daylight
Relative intensity:	Leads to 1.5 x 10^6 OH-radicals per cm^3
Spectrum of substance:	
Concentration of substance:	
Temperature:	25°C
Direct photolysis:	
Half life:	
Degradation:	
Quantum yield:	
Indirect photolysis:	
Type of sensitizer:	OH-radicals
Rate constant (radical):	27.3 x 10^{-12} cm^3 $molecule^{-1}$ sec^{-1}
Degradation:	Half-life: 4.7 hours
Method:	Calculated according to Atkinson (AOPWIN, Version 1.70, June 1995)
GLP:	Yes ( ) No (x) ? ( )
Test substance:	Vanillin
Remarks:	Validity of Estimation: 91% of estimations are within a factor of two of the experimental value.
Reference:	Völkel, 1995.

3.1.2 STABILITY IN WATER

Type:	Abiotic (hydrolysis) (x); Biotic (sediment) ( )
Half life:	
Degradation:	Stable
Method:	OECD TG 111: "Hydrolysis as a function of pH" 1981.
GLP:	Yes (x) No ( ) ? ( )
Test substance:	As prescribed by 1.1-1.2 Purity: 99.9%
Remarks:	The study was terminated after the preliminary test, since the hydrolysis did not reach > 10% in any of the pH-systems.
Reference:	Semb et al, 1996.

3.1.3 STABILITY IN SOIL

Type:	Field trial ( ); Laboratory (x); Other ( )
Radiolabel:	Yes ( ); No (x); ? ( )
Concentration:	1190 mg/kg
Soil temperature:	25°C
Soil humidity:	7 g water/100 g soil dry weight
Soil classification:	DIN19863 ( ); NF X31-107 ( ); USDA ( ); Other (x) Natural soil amended with 9% montmorillonite

Content of clay etc.:	Clay 9%, Silt 34%, Sand 57%
Organic Carbon:	5.8%
Soil pH:	5.6
Cation exchange capacity:	8.2 meq/100 g soil dry weight
Microbial biomass:	
Dissipation time:	
Dissipation:	41% after 21 days
Method:	Other: Carbon dioxide evolution measurements.
GLP:	Yes ( ) No ( ) ? (x)
Test substance:	Vanillin
Remarks:	As no significant amounts of CO_2 were detected after 1 week of incubation, a suspension of active "garden soil" was inoculated into each soil in an attempt to increase the number of organisms capable of utilizing Vanillin. The soils were then incubated for another 3 weeks. Other results: No clay added (pH 5,1): 9% biodegradation. 9% koaolinite added (pH 5,0): 11% biodegradation.
Reference:	Bewley et al, 1984.

3.2 MONITORING DATA (ENVIRONMENT)

(a)

Type of measurement:	Background (x); At contaminated site (x); Other ( )
Media:	Fog water and interstitial air
Result:	Among other compounds (guaiacol, syringol and their derivatives) Vanillin concentrations were found as: - On residental areas of Davis (Dec. 1990- Feb. 1991): 14. Dec.: 47 ug/l 18. Dec.: 44 ug/l 4. Jan.: 51 ug/l 5. Jan.: 29 ug/l 7. Feb.: 2 ug/l 8. Feb.: 6. 31 and 8 ug/l (3 samples) - On agricultural areas (Jan. 1991): 8. Jan.: Not detected (2 samples) 11. Jan.: 20. 7 and 17 ug/l (3 samples)
Remarks:	Vanillin evolved from wood burning. Fog water and interstitial air samples were collected on two sites in California: - City of Davis, population 50,000, in residental area, to determine the ambient concentrations of wood smoke markers. - Kearney Agricultural Research Center, typical agricultural area in the Central Valley: 8. Jan. 1991: ambient samples with nonusual activities. 11. Jan. 1991: while a waste pile of orchard purnings (primarily peach and almond) was burning, approx. 50 m from the sampler, to determine the local impact of such a waste disposal. Air samples, air samples filters and water filters were extracted by sonication with 3 aliquots of acetone for 30 minutes each. After

combination over anhydrous Na_2SO_4 and reduction by rotary evaporation, gas chromatography was performed.

Reference: Sagabiel et al, 1993.

(b)

Type of measurement: Background ( ); At contaminated site (x); Other ( )
Media: Ground water
Result: 54 mg/l
Remarks: Water collected from a contaminated monitoring well located at Nuclepore Corporation facility in Pleasanton, California.
Reference: California Regional Water Quality Control Board, 1983.

3.3 TRANSPORT AND DISTRIBUTION BETWEEN ENVIRONMENTAL COMPARTMENTS INCLUDING ESTIMATED ENVIRONMENTAL CONCENTRATIONS AND DISTRIBUTION PATHWAYS

3.3.1 TRANSPORT

Type: Adsorption ( ); Desorption ( ); Volatility ( ); Other ( )
Media:
Method:
Result:
Remarks: No data
Reference:

3.3.2. THEORETICAL DISTRIBUTION (FUGACITY CALCULATION)

Media: Air-biota ( ); Air-biota-sediment-soil-water (x); Soil-biota ( ); Water-air ( ); Water-biota ( ); Water-soil ( ); Other ( )
Method: Fugacity level I (x); Fugacity level II ( ); Fugacity level III ( ); Fugacity level IV ( ); Other (calculation) ( ); Other (measurement) ( )
Generic Models for Evaluating the Regional Fate of Chemicals (Mackay et al, 1992).
Result: The potential environmental distribution of Vanillin was calculated using a generic level I fugacity model. The distribution was:

Water:	98.5%
Air:	0.066%
Soil solids:	1.41%
Sediment soilds:	0.031%
Suspended sediments:	0.00098%
Fish:	0.000080%

Remarks: The calculation was based on a half-life of 4.7 hr in air and 1500 hr in water and soil.
Reference: Dahl, 1996.

3.4 IDENTIFICATION OF MAIN MODE OF DEGRADATION IN ACTUAL USE

Result: No data
Remarks:
Reference:

3.5 BIODEGRADATION

(a)
Type: Aerobic (x); Anaerobic ( )
Inoculum: Adapted (x); Non-adapted ( )
Aspergillus sp. (Aspergillus terreus)
Concentration of the chemical: Related to COD ( ); DOC ( ); Test substance ( )
Medium: Water ( ); Water-sediment ( ); Soil ( ); Sewage treatment (x)
Degradation: 62.5% after 6 days
Results: Readily biodeg. (x); Inherently biodeg. ( ); Under test condition no biodegradation observed ( ); Other ( )
Method: Assays in 5 litre batch reactor, steady flow of air 720 ml/min, stirring rate 200 rpm.
Incubation at 28 ^{o}C for 6 days.
GLP: Yes ( ) No ( ) ? (x)
Test substance: Vanillin in the waste produced by the olive-oil extraction industry.
Remarks: Biodegradation of Vanillin in the waste produced by the olive-oil extraction industry.
Reference: Martinez et al, 1993.

(b)
Type: Aerobic ( ); Anaerobic (x)
Inoculum: Adapted (x); Non-adapted ( )
Anaerobic sludge
Concentration of the chemical: 300 mg/l related to COD ( ); DOC ( ); Test substance (x)
Medium: Water (x); Water-sediment ( ); Soil ( ); Sewage treatment ( )
Containing $(NH_4)_2PO_4$, NH_4Cl, $MgCl_2$-$6H_2O$, KCl, $MnCl_2$-$4H_2O$, $CoCl_2$-$6H_2O$, H_3BO_3, $CaCl_2$-$2H_2O$, Na_2MoO_4-$2H_2O$, $ZnCl_2$, $FeCl_2$-$4H_2O$, $NaHCO_3$, Na_2S-$9H_2O$ and 1% (v/v) vitamin solution.
Degradation: 0% after 12 days
72% after 28 days
Results: Readily biodeg. (x); Inherently biodeg. ( ); Under test condition no biodegradation observed ( ); Other ( )
Method: A serum-bottle variation of the Hungate technique for growing anaerobic bacteria was adapted from Miller and Wolin (1974). The cultures were incubated in the dark at 35^{o}C.
GLP: Yes ( ) No (x) ? ( )
Test substance: Vanillin. No further data.
Remarks: 10 methanogenic enrichment cultures were found to degrade Vanillin after a lag phase of 12 +/- 1.2 days;
period of gas production (CO_2 and CH_4) 16 +/- 1.1 days;
conversion of substrate carbon to gas 72 +/- 1.4 days.
Vanillin is biodegradable to methane and carbon dioxide under strict anaerobic conditions.
Reference: Healy et al, 1979.

(c)

Type:	Aerobic ( ); Anaerobic (x)
Inoculum:	Adapted (x); Non-adapted ( ) Benthic microorganisms from an eutrophic lake (Microorganisms unable to grow on a medium without carbon source were picked)
Concentration of the chemical:	100 mg/l related to COD ( ); DOC ( ); Test substance (x)
Medium:	Water ( ); Water-sediment ( ); Soil ( ); Sewage treatment ( ) Bacto Yeast Nitrogen Base (Difco) without aminoacids.
Degradation:	From samples of bottom deposites at 2 sites of the eutrophic lake Jeziorak (Poland), 26% of the microorganisms were able to utilize Vanillin as sole source of carbon after 6 days of incubation.
Results:	Readily biodeg. ( ); Inherently biodeg. ( ); Under test condition no biodegradation observed ( ); Other (x)
Method:	After 6 days of incubation at 26^{o}C, colonial development was assessed by comparison of the cultures with control plates containing no carbon source. Plates containing ferrous gluconate (100 mg/l) were used to check the viability of the inoculum.
GLP:	Yes ( ) No (x) ? ( )
Test substance:	Vanillin. No further data.
Remarks:	
Reference:	Strzelczyc et al, 1972, Opdyke, 1977.

3.6 BOD_5, COD OR RATIO BOD_5/COD

BOD_5

Method:	ISO 5815 (Water quality - Determination of biochemical oxygen demand after 5 days (BOD_5) - Dilution and seeding method. 1989)
Concentration:	2 and 4 mg/l related to COD ( ); DOC ( ); Test substance (x)
Value:	1.26 mg O_2/mg
GLP:	Yes (x) No ( ) ? ( )

COD

Method:	ISO 6060 (Water quality - Determination of chemical oxygen demand. 1989)
Value:	1.76 mg O_2/mg
GLP:	Yes (x) No ( ) ? ( )

Ratio BOD_5/COD:	72 %
Test substance:	As prescribed by 1.1 - 1.2. Purity: 99.9%
Remarks:	Meets criteria of >50 % degradation
Reference:	Kälqvist, 1996a.

3.7 BIOACCUMULATION

Species:
Exposure period:
Temperature:
Concentration:

BCF:
Elimination: Yes ( ) No ( ) ? ( )
Method:
Type of test: Calculated ( ); Measured ( )
Static ( ); Semi-static ( ); Flow-through ( ); Other ( )
GLP: Yes ( ) No ( ) ? ( )
Test substance:
Remarks: According to Nordisk Miljøapport 1990 substances having log P_{ow} lower than or equal to 3.0 are not potentially bioaccumulative.
Reference: Nordisk Miljøapport, 1990.

3.8 ADDITIONAL REMARKS

A. Sewage treatment

Results: In case of disposal, pure Vanillin can be recirculated, e.g. recrystallized.
Do not release product into the environment. Decant and purify polluted waste water before it is released into the drains.
Incinerate product in licensed, suitable chemical incinerator, equipped with an after burner and a scrubber.
Remarks:
Reference: EuroVanillin KS, 1995c.

B. Other information

Results:
Remarks: When Vanillin 5-(14 C), 1 ul was injected in 5 soil invertebrates, 9-14% were oxidized to 14-CO_2 at 15^{o}C over 6 days.
Soil invertebrates:
- Isopod (Oniscus asellus)
- Millipede (Pseudopolydesmus serratus)
- Slug (Deroceras reticulatum)
- Snail (Oxychilus draparnaldi)
- Earthworm (Eisenia foetida)

Mortality:
- Isopod: 3/20
- Slug: 2/20
- Snail: 1/20
- Earthworm: 2/20

Approximately 2-10% of non-metabolized and 13-48% of metabolized Vanillin were present in the animal tissues after 6 days.
Correspondingly, 1-4% and 22-66% of these materials were found in egesta (in sand and feces).
Reference: Neuhauser et al, 1978.

4. ECOTOXICOLOGICAL DATA

4.1 ACUTE/PROLONGED TOXICITY TO FISH

(a)

Type of test: Static ( ); Semi-static ( ); Flow-through (x); Other ( )
Open-system ( ); Closed-system (x)

Species: Pimephales promelas (Fathead Minnow)

Exposure period: 96 hours

Results: LC_{50} (24h) =
LC_{50} (48h) =
LC_{50} (72h) = 123 (104-146) mg/l
LC_{50} (96h) = 123 (104-146) mg/l
NOEC =
LOEC =

Analytical monitoring: Yes (x) No ( ) ? ( )

Method: US EPA Environmental Research Laboratory - Duluth, MN, USA, 1981. (See Remarks).

GLP: Yes ( ) No (x) ? ( )

Test substance: Vanillin from Eastman Kodak Co, Rochester, NY, USA.
Purity: research grade.

Remarks: Fresh water.
Duplicate tests with 25 fish per concentration.
Test temperature: 24.7 (SD 0.72) ^{o}C.
Dissolved oxygen: 6.2 (SD 0.37) mg/l.
Hardness: 53.5 (SD 1.00) mg/l $CaCO_3$.
Alkalinity: 39.5 (SD 1.00) mg/l $CaCO_3$.
Tank volume: 5.5 l
Medium renewal: 13.1 l/day
pH: 7.00 (SD 0.09)
Fish mean length: 17.3 (SD 2.023) mm
Fish mean weight: 0.055 (SD 0.0182) g
Fish age: 31 days.
Fish loading: 0.2 g/l
95% confidence limits.
Affected fish lost equilibrium prior to death.

Reference: Brooke et al, 1984.

(b)

Type of test: Static ( ); Semi-static (x); Flow-through ( ); Other ( )
Open-system ( ); Closed-system (x)

Species: Pimephales promelas (Fathead Minnow)

Exposure period: 96 hours

Results: LC_{50} (24h) = 109.8 mg/l
LC_{50} (48h) = 63.8 mg/l
LC_{50} (72h) = 57 (53.0-61.3) mg/l
LC_{50} (96h) = 57 (53.0-61.3) mg/l
NOEC =
LOEC =

Analytical monitoring: Yes (x) No ( ) ? ( )
Method: US EPA Environmental Research Laboratory - Duluth, MN, USA, 1980. (See Remarks).
GLP: Yes ( ) No (x) ? ( )
Test substance: Vanillin from Eastman Kodak Co, Rochester, NY, USA.
Purity: research grade.
Remarks: Fresh water.
Duplicate tests with 25 fish per concentration.
Test temperature: 23.9 (SD 0.73) ^{o}C.
Dissolved oxygen: 7.2 (SD 0.93) mg/l.
Hardness: 51.0 (SD 0.00) mg/l $CaCO_3$.
Alkalinity: 40.5 (SD 0.00) mg/l $CaCO_3$.
Tank volume: 6.3 l.
Medium renewal: 5.7 l/day.
pH: 7.16 (SD 0.32).
Fish mean length: 20.0 (SD 1.67) mm.
Fish mean weight: 0.131 (SD 0.0323) g.
Fish age: 29 days.
Fish loading: 0.52 g/l.
95% confidence limits.
Affected fish stopped schooling, became hypoactive, swam at the surface, and lost equilibrium prior to death.
Reference: Brooke et al, 1984.

(c)
Type of test: Static (x); Semi-static ( ); Flow-through ( ); Other ( )
Open-system ( ); Closed-system (x)
Species: Pimephales promelas (Fathead Minnow)
Exposure period: 96 hours

	Test 1	Test 2	Test 3
Results: LC_{50}(1h) =	348 mg/l	>173 mg/l	>173 mg/l
LC_{50} (24h) =	100 mg/l	127 mg/l	125 mg/l
LC_{50} (48h) =	97 mg/l	121 mg/l	116 mg/l
LC_{50} (72h) =	88 mg/l	121 mg/l	116 mg/l
LC_{50} (96h) =	88 mg/l	121 mg/l	116 mg/l
NOEC =			
LOEC =			

Analytical monitoring: Yes ( ) No (x) ? ( )
Method: Static nonrenewal laboratory bioassay.
Test water: Reconstituted water.
Replicate tests with 10 fish per concentration.
Test temperature: 18-22 ^{o}C.
3 litres cylindrical glass battery jars containing 2 litres of test water.
Fish length: 11-31 mm.
Fish age: 4-8 weeks.
Fish acclimated 48 hours in flowing water (test 1 and 2 in Lake Superior water, test 3 in reconstituted water).
GLP: Yes ( ) No (x) ? ( )
Test substance: Vanillin from Curtin Metheson Scientific Inc.
Purity: reagent grade
Remarks: Dissolved oxygen measured during the test < 4 mg/l. This normally invalidates the test in standardised methods. Earlier work has shown

that at these concentrations low oxygen itself can cause adverse effects on fathead minnows in long-term toxicity tests.
Toxicant concentrations are nominal.

Reference: Mattson et al, 1976.

(d)

Type of test: Static (x); Semi-static ( ); Flow-through ( ); Other ( )
Open-system ( ); Closed-system (x)

Species: Pimephales promelas (Fathead Minnow)

Exposure period: 96 hours

Results:

	Test 1	Test 2
LC_{50} (1h) =	>173 mg/l	370 mg/l
LC_{50} (24h) =	131 mg/l	125 mg/l
LC_{50} (48h) =	123 mg/l	116 mg/l
LC_{50} (72h) =	121 mg/l	112 mg/l
LC_{50} (96h) =	121 mg/l	112 mg/l

NOEC =
LOEC =

Analytical monitoring: Yes ( ) No (x) ? ( )

Method: Static nonrenewal laboratory bioassay.
Test water: Lake Superior water.
Duplicate tests with 10 fish per concentration.
Test temperature: 18-22 ^{o}C.
3 litres cylindrical glass battery jars containing 2 litres of test water.
Fish length: 11-31 mm.
Fish age: 4-8 weeks.
Fish acclimated 48 hours in flowing water (test 1 and 2 in Lake Superior water, test 3 in reconstituted water).

GLP: Yes ( ) No (x) ? ( )

Test substance: Vanillin from Curtin Metheson Scientific Inc.
Purity: reagent grade

Remarks: Dissolved oxygen measured during the test < 4 mg/l. This normally invalidates the test in standardised methods. Earlier work has shown that at these concentrations low oxygen itself can cause adverse effects on fathead minnows in long-term toxicity tests.
Toxicant concentrations are nominal.

Reference: Mattson et al, 1976.

4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES (e.g. DAPHNIA)

A. Daphnia

Type of test: Static ( ); Semi-static ( ); Flow-through ( ); Other ( )
Open-system ( ); Closed-system ( )

Species: Daphnia magna (Crustacea)

Exposure period: 24 hours

Results:
EC_{50} (24h) = 180 mg/l
EC_{50} (48h) =
EC_{xx} (...h) =
NOEC =

LOEC =

Analytical monitoring: Yes ( ) No (x) ? ( )

Method: ISO 6341 15 "Water quality - Determination of the inhibition of the mobility of Daphnia magna Straus (Cladocera, Crustacea)".

GLP: Yes ( ) No (x) ? ( )

Test substance: As prescribed by 1.1 - 1.2.
Purity: >= 99.6% w/w.

Remarks:

Reference: Rhône-Poulenc, 1983.

B. Other aquatic organisms

Type of test: Static ( ); Semi-static ( ); Flow-through ( ); Other ( )
Open-system ( ); Closed-system ( )

Species:

Exposure period:

Results: EC_{50} (24h) =
EC_{50} (48h) =
EC_{xx} (...h) =
NOEC =
LOEC =

Analytical monitoring: Yes ( ) No ( ) ? ( )

Method:

GLP: Yes ( ) No ( ) ? ( )

Test substance:

Remarks: No data.

Reference:

4.3 TOXICITY TO AQUATIC PLANTS (E.G. ALGAE)

(a)

Species: Scenedesmus obliquus (green algae)

End point: Biomass (x); Growth rate ( ); Other ( )

Exposure period: 3, 7, 14 and 21 days

Results: EC_{50} (...h) =
EC_{xx} (...h) =
NOEC = 2 mg/l (7, 14 and 21 days)
LOEC = 2 mg/l (3 days)
Partially toxic after 3 days exposure (growth occured in the presence of Vanillin, but the amount was not as great as that in the control flask).
Non-toxic after 7, 14 and 21 days exposure (growth in the presence of Vanillin was similar to that in the control flask).

Analytical monitoring: Yes ( ) No (x) ? ( )

Method: Static - LAB.
Culture medium approximated Gerloff's modification of Chu No 10 with the amount of nitrate doubled (Palmer and Maloney, 1953).
Tests carried out in 25 ml Erlenmeyer flasks, incubated at 22°C.
Sterile, double strength medium with 1/15 to 1/30 by volume of an actively growing culture of the alga.

7.5 ml of this inoculated medium was combined with a like quantity of distilled water containing 4 ppm by weight of Vanillin (final concentration of 2 ppm Vanillin in normal strength medium, inoculum of 1/30 to 1/60 the volume of the original algal culture).
Number of algal cells in the medium at the start of the test: Approx. 125,000 cells/ml.
Visible algal growth was recorded at 3, 7, 14 and 21 days and compared with the growth in a control flask containing 15 ml of normal strength inoculated culture medium without Vanillin.

GLP: Yes ( ) No (x) ? ( )
Test substance: Vanillin; no further data.
Remarks: Only concentration tested.
Reference: Palmer et al, 1955.

(b)
Species: Chlorella variegata (green algae)
End point: Biomass (x); Growth rate ( ); Other ( )
Exposure period: 3, 7, 14 and 21 days
Results: EC_{50} (...h) =
EC_{xx} (...h) =
NOEC = 2 mg/l (7, 14 and 21 days)
LOEC = 2 mg/l (3 days)
Partially toxic after 3 days exposure (growth occured in the presence of Vanillin, but the amount was not as great as that in the control flask).
Non-toxic after 7, 14 and 21 days exposure (growth in the presence of Vanillin was similar to that in the control flask).
Analytical monitoring: Yes ( ) No (x) ? ( )
Method: Static - LAB.
Culture medium approximated Gerloff's modification of Chu No 10 with the amount of nitrate doubled (Palmer and Maloney, 1953).
Tests carried out in 25 ml Erlenmeyer flasks, incubated at 22^{o}C.
Sterile, double strength medium with 1/15 to 1/30 by volume of an actively growing culture of the alga.
7.5 ml of this inoculated medium was combined with a like quantity of distilled water containing 4 ppm by weight of Vanillin (final concentration of 2 ppm Vanillin in normal strength medium, inoculum of 1/30 to 1/60 the volume of the original algal culture).
Number of algal cells in the medium at the start of the test: Approx. 125,000 cells/ml.
Visible algal growth was recorded at 3, 7, 14 and 21 days and compared with the growth in a control flask containing 15 ml of normal strength inoculated culture medium without Vanillin.

GLP: Yes ( ) No (x) ? ( )
Test substance: Vanillin; no further data.
Remarks: Only concentration tested.
Reference: Palmer et al, 1955.

(c)
Species: Gomphonema parvulum (diatom)
End point: Biomass (x); Growth rate ( ); Other ( )
Exposure period: 3, 7, 14 and 21 days

Results:	EC_{50} (...h) = EC_{xx} (...h) = NOEC = LOEC = 2mg/l Toxic after 3 days exposure (no growth occured in the presence of Vanillin, but occured in the control flask). Partially toxic after 7, 14 and 21 days exposure (growth occured in the presence of Vanillin, but the amount was not as great as that in the control flask).
Analytical monitoring:	Yes ( ) No (x) ? ( )
Method:	Static - LAB. Culture medium approximated Gerloff's modification of Chu No 10 with the amount of nitrate doubled (Palmer and Maloney, 1953). Tests carried out in 25 ml Erlenmeyer flasks, incubated at 22°C. Sterile, double strength medium with 1/15 to 1/30 by volume of an actively growing culture of the alga. 7.5 ml of this inoculated medium was combined with a like quantity of distilled water containing 4 ppm by weight of Vanillin (final concentration of 2 ppm Vanillin in normal strength medium, inoculum of 1/30 to 1/60 the volume of the original algal culture). Number of algal cells in the medium at the start of the test: Approx. 125,000 cells/ml. Visible algal growth was recorded at 3, 7, 14 and 21 days and compared with the growth in a control flask containing 15 ml of normal strength inoculated culture medium without Vanillin.
GLP:	Yes ( ) No (x) ? ( )
Test substance:	Vanillin; no further data.
Remarks:	Only concentration tested.
Reference:	Palmer et al, 1955.

(d)

Species:	Nitzschia palea (diatom)
End point:	Biomass (x); Growth rate ( ); Other ( )
Exposure period:	3, 7, 14 and 21 days
Results:	EC_{50} (...h) = EC_{xx} (...h) = NOEC = 2 mg/l (3, 14 and 21 days) LOEC = 2 mg/l (7 days) Partially toxic after 7 days exposure (growth occured in the presence of Vanillin, but the amount was not as great as that in the control flask). Non-toxic after 3, 14 and 21 days exposure (growth in the presence of Vanillin was similar to that in the control flask).
Analytical monitoring:	Yes ( ) No (x) ? ( )
Method:	Static - LAB. Culture medium approximated Gerloff's modification of Chu No 10 with the amount of nitrate doubled (Palmer and Maloney, 1953). Tests carried out in 25 ml Erlenmeyer flasks, incubated at 22°C. Sterile, double strength medium with 1/15 to 1/30 by volume of an actively growing culture of the alga. 7.5 ml of this inoculated medium was combined with a like quantity of distilled water containing 4 ppm by weight of Vanillin (final

	concentration of 2 ppm Vanillin in normal strength medium, inoculum of 1/30 to 1/60 the volume of the original algal culture). Number of algal cells in the medium at the start of the test: Approx. 125,000 cells/ml. Visible algal growth was recorded at 3, 7, 14 and 21 days and compared with the growth in a control flask containing 15 ml of normal strength inoculated culture medium without Vanillin.
GLP:	Yes ( ) No (x) ? ( )
Test substance:	Vanillin; no further data.
Remarks:	Only concentration tested.
Reference:	Palmer et al, 1955.

(e)

Species:	Chlorella vulgaris (algae)
End point:	Biomass (x); Growth rate ( ); Other ( )
Exposure period:	80 hours
Results:	EC_{50} (80h) = ca. 1 mmol/l EC_{xx} (...h) = NOEC = LOEC =
Analytical monitoring:	Yes (x) No ( ) ? ( )
Method:	Other
GLP:	Yes ( ) No (x) ? ( )
Test substance:	Vanillin from Fluka, Buchs, Switzerland.
Remarks:	Test temperature: 26^{o}C. Growth inhibition: - after 80h: 50% at 1 mmol/l - after 160h: 30% at 1 mmol/l.
Reference:	Dedonder et al, 1971.

4.4 TOXICITY TO BACTERIA

(a)

Type:	Aquatic (x); Field ( ); Soil ( ); Other ( )
Species:	Anaerobic sludge
Exposure period:	49 hours
Results:	EC_{10} (...h) = EC_{50} (49h) = 1800 mg/l EC_{80} (49h) = 1880 mg/l EC_{100}(...h) =
Analytical monitoring:	Yes ( ) No (x) ? ( )
Method:	The methanogenic inhibition was determined at 30^{o}C in standard toxicity assays, utilizing anaerobic granular sludge as inoculum.
GLP:	Yes ( ) No ( ) ? (x)
Test substance:	Vanillin - commercially available.
Remarks:	50% and 80% methane production inhibition concentrations. Specific methanogenic activity measurements were performed in 0.3 dm^3 glass serum flasks. Granular sludge (1g VSS (volatile suspended solids)/dm^3, from a full scale reactor treating distillery wastewater, not acclimated to the toxicants) was transferred to 0.1 l of the basal medium, and acetate was added from a neutralized at pH7 stock solution

to obtain a final concentration of 2 g COD/l. Subsequently, the flasks were sealed and placed in a reciprocating shaker at 30 +/- 2°C.
After 1 day of incubation, the acetate concentration was measured and replenished to obtain 2 g COD/l. The required amount of inhibitory compound was added.
After 2 days of exposure, the acetate concentration was replenished to 2 g COD/l, and the bottles were reincubated for 1 hour prior to the determination of the methane production rate. The methane composition in the head space content of each serum flask was determined periodically during the subsequent 4 to 5 hours.

Reference: Sierra-Alvarez et al, 1991.

(b)

Type: Aquatic (x); Field ( ); Soil ( ); Other ( )
Species: Photobacterium phosphoreum (bacteria)
Exposure period: 5 minutes
Results: EC_{10} (...h) =
EC_{50} (5 min) = 0.38 mmol/l
EC_{100}(...h) =
Analytical monitoring: Yes ( ) No (x) ? ( )
Method: Microtox Test
GLP: Yes ( ) No ( ) ? (x)
Test substance: Vanillin. (Determined according to the standard method defined by Beckman Instruments Inc. (1982)). No further data.
Remarks: Mean of replicate tests.
Reference: Cronin et al, 1991.

(c)

Type: Aquatic (x); Field ( ); Soil ( ); Other ( )
Species: Saccharomyces cerevisiae
Exposure period: 210 minutes
Results: EC_{10} (...h) =
EC_{50} (210 min) = 179 mg/l
EC_{100}(...h) =
Analytical monitoring: Yes ( ) No (x) ? ( )
Method: The Yeast Test
GLP: Yes ( ) No ( ) ? (x)
Test substance: Vanillin. Of highest grade purity, checked by chromatographic and spectrophotometric methods.
Remarks: Test temperature: 30°C.
The initial density was adjusted to approx. E+8 cells per ml.
The inhibition of the growth rate is evaluated by counting the cell number with a microscope or a Coulter counter.
Reference: Koch et al. 1993.

(d)

Type: Aquatic ( ); Field ( ); Soil (x); Other ( )
Species: White-rot fungi
Exposure period: 96 hours
Results: EC_{10} (...h) =
EC_{50} (...h) =
EC_{100}(...h) =

Vanillin inhibited fungal growth by:
- 0-33% at 1 mmol/l
- 76-100% at 5 mmol/l
- no growth at 10 mmol/l

Analytical monitoring: Yes ( ) No (x) ? ( )

Method: Fungal growth measurements:
Radial growth was measured at four equidistant points, on plates inoculated in the centre with a 3 mm diam. agar disc taken from the growing edge of each fungal culture

GLP: Yes ( ) No ( ) ? (x)

Test substance: Vanillin. No further data.

Remarks: Strains tested 96h:
- Bjerkandera adusta
- Coriolus versicolor
- Phlebia radiata
- Polyporus dichrous
- Pycnoporus cinnabarinus

Strains tested 42h:
- Pleorotus ostreatus

Strains tested 30h:
- Phanerochaete chrysosporium K3 and PHE3.

Fungal growth rate were linear with time.

Reference: Buswell et al, 1994.

(e)

Type: Aquatic (x); Field ( ); Soil ( ); Other ( )

Species: Microcystis aeruginosa (blue-green algae)

Exposure period: 3, 7, 14 and 21 days

Results: EC_{10} (...h) =
EC_{50} (...h) =
EC_{100}(...h) =
NOEC = 2 mg/l
LOEC =
Non-toxic after 3, 7, 14 and 21 days exposure (growth in the presence of Vanillin was similar to that in the control flask).

Analytical monitoring: Yes ( ) No (x) ? ( )

Method: Static - LAB.
Culture medium approximated Gerloff's modification of Chu No 10 with the amount of nitrate doubled (Palmer and Maloney, 1953).
Tests carried out in 25 ml Erlenmeyer flasks, incubated at 22^{o}C.
Sterile, double strength medium with 1/15 to 1/30 by volume of an actively growing culture of the alga.
7.5 ml of this inoculated medium was combined with a like quantity of distilled water containing 4 ppm by weight of Vanillin (final concentration of 2 ppm Vanillin in normal strength medium, inoculum of 1/30 to 1/60 the volume of the original algal culture).
Number of algal cells in the medium at the start of the test: Approx. 125,000 cells/ml.
Visible algal growth was recorded at 3, 7, 14 and 21 days and compared with the growth in a control flask containing 15 ml of normal strength inoculated culture medium without Vanillin.

GLP: Yes ( ) No (x) ? ( )

Test substance: Vanillin; no further data.
Remarks: Only concentration tested.
Reference: Palmer et al, 1955.

(f)
Type: Aquatic (x); Field ( ); Soil ( ); Other ( )
Species: Cylindrospermium licheniforme (blue-green algae)
Exposure period: 3, 7, 14 and 21 days
Results: EC_{10} (...h) =
EC_{50} (...h) =
EC_{100}(...h) =
NOEC = 2 mg/l
LOEC =
Non-toxic after 3, 7, 14 and 21 days exposure (growth in the presence of Vanillin was similar to that in the control flask).
Analytical monitoring: Yes ( ) No (x) ? ( )
Method: Static - LAB.
Culture medium approximated Gerloff's modification of Chu No 10 with the amount of nitrate doubled (Palmer and Maloney, 1953).
Tests carried out in 25 ml Erlenmeyer flasks, incubated at 22^{o}C.
Sterile, double strength medium with 1/15 to 1/30 by volume of an actively growing culture of the alga.
7.5 ml of this inoculated medium was combined with a like quantity of distilled water containing 4 ppm by weight of Vanillin (final concentration of 2 ppm Vanillin in normal strength medium, inoculum of 1/30 to 1/60 the volume of the original algal culture).
Number of algal cells in the medium at the start of the test: Approx. 125,000 cells/ml.
Visible algal growth was recorded at 3, 7, 14 and 21 days and compared with the growth in a control flask containing 15 ml of normal strength inoculated culture medium without Vanillin.
GLP: Yes ( ) No (x) ? ( )
Test substance: Vanillin; no further data.
Remarks: Only concentration tested.
Reference: Palmer et al, 1955.

4.5 CHRONIC TOXICITY TO AQUATIC ORGANISMS

4.5.1 CHRONIC TOXICITY TO FISH

Type of test: Static ( ); Semi-static ( ); Flow-through ( ); Other ( )
Open-system ( ); Closed-system ( )
Species:
End point: Length of young fish ( ); Weight of young fish ( );
Reproduction rate ( ); Other ( )
Exposure period:
Results: EC_{50} (...d) =
EC_{xx} (...d) =
NOEC =
LOEC =
Analytical monitoring: Yes ( ) No ( ) ? ( )

Method:
GLP: Yes ( ) No ( ) ? ( )
Test substance:
Remarks: No data
Reference:

4.5.2 CHRONIC TOXICITY TO AQUATIC INVERTEBRATES

Type of test: Static ( ); Semi-static (x); Flow-through ( ); Other ( )
Open-system ( ); Closed-system (x)
Species: Daphnia magna (strain A)
End point: Mortality (x); Reproduction rate (x); Other ( )
Exposure period: 21 days
Results: Immobilisation of all animals occured at the highest concentration tested (100 mg/l) within 13 days exposure.
No immobilised animals were recorded in the controls and at concentrations up to 56 mg/l.
Hence the EC_{50} value for immobilisation estimated as the geometric mean concentration for 0 and 100% immobilisation was 75 mg/l from 13 days to 21 days exposure.

Nominal conc. Measured	conc.	EC_{50} (21d)
(reproduction) =	24 mg/l	16 mg/l
NOEC (reproduction) =	10 mg/l	5.9 mg/l
LOEC (reproduction) =	18 mg/l	10 mg/l

Analytical monitoring: Yes (x) No ( ) ? ( )
Method: OECD TG 202 (1984) "Daphnia sp., acute Immobilisation Test and Reproduction Test.
GLP: Yes (x) No ( ) ? ( )
Test substance: As prescribed by 1.1 -1.2.
Purity: 99.9%
Remarks: Weighted mean concentrations of Vanillin in the test medium was calculated from the measured concentrations as described in the draft revised OECD TG 202 part II (1996).
Reference: Kllqvist, 1996b.

4.6 TOXICITY TO TERRESTRIAL ORGANISMS

4.6.1 TOXICITY TO SOIL DWELLING ORGANISMS

Type: Artificial Soil ( ); Filter paper ( ); Other (x)
Species: Eisenia foetida (Earthworm)
End point: Mortality (x); Weight (x); Other ( )
Exposure period: 42 days
Results: EC_{50} (...d) =
EC_{xx} (...d) =
NOEC = approx. 10,000 mg/kg soil dw
LOEC = approx. 40,000 mg/kg soil dw
Method: Activated sludge (ca. 13% solids) with test substance were placed over a ca. 4 mm depth of silt loam in a Petri dish.

	The amount of substance tested was mixed with the activated sludge. There were 2 hatchlings per concentration and 5 replicates per concentration. Concentrations: approx. 0, 0.1, 1.0, 4 and 8% (w/w). Stored at 24°C.
GLP:	Yes ( ) No ( ) ? (x)
Test substance:	Vanillin from Aldrich Chemical Co., Milwaukee, WI, USA. Purity: No data.
Remarks:	The mortality at LOEC was 80%.
Reference:	Hartenstein, 1982.

4.6.2 TOXICITY TO TERRESTRIAL PLANTS

(a)

Species:	Lactuca sativa (Lettuce)
End point:	Emergence (x); Growth ( ); Other ( )
Exposure period:	3 days
Results:	EC_{50} /LC_{50}(7d) = EC_{50}/LC_{50}(14d) = EC_{xx}/LC_{50}(3d) =4.26 (+/-0.20) mmol/l NOEC = LOEC =
Method:	Germination tests carried out on agar at 30°C with Lactuca sativa L. cv. Great Lakes as described by Reynolds (1957, 1977) using aqueous solution of Vanillin and inhibitory activity expressed as the millimolar concentration producing 50% reduction in percentage germination compared with water controls at given temperature.
GLP:	Yes ( ) No (x) ? ( )
Test substance:	Vanillin. No further data.
Remarks:	95% confidence limits.
Reference:	Reynolds, 1978.

(b)

Species:	Triticum aestivum L. (Wheat) Other terrestrial plant: Gossypium hirsutum L. (Cotton)
End point:	Emergence ( ); Growth (x); Other ( )
Exposure period:	No data
Results:	EC_{50} /LC_{50}(7d) = EC_{50}/LC_{50}(14d) = EC_{xx}/LC_{xx}(...d) = NOEC = LOEC =
Method:	Petri dish bioassay The potential allelopathic activity of devil's-claw essential oil and a few of the components it contains (incl. Vanillin) on the elongation of cotton and wheat radicles was studied using a Petri dish bioassay.
GLP:	Yes ( ) No ( ) ? (x)
Test substance:	Vanillin from Sigma Chemical Co., St. Louis, Missouri 63178, USA. Purity: No data

Remarks: Vanillin, identified by CGC-MS-DS in the root and pod, was found to be visually observable inhibitory to cotton, but not to wheat at a concentration of 1 mmol in methanol.
30 mg Vanillin/dish (2 ml of a 1mmol solution added to the dish) was 11% inhibitory to cotton radicles, but not inhibitory to wheat.
Reference: Riffle et al, 1990.

4.6.3 TOXICITY TO OTHER NON-MAMMALIAN TERRESTRIAL ORGANISMS

Species:
End point: Mortality ( ); Reproduction rate ( ); Weight ( ); Other ( )
Exposure period:
Results: LD_{xx} /LC_{xx}(...d) =
NOEC =
LOEC =
Method:
GLP: Yes ( ) No ( ) ? ( )
Test substance:
Remarks: No data
Reference:

4.7 BIOLOGICAL EFFECTS MONITORING (INCLUDING BIOMAGNIFICATION)

Results: Substance:
Species or ecosystem studied:
Effects monitored:
Results:
Chemical analysis:
Remarks: No data
Reference:

4.8 BIOTRANSFORMATION AND KINETICS IN ENVIRONMENTAL SPECIES

Type: Animal ( ); Aquatic ( ); Plant ( ); Terrestrial ( ); Other ( )
Results: No data
Remarks:
Reference:

4.9 ADDITIONAL REMARKS

Results: No data
Remarks:
Reference:

5. TOXICITY

5.1 ACUTE TOXICITY

5.1.1 ACUTE ORAL TOXICITY

(a)

Type:	LD_0 ( ); LD_{100} ( ); LD_{50} (x); LDL_0 ( ); Other ( )
Species/strain:	Rat, Sprague Dawley
Value:	3925[1] and 3978[2] mg/kg
Method:	OECD TG 401 (1987) and Directive 84/449/EEC, B.1 (1984) "Acute toxicity (oral)"
GLP:	Yes (x) No ( ) ? ( )
Test substance:	As prescribed by 1.1-1.2. (Lot no. 90-24-701 from Rhône-Poulenc, France) Purity: 99.9%
Remarks:	Single ingastric intubation as a suspension in aqueous solution of 1% (w/v) carboxymethylcellulose at the dose levels 2000, 2510, 3160 and 3980 mg/kg. 5 males and 5 females per group. (1) Bliss' method: LD_{50} = 3978 mg/kg (2484-6368) (2) Litchfield & Wilcoxon's method: LD_{50} = 3925 mg/kg (2834-5435) Body weight gained similar to control; no macroscopical anomali observed, except some congestive lungs in dead animals.
Reference:	Lheritier, 1992.

(b)

Type:	LD_0 ( ); LD_{100} ( ); LD_{50} (x); LDL_0 ( ); Other ( )
Species/strain:	Rat, Sprague Dawley
Value:	4200[1], 3800[2] and 4600[3] mg/kg bw
Method:	OECD TG 401 and Directive 79/831/EEC Annex V, B1.
GLP:	Yes ( ) No ( ) ? (x)
Test substance:	As prescribed by 1.1-1.2. (Batch no. 132 dated 18.09.86, from EuroVanillin KS, Norway) Purity: 99.8%
Remarks:	Dose levels: 2500, 3200, 4000 and 5000 mg/kg. 5 males and 5 females per group. 95% confidence limits in parenthesis: (1) Males and females combined: LD_{50} = 4200 mg/kg (3600-5400) (2) Males only: LD_{50}= 3800 mg/kg (2900-5000) (3) Females only: LD_{50} = 4600 mg/kg (3700-6900)
Reference:	Gardner, 1987.

(c)

Type:	LD_0 ( ); LD_{100} ( ); LD_{50} (x); LDL_0 ( ); Other ( )
Species/strain:	Rat, Sprague Dawley
Value:	3300 mg/kg
Method:	Similar to OECD TG 401.
GLP:	Yes ( ) No (x) ? ( )

Test substance: Vanillin from Monsanto Chemical Company (USA)
Remarks: Vanillin in suspension of 10% corn oil.
5 rats per group.
Dose levels: 2510, 3160 and 3980 mg/kg.
LD_{50}: 3300 mg/kg (3100-3530).
At autopsy, lung and liver hypermia and gastrointestinal inflammation in dead animals. Viscera appeared normal in survivors.
Reference: Younger Laboratories Inc., 1976.

(d)
Type: LD_0 ( ); LD_{100} ( ); LD_{50} (x); LDL_0 ( ); Other ( )
Species/strain: Rat, Osborne Mendel
Value: 1580 mg/kg
Method: Litchfield & Wilcoxon (1949)
GLP: Yes ( ) No (x) ? ( )
Test substance: Vanillin; commercially available material.
Remarks: Vanillin was diluted in propyleneglycol to a 20% (w/v) solution.
10 young adult rats, evenly divided by sex.
Were fasted approx. 18 hours prior to treatment.
Observation period: 2 weeks.
Coma soon after treatment.
Death time: 4 hours to 4 days.
Reference: Jenner et al, 1964.

(e)
Type: LD_0 ( ); LD_{100} ( ); LD_{50} (x); LDL_0 ( ); Other ( )
Species/strain: Rat
Value: 2000 mg/kg
Method: No data
GLP: Yes ( ) No (x) ? ( )
Test substance: Vanillin; no further data
Remarks: Single doses of Vanillin as a suspension in corn oil.
95% confidence limits in parenthesis:
LD_{50} = 2000 mg/kg (1600-2500).
Reference: Hake et al, 1963.

(f)
Type: LD_0 ( ); LD_{100} ( ); LD_{50} (x); LDL_0 ( ); Other ( )
Species/strain: Rat, albino
Value: 3830 mg/kg
Method: Thompson moving average method.
GLP: Yes ( ) No (x) ? ( )
Test substance: Vanillin from Monsanto Chemical Company, USA.
Remarks: Administration as a 20% or 40% suspension in 0,5% solution of methyl cellulose.
5 male rats per group.
Dose levels: 2150, 3160, 4640, 6810 and 10,000 mg/kg.
Observation period: 7 days.
LD_{50} = 3830 mg/kg (2930-5000).
Hemorragic lungs, irritation gastrointestinal, congested kidneys and adrenals in dead animals.
No gross pathology in survivors.

Reference: Hazleton Laboratory, 1955.

(g)

Type: LD_0 ( ); LD_{100} ( ); LD_{50} (x); LDL_0 ( ); Other ( )
Species/strain: Guinea Pig
Value: 1400 mg/kg (1310-1500 mg/kg)
Method: Litchfield & Wilcoxon (1949).
GLP: Yes ( ) No (x) ? ( )
Test substance: Vanillin; commercially available material.
Remarks: Vanillin was diluted in propyleneglycol to a 20% (w/v) solution.
10 guinea pigs, evenly divided by sex.
Were fasted approx. 18 hours prior to treatment.
Observation period: 2 weeks.
Depression within 1 hour.
Death time: 1-3 days.
Reference: Jenner et al, 1964.

5.1.2 ACUTE INHALATION TOXICITY

Type:
Species/strain:
Exposure time:
Value: No data
Method:
GLP:
Test substance:
Remarks:
Reference:

5.1.3 ACUTE DERMAL TOXICITY

(a)

Type: LD_0 (x); LD_{100} ( ); LD_{50} ( ); LDL_0 ( ); Other ( )
Species/strain: Rat, Sprague Dawley
Value: >= 2000 mg/kg
Method: OECD TG 402 (1987), Directive 84/449/EEC (1984), E.P.A. guideline no. 798.1100 (1985) and M.A.F.F. guideline no. 4200 (1985).
GLP: Yes (x) No ( ) ? ( )
Test substance: As prescribed by 1.1-1.2. (Lot no. 90-24-701 from Rhône-Poulenc, France)
Purity: 99.9%
Remarks: Limit test.
5 male and 5 female.
Unique dose 2000 mg/kg.
A paste of ca. 70% Vanillin in purified water was applied on the shaved skin (10% body area) for 24 hours using semi-occusive patch.
Examination after 15 minutes, 1,2 and 4 hours and daily for 14 days.
No mortality or pathological clinical sign.
No cutaneous lesions.
No macroscopic anomali at necropsy.

Reference: Lheritier, 1991.

(b)
Type: LD_0 ( ); LD_{100} ( ); LD_{50} (x); LDL_0 ( ); Other ( )
Species/strain: Rabbit
Value: > 5010 mg/kg
Method: Other
GLP: Yes ( ) No (x) ? ( )
Test substance: Vanillin from Monsanto Chemical Company, USA.
Remarks: Applied as a 40% solution suspended in corn oil.
Exposure for 24 hours.
1 rabbit per dose level.
No mortality after 14 days at 3160 and 5010 mg/kg.
Mortality after 3 days at 7940 mg/kg.
Reference: Younger Laboratories Inc, 1976.

5.1.4 ACUTE TOXICITY, OTHER ROUTES OF ADMINISTRATION

(a)
Type: LC_0 ( ); LC_{100} ( ); LC_{50} ( ); LCL_0 ( ); Other ( )
LD_0 ( ); LD_{100} ( ); LD_{50} (x); LDL_0 ( ); Other ( )
Species/strain: Rat
Route of administration: i.m. ( ); i.p. (x); i.v. ( ); Infusion ( ); s.c. ( ); Other ( )
Exposure time: No data
Value: 1160 mg/kg
Method: No data
GLP: Yes ( ) No (x) ? ( )
Test substance: No data
Remarks:
Reference: Caujolle et al, 1956.

(b)
Type: LC_0 ( ); LC_{100} ( ); LC_{50} ( ); LCL_0 ( ); Other ( )
LD_0 ( ); LD_{100} ( ); LD_{50} (x); LDL_0 ( ); Other ( )
Species/strain: Mouse
Route of administration: i.m. ( ); i.p. (x); i.v. ( ); Infusion ( ); s.c. ( ); Other ( )
Exposure time: No data
Value: 780 mg/kg
Method: No data
GLP: Yes ( ) No (x) ? ( )
Test substance: No data
Remarks:
Reference: Caujolle et al, 1954.

(c)
Type: LC_0 ( ); LC_{100} ( ); LC_{50} ( ); LCL_0 ( ); Other ( )
LD_0 ( ); LD_{100} ( ); LD_{50} (x); LDL_0 ( ); Other ( )
Species/strain: Mouse
Route of administration: i.m. ( ); i.p. (x); i.v. ( ); Infusion ( ); s.c. ( ); Other ( )
Exposure time: No data
Value: 475 mg/kg

Method: No data
GLP: Yes ( ) No (x) ? ( )
Test substance: No data
Remarks:
Reference: Frazer, 1967.

(d)
Type: LC_0 ( ); LC_{100} ( ); LC_{50} ( ); LCL_0 ( ); Other ( )
LD_0 ( ); LD_{100} ( ); LD_{50} (x); LDL_0 ( ); Other ( )
Species/strain: Guinea pig
Route of administration: i.m. ( ); i.p. (x); i.v. ( ); Infusion ( ); s.c. ( ); Other ( )
Exposure time: No data
Value: 1190 mg/kg
Method: No data
GLP: Yes ( ) No (x) ? ( )
Test substance: No data
Remarks:
Reference: Caujolle et al, 1954.

(e)
Type: LC_0 ( ); LC_{100} ( ); LC_{50} ( ); LCL_0 ( ); Other ( )
LD_0 ( ); LD_{100} ( ); LD_{50} (x); LDL_0 ( ); Other ( )
Species/strain: Rat, albino
Route of administration: i.m. ( ); i.p. ( ); i.v. ( ); Infusion ( ); s.c. (x); Other ( )
Exposure time: Untill death
Value: 2600 mg/kg bw.
Method: Vanillin was dissolved in milk (4%) by slow heating to 90^{o}C, and cooling to 37^{o}C. Injected subcutaneously into young albino rats.
GLP: Yes ( ) No (x) ? ( )
Test substance: Vanillin.
Purity: high degree of purity, obtained from commercial sources.
Remarks: Time till death: 2,5 hours - 4 days.
1000 and 1400 mg/kg had no lethal effect, 1800 mg/kg killed 20% of the rats, and 2200 mg/kg killed 40% of the rats.
Reference: Deichman et al, 1940.

(f)
Type: LC_0 ( ); LC_{100} ( ); LC_{50} ( ); LCL_0 ( ); Other ( )
LD_0 ( ); LD_{100} ( ); LD_{50} ( ); LDL_0 (x); Other ( )
Species/strain: Dog
Route of administration: i.m. ( ); i.p. ( ); i.v. (x); Infusion ( ); s.c. ( ); Other ( )
Exposure time: No data
Value: 1320 mg/kg
Method: Slow i.v. infusion
GLP: Yes ( ) No (x) ? ( )
Test substance: No data
Remarks:
Reference: Caujolle et al, 1953.

5.2 CORROSIVENESS/IRRITATION

5.2.1 SKIN IRRITATION/CORROSION

(a)

Species/strain: Rabbit

Results: Highly corrosive ( ); Corrosive ( ); Highly irritating ( ); Irritating ( ); Moderate irritating ( ); Slightly irritating ( ); Not irritating (x)

Classification: Highly corrosive (causes severe burns) ( );
Corrosive (caused burns) ( ); Irritating ( ); Not irritating (x)

Method: No data

GLP: Yes ( ) No (x) ? ( )

Test substance: Vanillin from Monsanto Chemical Company, USA

Remarks: 6 rabbits.
Applicated as a finely ground sample moistened with water.
Exposure for 24 h.
No irritation.

Reference: Younger Laboratories Inc, 1976.

(b)

Species/strain: Human

Results: Highly corrosive ( ); Corrosive ( ); Highly irritating ( ); Irritating ( ); Moderate irritating ( ); Slightly irritating ( ); Not irritating (x)

Classification: Highly corrosive (causes severe burns) ( );
Corrosive (caused burns) ( ); Irritating ( ); Not irritating (x)

Method: Closed patch test (24-72 hours)

GLP: Yes ( ) No (x) ? ( )

Test substance: Vanillin; no further data

Remarks: No primary irritation when tested in the following closed patch tests:
1)
Vanillin concentration: 20%
Media: Vaseline Aldum, Unguentum Hydrophilicum
Number of subjects: 29 (normal health)
Site of application: small of back
Duration: 48 hours
Result: Negative in 29 sucjects
2)
Vanillin concentration: 2%
Media: Unguentum Simplex, Unguentum Hydrophilicum
Number of subjects: 30 (normal health)
Site of application: upper inside of arm
Duration: 24-72 hours
Result: Negative in 30 subjects
3)
Vanillin concentration: 0.4 %
Media: 99% Ethanol, Non-irritative Cream base
Number of subjects: 35 (with dermatoses)
Site of application: upper inside of arm
Duration: 24-48 hours
Result: negative in 35 subjects

Reference: Fujii et al, 1972.

(c)

Species/strain: Human

Results: Highly corrosive ( ); Corrosive ( ); Highly irritating ( ); Irritating ( ); Moderate irritating ( ); Slightly irritating ( ); Not irritating (x)

Classification: Highly corrosive (causes severe burns) ( );
Corrosive (caused burns) ( ); Irritating ( ); Not irritating ( )

Method: Closed patch test 48 h.

GLP: Yes ( ) No (x) ? ( )

Test substance: Vanillin; no further data

Remarks: Closed patch tests.
Exposure: 48 hours (reading 1 hour later) with pure Vanillin.
30 employees (in packaging section, packaging done manually), some of which have/had dermatitis and 15 healthy workers from other units.
Negative results only.

Reference: Wang et al, 1987.

(d)

Species/strain: Guinea pig

Results: Highly corrosive ( ); Corrosive ( ); Highly irritating ( ); Irritating ( ); Moderate irritating ( ); Slightly irritating ( ); Not irritating (x)

Classification: Highly corrosive (causes severe burns) ( );
Corrosive (caused burns) ( ); Irritating ( ); Not irritating ( )

Method: Closed patch test.

GLP: Yes ( ) No (x) ? ( )

Test substance: Vanillin; no further data

Remarks: Albino guinea pigs (300-450g), 6-8 weeks old, male and female.
5 pigs:
- 1, 2, 5 and 10% preparations of Vanillin in petrolatum.
- 0.1 g applied.
- Removed after 48 hours, reading 1, 24 and 48 hours later.
- Result: negative.

10 pigs:
- 10% preparation of Vanillin in petrolatum.
- 0.1 g applied.
- Exposure 24 hours.
- Repeated 3 times a week for 2 weeks.
- 2 weeks after 2 and 5% preparations and pure Vanillin were applied and removed 48 hours later.
- Reading after 1, 24 and 48 hours.
- All negative results.

Reference: Wang et al, 1987.

5.2.2 EYE IRRITATION/CORROSION

(a)

Species/strain: Rabbit

Results: Highly corrosive ( ); Corrosive ( ); Highly irritating ( ); Irritating ( ); Moderate irritating ( ); Slightly irritating (x); Not irritating ( )

Classification: Irritating ( ); Not irritating (x); Risk of serious damage to eyes ( )

Method: No data

GLP: Yes ( ) No (x) ? ( )

Test substance:	Vanillin from Monsanto Chemical Company, USA.
Remarks:	6 rabbits. Application as finely ground powder (dose equivalent to 0.1 ml volume: 55 mg sample). Exposure for 24 hours. Irritating score: 18.8/110. Gradually improvement from 48 to 120 hours. All scored zero after 168 hours.
Reference:	Younger Laboratories Inc, 1976.

5.3 SKIN SENSITISATION

(a)

Type:	Buehler test
Species/strain:	Guinea pig
Results:	Sensitizing ( ); Not sensitizing (x); Ambigous ( )
Classification:	Sensitizing ( ); Not sensitizing ( )
Method:	Closed patch test
GLP:	Yes ( ) No (x) ? ( )
Test substance:	Vanillin; no further data
Remarks:	Albino guinea pigs (300-450g), 6-8 weeks old, male and female. 5 pigs: - 1, 2, 5 and 10% preparations of Vanillin in petrolatum. - 0.1 g applied. - Removed after 48 hours, reading 1, 24 and 48 hours later. - Result: negative for all animals. 10 pigs: - 10% preparation of Vanillin in petrolatum. - 0.1 g applied. - Exposure 24 hours. - Repeated 3 times a week for 2 weeks. - 2 weeks after 2 and 5% preparations and pure Vanillin were applied and removed 48 hours later. - Reading after 1, 24 and 48 hours. - Negative results for all animals.
Reference:	Wang et al, 1987.

(b)

Type:	Draize-test
Species/strain:	Guinea pig
Results:	Negative. Sensitizing ( ); Not sensitizing (x); Ambigous ( )
Classification:	Sensitizing ( ); Not sensitizing (x)
Method:	Similar to Directive 84/449/EEC, B.6. A dose of 0.05 ml of a 0.1 per cent solution of the compound tested in isotonic saline was injected intradermally on day 0 and further doses of 0.1 ml each were injected on 9 alternate days (total dose 0.95 mg). The treated animals and untreated controls were challenged intradermally with 0.05 ml of a 0.1 per cent solution on days 35 and 49. The evaluation criterion was the mean diameter of the papular reactions.
GLP:	Yes ( ) No (x) ? ( )

Test substance:	Vanillin; no further data
Remarks:	6 to 8 guinea pigs per concentration group. Weight 400-500 g.
Reference:	Klecak et al, 1977.

(c)

Type:	Freund's complete adjuvant test
Species/strain:	Guinea pig
Results:	Sensitizing (x); Not sensitizing ( ); Ambigous ( )
Classification:	Sensitizing ( ); Not sensitizing ( )
Method:	Doses of 0.05 ml of the undiluted compound mixed with the same volume of FCA (Freund's complete adjuvant) were injected into the neck on days 0, 2, 4, 7 and 9 (total dose: 250 mg). The control animals were similarly treated with 5x0.05 ml of FCA alone. All the animals were subjects to epicutaneous test on days 21 and 35.
GLP:	Yes ( ) No (x) ? ( )
Test substance:	Vanillin; no further data
Remarks:	6 to 8 guinea pigs per concentration group. Weight: 400-500 g. No further data on percentage of animals with positive result.
Reference:	Klecak et al, 1977.

(d)

Type:	Freund's complete adjuvant test (modified)
Species/strain:	Guinea pig
Results:	Weak sensitizing capacity at 10% concentration; mean response: 1.17 (quotient of the sum of all reactions obtained, divided by the total number of animals tested). Sensitizing ( ); Not sensitizing ( ); Ambigous (x)
Classification:	Sensitizing ( ); Not sensitizing ( )
Method:	See Remarks
GLP:	Yes ( ) No ( ) ? (x)
Test substance:	Vanillin from Merck; purified by preparative TLC (thin-layer chromatography), eluent: chloroform-methanol (100+2) (Haarmann & Reimer, Holzminden, Germany).
Remarks:	10 guinea pigs. Challenge concentration: 10% in acetone. The compounds tested were divided into groups of weak, moderate or strong sensitizing capacity. No further data on percentage of animals with positive result.
Reference:	Hausen et al, 1992.

(e)

Type:	Guinea pig maximization test
Species/strain:	Guinea pig, Dunkin Hartley
Results:	Sensitizing ( ); Not sensitizing (x); Ambigous ( )
Classification:	Sensitizing ( ); Not sensitizing (x)
Method:	OECD TG 406 (1981), Directive 84/499/EEC Annex V, B.6 (1984) and E.P.A. guideline no 798.4100 (1985).
GLP:	Yes (x) No ( ) ? ( )
Test substance:	As prescribed by 1.1-1.2 (Lot no. 90-24-701 from Rhône-Poulenc, France) Purity: 99.9%

Remarks:	40 albino guinea pigs of both sexes; one control group and one treated group. Control group: Induction: vehicle, Challenge: test article (Vanillin crystals). Treated group: Induction and challenge: test article. 1) Induction by 3 series of 2 intradermal injections: - FCA - Vanillin 35% in ethanol solution - Vanillin 17.5% in ethanol and FCA 2) Topical occlusive application for 48 hours - Vanillin as a 73% paste in ethanol 3) Rest period for 11 days 4) Topical occlusive application for 24 hours as a 73% paste in ethanol. Signs of irritation were noted during the induction. The test article did not provoke any reaction of cutaneous sensitization in the animals examined.
Reference:	Mercier, 1992.

(f)

Type:	Guinea pig maximization test
Species/strain:	Guinea pig, Hartley
Results:	Sensitizing (x); Not sensitizing ( ); Ambigous ( )
Classification:	Sensitizing ( ); Not sensitizing ( )
Method:	See Remarks
GLP:	Yes ( ) No ( ) ? (x)
Test substance:	Vanillin from Allied Corporation. Purity: >98%
Remarks:	15 guinea pigs, 6 controls. Vehicle: petrolatum Concentrations: Intradermal injection: 50% Topical induction: 50% Challenge (Split adjuvant (Klecak, 1983)): 50%. Induction: 2 intradermal injections, followed by (day 7) closed patch application to id injected skin site for 48 hours. Challenge: Day 21, closed patch application for 24 hours to naive skin site. Positive results in 60% of animals.
Reference:	Gad et al, 1986, Gad, 1988.

(g)

Type:	Guinea pig maximization test
Species/strain:	Guinea pig
Results:	Sensitizing (x); Not sensitizing ( ); Ambigous ( )
Classification:	Sensitizing ( ); Not sensitizing ( )
Method:	No data
GLP:	Yes ( ) No ( ) ? (x)
Test substance:	No data
Remarks:	Intradermal injection and/or topical application. Positive result; 10-50% Vanillin. No further data on percentage of animals with positive result. Moderate sensitizer.
Reference:	Ishihara et al, 1986.

(h)

Type:	Guinea pig maximization test
Species/strain:	Guinea pig
Results:	Sensitizing (x); Not sensitizing ( ); Ambigous ( )
Classification:	Sensitizing ( ); Not sensitizing ( )
Method:	Induction: Day 0: Intradermal injection of 0.1 ml of a 5% solution of the compound, of 0.1 ml of a 5% emulsion of the compound in FCA (Freund's complete adjuvant) and of 0.1 ml of FCA alone. Each injection was given twice. Day 8: Application, for 2 days under occlusive bandage to a clipped skin area of the neck, of 250 mg of the compound dissolved in petrolatum at a concentration of 25% (wich always causes mild to moderate skin irritation under occlusion). Total dose: 20 mg intradermally plus 250 mg epicutaneously. Challenge: Day 21: Occlusive patch test at a subirritant concentration (unspecified) in petrolatum, applied to the flank for 24 hours. Reading 24 and 48 hours after removing the patch.
GLP:	Yes ( ) No (x) ? ( )
Test substance:	Vanillin; no further data
Remarks:	6 to 8 guinea pigs per concentration group. No further data on percentage of animals with positive result.
Reference:	Klecak et al, 1977.

(i)

Type:	Open epicutaneous test
Species/strain:	Guinea pig
Results:	Not sensitizing. Undiluted Vanillin: positive. Sensitizing ( ); Not sensitizing ( ); Ambigous (x)
Classification:	Sensitizing ( ); Not sensitizing ( )
Method:	Induction: Application of 0.1 ml undiluted compound and diluted solutions to shaved skin, repeated daily for 21 days, using the same skin site. Applications left uncovered. Reading 24 hours after each application. Minimum irritation concentration was determined. Minimum irritation concentration: 30% after 1 application, 3% after 21 applications. The same guinea pigs (+6-8 untreated controls), were tested on days 21 and 35 on contralateral flank, at the minimal irritating concentration and at some lower non-irritant concentrations (unspecified). Reading after 24, 48 and 72 hours. The minimal sensitizing concentration necessary to induce contact hypersensitivity was determined. A concentration was concidered allergenic when at least 2 out of 8 animals showed positive reactions with non-irritant concentrations used for challenge, based on practical experience.
GLP:	Yes ( ) No (x) ? ( )
Test substance:	Vanillin: no further data
Remarks:	Test concentrations: Undiluted, dissolved at concentrations of 30, 10, 3, 1, 0.3, 0.1 and 0.03 %. 6 to 8 guinea pigs per concentration group.

Reference: Klecak et al, 1977.

(j)

Type: Other: Maximization test
Species/strain: Human
Results: Sensitizing ( ); Not sensitizing (x); Ambigous ( )
Classification: Sensitizing ( ); Not sensitizing ( )
Method: Kligman Maximization Test
GLP: Yes ( ) No ( ) ? (x)
Test substance: Vanillin; no selections for pure grades, materials were picked as actually used.
Remarks: 25 healthy adults.
Patch test: 1.0 ml of 5% aqueous sodium lauryl sulfate solution for 24 hours to produce a moderate inflammatory reaction and render the skin more permeable to the test agent (1).
Same site: 48 hour occlusive patch applied with test material (2).
(1) and (2) are alternated for a total 5 exposures of each, period of 15 days, 10 days rest period.
Challenge.
A new site: 10% solution was applied for 1 hour. Washed off. Test material was applied to this new pretreated area; occlusive patch for 48 hours.
Test concentration: 2%.
Test area examined immediately and at 2 successive days.
No sensitizing reactions were induced in groups of 25 volunteers.
Reference: Greif, 1967.

(k)

Type: Mouse ear swelling test
Species/strain: Mouse, CF-1
Results: No animal sensitized.
Sensitizing ( ); Not sensitizing (x); Ambigous ( )
Classification: Sensitizing ( ); Not sensitizing (x)
Method: Alternative OECD method
GLP: Yes ( ) No ( ) ? (x)
Test substance: Vanillin from Allied Corp.
Purity: > 98%
Remarks: 10-15 female mice, 5-10 in control group.
Induction: Topical application at days 0, 1, 2, 3 and 4 to abdominal skin prepared by FCA intradermal injection.
Challenge: Day 10, topical application of test substance to one ear, of vehicle to the other.
Ear thickness measurement after 24 and 48 hours.
Vanillin 50% in ethanol 70%.
Reference: Gad et al, 1986, Gad, 1988.

5.4 REPEATED DOSE TOXICITY

(a)

Species/strain: Rat, Osborne-Mendel
Sex: Female ( ); Male ( ); Male/Female (x); No data ( )

Route of administration:	Oral feed
Exposure period:	16 weeks
Frequency of treatment:	Daily
Postexposure observ. period:	None
Dose:	10,000 ppm
Control group:	Yes (x); No ( ); No data ( )
	Concurrent no treatment ( ); Concurrent vehicle (x); Historical ( )
NOEL:	>= 10,000 ppm
LOEL:	
Results:	No effect on growth or haematology.
	No macroscopic or microscopic changes in the tissues (incl. testes).
Method:	5 male and 5 female rats (test and control groups).
	Vanillin was mixed in the diet. Fresh diets were made and distributed weekly. The concentration was adjusted so that all rats received a constant volume of 1 ml of solution/kg daily.
	The rat's weight, food intake and general condition were recorded every week.
	Haematological examinations were made at termination of the study. These examinations included white cell counts, red cell counts, haemoglobins and haematocrits.
	At the termination of the experiments the rats were sacrificed and exsaguinated. The tissues of all the rats were examined macroscopically at the time of sacrifice.
	The viscera were removed and the liver, kidneys, spleen, heart and testes were weighed.
	These organs, the remaining abdominal and thoracic viscera, and one hind leg, for bone, bone marrow, and muscle, were preserved in 10% buffered formalin-saline solution for histopathological examination.
	For routine histopathology, sections were embedded in paraffin wax and stained with haematoxylin and eosin.
	Detailed microscopic examinations in the subacute studies were generally done on 6 or 8 rats, evenly devided by sex, from the high dose group and the control group.
GLP:	Yes ( ) No (x) ? ( )
Test substance:	Vanillin; commercially available.
Reference:	Hagan et al, 1967.

(b)

Species/strain:	Rat, Osborne-Mendel
Sex:	Female ( ); Male ( ); Male/Female (x); No data ( )
Route of administration:	Oral feed
Exposure period:	27-28 weeks
Frequency of treatment:	Daily
Postexposure observ. period:	None
Dose:	1000 ppm
Control group:	Yes (x); No ( ); No data ( )
	Concurrent no treatment ( ); Concurrent vehicle (x); Historical ( )
NOEL:	>= 1000 ppm
LOEL:	
Results:	No effect on growth or haematology.
	No macroscopic or microscopic changes in the tissues (incl. testes).
Method:	5 male and 5 female rats (test and control groups).

	Vanillin was mixed in the diet. Fresh diets were made and distributed weekly. The concentration was adjusted so that all rats received a constant volume of 1 ml of solution/kg daily. The rat's weight, food intake and general condition were recorded every week. Haematological examinations were made after 3 months and at termination of the study. These examinations included white cell counts, red cell counts, haemoglobins and haematocrits. At the termination of the experiments the rats were sacrificed and exsaguinated. The tissues of all the rats were examined macroscopically at the time of sacrifice. The viscera were removed and the liver, kidneys, spleen, heart and testes were weighed. These organs, the remaining abdominal and thoracic viscera, and one hind leg, for bone, bone marrow, and muscle, were preserved in 10% buffered formalin-saline solution for histopathological examination. For routine histopathology, sections were embedded in paraffin wax and stained with haematoxylin and eosin. Detailed microscopic examinations in the subacute studies were generally done on 6 or 8 rats, evenly devided by sex, from the high dose group and the control group.
GLP:	Yes ( ) No (x) ? ( )
Test substance:	Vanillin; commercially available
Reference:	Hagan et al, 1967.

(c)

Species/strain:	Rat, Osborne-Mendel
Sex:	Female ( ); Male (x); Male/Female ( ); No data ( )
Route of administration:	Oral feed
Exposure period:	1 year
Frequency of treatment:	Daily
Postexposure observ. period:	None
Dose:	20,000 and 50,000 ppm
Control group:	Yes (x); No ( ); No data ( ) Concurrent no treatment ( ); Concurrent vehicle (x); Historical ( )
NOEL:	>= 50,000 ppm
LOEL:	
Results:	No effect on growth or haematology. No macroscopic or microscopic changes in the tissues (incl. testes).
Method:	5 male rats (test and control groups). Vanillin was mixed in the diet. Fresh diets were made and distributed weekly. The concentration was adjusted so that all rats received a constant volume of 1 ml of solution/kg daily. 3% (w/w) corn oil added to control or test diets as a binder to reduce evaporation of the flavouring. The rat's weight, food intake and general condition were recorded every week. Haematological examinations were made after 3, 6, and 12 months. These examinations included white cell counts, red cell counts, haemoglobins and haematocrits.

	At the termination of the experiments the rats were sacrificed and exsaguinated. The tissues of all the rats were examined macroscopically at the time of sacrifice. The viscera were removed and the liver, kidneys, spleen, heart and testes were weighed. These organs, the remaining abdominal and thoracic viscera, and one hind leg, for bone, bone marrow, and muscle, were preserved in 10% buffered formalin-saline solution for histopathological examination. For routine histopathology, sections were embedded in paraffin wax and stained with haematoxylin and eosin. Detailed microscopic examinations in the subacute studies were generally done on 6 or 8 rats, evenly devided by sex, from the high dose group and the control group.
GLP:	Yes ( ) No (x) ? ( )
Test substance:	Vanillin; commercially available.
Reference:	Hagan et al, 1967.
(d)	
Species/strain:	Rat, Osborne-Mendel
Sex:	Female ( ); Male ( ); Male/Female (x); No data ( )
Route of administration:	Oral feed
Exposure period:	2 years
Frequency of treatment:	Daily
Postexposure observ. period:	None
Dose:	5000, 10,000 and 20,000 ppm
Control group:	Yes (x); No ( ); No data ( ) Concurrent no treatment ( ); Concurrent vehicle (x); Historical ( )
NOEL:	>= 20,000 ppm
LOEL:	
Results:	No effect on growth or haematology. No macroscopic or microscopic changes in the tissues (incl. testes).
Method:	12 male and 12 female rats (test and control groups). Vanillin was mixed in the diet. Fresh diets were made and distributed weekly. The concentration was adjusted so that all rats received a constant volume of 1 ml of solution/kg daily. 3% (w/w) propylene glycol added to control or test diets as a binder to reduce evaporation of the flavour. The rat's weight, food intake and general condition were recorded every week. Haematological examinations were made at 3, 6, 12 and 22 months. These examinations included white cell counts, red cell counts, haemoglobins and haematocrits. At the termination of the experiments the rats were sacrificed and exsaguinated. The tissues of all the rats were examined macroscopically at the time of sacrifice. The viscera were removed and the liver, kidneys, spleen, heart and testes were weighed. These organs, the remaining abdominal and thoracic viscera, and one hind leg, for bone, bone marrow, and muscle, were preserved in 10% buffered formalin-saline solution for histopathological examination. For routine histopathology, sections were embedded in paraffin wax and stained with haematoxylin and eosin.

	Detailed microscopic examinations in the subacute studies were generally done on 6 or 8 rats, evenly devided by sex, from the high dose group and the control group.
GLP:	Yes ( ) No (x) ? ( )
Test substance:	Vanillin; commercially available.
Reference:	Hagan et al, 1967.

(e)

Species/strain:	Rat
Sex:	Female ( ); Male ( ); Male/Female (x); No data ( )
Route of administration:	Oral feed
Exposure period:	91 days
Frequency of treatment:	No data
Postexposure observ. period:	No data
Dose:	3000, 10,000 and 50,000 ppm (ca. 150, 500 and 2500 mg/kg/day)
Control group:	Yes ( ); No ( ); No data (x) Concurrent no treatment ( ); Concurrent vehicle ( ); Historical ( )
NOEL:	>= 3000 ppm
LOEL:	>= 10,000 ppm
Results:	When judged by appearance, behaviour, growth, mortality, final body and organ weights, terminal hematological examination, and histologigal studies, no adverse effects were detected at 3000 ppm. Mild adverse effects at 10 000 ppm, Growth depression and enlargement of liver, kidney and spleen at 50,000 ppm.
Method:	10 male and 10 female rats. 4 to 6 weeks of age. No further data.
GLP:	Yes ( ) No (x) ? ( )
Test substance:	Vanillin; no further data
Reference:	Hake et al, 1963.

(f)

Species/strain:	Rat
Sex:	Female ( ); Male (x); Male/Female ( ); No data ( )
Route of administration:	Oral feed
Exposure period:	26 weeks
Frequency of treatment:	
Postexposure observ. period:	None
Dose:	1000, 5000 and 10,000 ppm (or 0.1%, 0.5% and 1.0%)
Control group:	Yes (x); No ( ); No data ( ) Concurrent no treatment ( ); Concurrent vehicle ( ); Historical ( )
NOEL:	>= 10,000 ppm
LOEL:	
Results:	No significant difference in body weight gain. Autopsies and microscopic examinations of tissues revealed no pathology.
Method:	10 male rats per group. No further data.
GLP:	Yes ( ) No (x) ? ( )
Test substance:	Vanillin from Monsanto Chemical Company, USA.
Reference:	Hazleton Laboratory, 1955.

(g)

Species/strain:	Rat
Sex:	Female ( ); Male ( ); Male/Female ( ); No data (x)

Route of administration: Gavage
Exposure period: 14 weeks
Frequency of treatment: Twice a week
Postexposure observ. period: No data
Dose: 300 mg/kg
Control group: Yes ( ); No ( ); No data (x)
Concurrent no treatment ( ); Concurrent vehicle ( ); Historical ( )
NOEL: >= 300 mg/kg
LOEL:
Results: No adverse effects.
Appearance, behaviour and gain in weight were normal.
Method: 12 young albino rats.
4% solution in olive oil. Blunt hypodermic needle introduced into the esophagus.
GLP: Yes ( ) No (x) ? ( )
Test substance: Vanillin; high degree of purity from commercial sources.
Reference: Deichman et al, 1940.

(h)
Species/strain: Rat
Sex: Female ( ); Male ( ); Male/Female ( ); No data (x)
Route of administration: Oral feed
Exposure period: 18 weeks
Frequency of treatment: Daily
Postexposure observ. period: No data
Dose: approx. 20 mg/kg/day
Control group: Yes ( ); No ( ); No data (x)
Concurrent no treatment ( ); Concurrent vehicle ( ); Historical ( )
NOEL: >= 20 mg/kg bw/day
LOEL:
Results: No adverse effects.
Appearance, behaviour and gain in weight were normal.
Method: 16 young albino rats.
4% solution in milk, in a normal diet.
GLP: Yes ( ) No (x) ? ( )
Test substance: Vanillin; high degree of purity from commercial sources.
Reference: Deichman et al, 1940.

(i)
Species/strain: Rat
Sex: Female ( ); Male ( ); Male/Female ( ); No data (x)
Route of administration: Oral feed
Exposure period: 10 weeks
Frequency of treatment: Daily
Postexposure observ. period: No data
Dose: 64 mg/kg/day
Control group: Yes ( ); No ( ); No data (x)
Concurrent no treatment ( ); Concurrent vehicle ( ); Historical ( )
NOEL:
LOEL: >= 64 mg/kg bw/day
Results: Appeared normal and lively, but the rate of increase in weight was retarded.

	Histopathological changes in the myocardium, liver, kidney, lung, spleen and stomach.
Method:	16 young albino rats. 4% solution in milk, in a normal diet.
GLP:	Yes ( ) No (x) ? ( )
Test substance:	Vanillin; high degree of purity from commercial sources.
Reference:	Deichman et al, 1940.

(j)

Species/strain:	Dog
Sex:	Female ( ); Male ( ); Male/Female (x); No data ( )
Route of administration:	Other: capsule
Exposure period:	26 weeks and 4 days
Frequency of treatment:	5 days a week
Postexposure observ. period:	None
Dose:	0, 25 and 100 mg/kg.
Control group:	Yes (x); No ( ); No data ( ) Concurrent no treatment ( ); Concurrent vehicle ( ); Historical ( )
NOEL:	>= 100 mg/kg
LOEL:	
Results:	1 male and 1 female dog per dose. Normal behaviour and body weight gains. Hematological or biochemical values and urine analysis for all treated animals were within normal limits and comparable to the control values. Gross autopsies and microscopic examinations of tissues revealed no pathology.
Method:	No data
GLP:	Yes ( ) No (x) ? ( )
Test substance:	Vanillin from Monsanto Chemical Company, USA.
Reference:	Hazleton Laboratory, 1955.

5.5 GENETIC TOXICITY IN VITRO

A. Bacterial test

(a)

Type:	Ames test (Salmonella/mammalian-microsome reverse mutation assay)
System for testing:	Salmonella typhimurium TA98, TA100, TA1535, TA1537, TA1538
Concentration:	100, 333, 667, 1000, 3330 and 5000 ug/plate
Metabolic activation:	With ( ); Without ( ); With and without (x); No data ( )
Results:	Vanillin did not cause a positive increase in the number of histidine revertants per plate of any of the tested strains either in the presence or absence of microsomal enzymes prepared from Aroclor-induced rat liver (S9).
Cytotoxicity conc.:	With metabolic activation: > 5000 ug/plate Without metabolic activation: > 5000 ug/plate
Precipitation conc.:	
Genotoxic effects:	With metabolic activation: + ( ) ? ( ) - (x) Without metabolic activation: + ( ) ? ( ) - (x)
Method:	OECD TG 471 "Genetic Toxicology: Salmonella thyphimurium Reverse Mutation Assay".

GLP: Yes (x) No ( ) ? ( )
Test substance: As prescibed by 1.1-1.2 (Sample from Rhône Poulenc)
Purity: no data (most probably > 99.6%)
Remarks:
Reference: Lawlor, 1991.

(b)
Type: Ames test (Bacterial reverse gene mutation assay)
System for testing: Salmonella typhimurium TA98, TA100, TA1535, TA1537, TA1538
Concentration: 50, 150, 500, 1500 and 5000 ug/plate
Metabolic activation: With ( ); Without ( ); With and without (x); No data ( )
Results: No substantial increases in revertant colony numbers of any of the five tester strains were observed following treatment with Vanillin at any dose level, either in the presence or absence of metabolic activation (S-9 mix).
It was concluded that no evidence of mutagenic potential of Vanillin was obtained in this bacterial test system at the dose levels used.
Cytotoxicity conc.: With metabolic activation: > 5000 ug/plate
Without metabolic activation: > 5000 ug/plate
Precipitation conc.:
Genotoxic effects: With metabolic activation: + ( ) ? ( ) - (x)
Without metabolic activation: + ( ) ? ( ) - (x)
Method: Bacterial reverse gene mutation assay (Ames test)
GLP: Yes (x) No ( ) ? ( )
Test substance: As prescribed by 1.1-1.2 (Batch no. 132 dated 18.09.86 from EuroVanillin KS, Norway)
Purity: 99.8%
Remarks:
Reference: Jones et al, 1986.

(c)
Type: Ames test (Salmonella/mammalian-microsome reverse mutation assay)
System for testing: Salmonella typhimurium TA98, TA100, TA1535, TA1537
Concentration: 100, 333, 1000, 3333 and 10,000 ug/plate
Metabolic activation: With ( ); Without ( ); With and without (x); No data ( )
Results: Negative
Cytotoxicity conc.: With metabolic activation: > 10,000 ug/plate
Without metabolic activation: > 10,000 ug/plate
Precipitation conc.:
Genotoxic effects: With metabolic activation: + ( ) ? ( ) - (x)
Without metabolic activation: + ( ) ? ( ) - (x)
Method: Salmonella Mutagenicity Test.
With and without Aroclor 1254-induced rat and hamster metabolic activation systems.
GLP: Yes ( ) No ( ) ? (x)
Test substance: Vanillin from Aldrich
Purity: 99%
Remarks: Cytotoxicity at 10,000 ug/plate.
Reference: Mortelmans et al. 1986.

(d)
Type: Ames test

System for testing: Salmonella typhimurium TA98, TA100, TA1535, TA1537
Concentration: 8, 40, 200 and 1000 ug/plate
Metabolic activation: With ( ); Without ( ); With and without (x); No data ( )
Results: Negative
Cytotoxicity conc.: With metabolic activation:
Without metabolic activation:
Precipitation conc.:
Genotoxic effects: With metabolic activation: + ( ) ? ( ) - ( )
Without metabolic activation: + ( ) ? ( ) - ( )
Method: OECD TG 471 "Genetic Toxicology: Salmonella thyphimurium Reverse Mutation Assay".
GLP: Yes ( ) No (x) ? ( )
Test substance: As prescribed by 1.1-1.2
Purity: no exact data
Remarks:
Reference: Marzin, 1979a.

(e)
Type: Ames test
System for testing: Salmonella typhimurium TA98, TA100, TA1535, TA1537, TA1538
Concentration: 0,5, 5, 50, 500 and 5000 ug/plate
Metabolic activation: With ( ); Without ( ); With and without (x); No data ( )
Results: Negative at 0,5, 5, 50 and 500 ug/plate.
At 5000 ug/plate; toxicity as evidenced by a thinning of the background lawn.
Vanillin did not show any mutagenicity in the Ames assay.
Cytotoxicity conc.: With metabolic activation:
Without metabolic activation:
Precipitation conc.:
Genotoxic effects: With metabolic activation: + ( ) ? ( ) - (x)
Without metabolic activation: + ( ) ? ( ) - (x)
Method: Salmonella thyphimurium Assay.
GLP: Yes ( ) No (x) ? ( )
Test substance: Vanillin from Schuchardt, Munich.
Purity: no data
Remarks: Tested in quadruplicate at five concentrations on each of the 5 bacterial strains, both in presence and absence of the S9-mix.
Reference: Pool et al, 1982.

(f)
Type: Ames test
System for testing: Salmonella typhimurium TA98, TA100, TA1535, TA1537
Concentration: 3 umol/plate (or 456 ug/plate)
Metabolic activation: With ( ); Without ( ); With and without (x); No data ( )
Results: Negative.
Not mutagenic.
Cytotoxicity conc.: With metabolic activation: > 456 ug/plate
Without metabolic activation: > 456 ug/plate
Precipitation conc.: Did not precipitate at 456 ug/plate.
Genotoxic effects: With metabolic activation: + ( ) ? ( ) - (x)
Without metabolic activation: + ( ) ? ( ) - (x)
Method: Spot test

GLP: Yes ( ) No (x) ? ( )
Test substance: Vanillin; commercially available, checked for purity using thin-layer chromatography, gas chromatography and NMR.
Remarks: Metabolic activation: S-9, Aroclor 1254.
Reference: Florin et al. 1980.

(g)
Type: Ames test (Salmonella/microsome test - reverse mutation assay)
System for testing: Salmonella typhimurium TA92, TA94, TA98, TA100, TA1535, TA1537
Concentration: 6 concentrations up to 10,000 ug/plate
Metabolic activation: With ( ); Without ( ); With and without (x); No data ( )
Results: Negative.
No significant increase in the numbers of revertant colonies were detected in any of the strains at the maximum dose (10,000 ug/plate).
Cytotoxicity conc.: With metabolic activation: > 10,000 ug/plate
Without metabolic activation: > 10,000 ug/plate
Precipitation conc.:
Genotoxic effects: With metabolic activation: + ( ) ? ( ) - (x)
Without metabolic activation: + ( ) ? ( ) - (x)
Method: Salmonella/microsome test - reverse mutation assay. Ames test.
Liver microsome fraction (S-9) was prepared from the liver of Fischer rats.
GLP: Yes ( ) No ( ) ? (x)
Test substance: Vanillin from the Japan Food Additives Association, Tokyo.
Purity: checked, no further data
Remarks:
Reference: Ishidate et al, 1984.

(h)
Type: Ames test (bacterial mutation test in the Salmonella/microsome system)
System for testing: Salmonella typhimurium TA98, TA100
Concentration: 0.05 to 1000 ug/plate
Metabolic activation: With ( ); Without ( ); With and without (x); No data ( )
Results: Negative.
Did not induce a number of revertants that was over half the number of spontaneous revertants of TA98 and TA100 at the indicated dose ranges, either with or without S9 mix.
Cytotoxicity conc.: With metabolic activation: > 1000 ug/plate
Without metabolic activation: > 1000 ug/plate
Precipitation conc.:
Genotoxic effects: With metabolic activation: + ( ) ? ( ) - (x)
Without metabolic activation: + ( ) ? ( ) - (x)
Method: Bacterial mutation test in the Salmonella/microsome system.
Rat liver microsome (S-9) was prepared from Sprague-Dawley rats treated with Aroclor 1254 by the metod of Ames.
GLP: Yes ( ) No ( ) ? (x)
Test substance: Vanillin from Nakarai Pharmaceutical Co. Ltd., Japan.
Purity: 90-95%
Remarks: Metabolic activation: Rat-liver microsome (S 9) from Sprague-Dawley rats treated with Aroclor 1254.
Reference: Kasamaki et al, 1982.

(i)

Type:	Salmonella typhimurium reverse mutation assay (Modified Ames test)
System for testing:	Salmonella typhimurium TA102, TA104
Concentration:	0, 2, 4 6, 8, and 10 umol/plate (10 uM = 1520 ug)
Metabolic activation:	With ( ); Without (x); With and without ( ); No data ( )
Results:	Negative. Vanillin was a powerful inhibitor of the spontaneous mutagenicity in both TA104 (ca. 50% decrease) and TA102 (ca. 40% decrease).
Cytotoxicity conc.:	With metabolic activation: Without metabolic activation: > 10 umol/plate
Precipitation conc.:	
Genotoxic effects:	With metabolic activation: + ( ) ? ( ) - ( ) Without metabolic activation: + ( ) ? ( ) - (x)
Method:	Inhibition of the spontaneous mutagenicity
GLP:	Yes ( ) No ( ) ? (x)
Test substance:	Vanillin from Aldrich. Purity: no data
Remarks:	
Reference:	De Flora et al, 1994.

(j)

Type:	Salmonella typhimurium reverse mutation assay (modified Ames test)
System for testing:	Salmonella typhimurium TA98, TA100, TA1535
Concentration:	No data
Metabolic activation:	With (x); Without ( ); With and without ( ); No data ( )
Results:	Negative. Vanillin was non-mutagenic.
Cytotoxicity conc.:	With metabolic activation: Without metabolic activation:
Precipitation conc.:	
Genotoxic effects:	With metabolic activation: + ( ) ? ( ) - (x) Without metabolic activation: + ( ) ? ( ) - (x)
Method:	Salmonella typhimurium reverse mutation assay . S9 fraction from the livers of Aroclor-induced Wistar rats and S9 activation mix were prepared according to the method of McCann et al (1975).
GLP:	Yes ( ) No (x) ? ( )
Test substance:	Vanillin from Sisco Laboratories, Bombay Purity: 90%
Remarks:	Metabolic activation: S9 from livers of Aroclor-induced Wistar rats.
Reference:	Nagabhushan et al, 1985.

(k)

Type:	Bacterial reverse mutation assay
System for testing:	Escherichia coli WP2s (uvrA, trpE), Salmonella typhimurium TA98 (uvrB, hisD)
Concentration:	50, 100 and 150 ug/plate
Metabolic activation:	With (x); Without ( ); With and without ( ); No data ( )
Results:	#Antimutagenic effects of Vanillin post-treatment on mutagenesis induced in E. coli: - by 4-Nitroquinoline 1-oxide (at 2 ug/ml for 15 minutes):

Strong suppressing activity, marked increase in the survival of treated cells.
- by Furylfuramide (at 0.4 ug/ml for 60 minutes):
Strong suppressive activity.
- by Captan (at 2 ug/ml for 15 minutes):
Strong suppressive activity.
- by Methylglyoxal (at 100 ug/ml for 60 minutes):
Strong suppressive activity.
#In Salmonella typh. TA98 Vanillin was not effective against mutations provoked by:
- 3-Amino-1-methyl-5H-pyrido(3-4-b) indole
- 2-Amino-3-methylimidazo (4-5-f) quinoline
in Salmonella.

Cytotoxicity conc.: With metabolic activation: >150 ug/plate
Without metabolic activation: > 150 ug/plate

Precipitation conc.:

Genotoxic effects: With metabolic activation: + ( ) ? ( ) - (x)
Without metabolic activation: + ( ) ? ( ) - (x)

Method: Assay for antimutagenic effects

GLP: Yes ( ) No ( ) ? (x)

Test substance: Vanillin from Tokyo Kasei Kogyo, Tokyo Japan
Purity: no data

Remarks: Metabolic activation: S-9.

Reference: Ohta et al, 1986.

(l)

Type: DNA damage and repair assay

System for testing: Escherichia coli K-12 and PJ5

Concentration: 0,5, 1, 3, 5 and 10 mmol/l (1520 mg/l)

Metabolic activation: With ( ); Without (x); With and without ( ); No data ( )

Results: Negative.
Vanillin has an effect on the adaptive and SOS responses, as well as mutagenesis, induced in E.coli by N-methyl-N-nitroso urea (MNU) and UV-irradiation.
Vanillin, to some extent, suppressed MNU-induced mutagenesis in both DNA repair-proficient strain (AB1157) and the ada-5 mutant (PJ5) of E. coli K-12. However, when E.coli K-12 AB1157 cells were treated with MNU in a buffer followed by incubation in a growth medium containing vanillin, MNU-induced mutagenesis was not suppressed.
It is therefore suggested that Vanillin might suppress the methyl-action of DNA by MNU.
Concerning UV-induced mutagenesis, vanillin suppressed mutagenesis under the assay conditions in E.coli K-12 AB1157. Vanillin alone are incapable of inducing any revertants over the background level under the present assay conditions in either DNA repair-proficient (AB1157) or the ada-5 mutant (PJ5).

Cytotoxicity conc.: With metabolic activation:
Without metabolic activation: > 1520 mg/l

Precipitation conc.:

Genotoxic effects: With metabolic activation: + ( ) ? ( ) - ( )
Without metabolic activation: + ( ) ? ( ) - (x)

Method: Effects on MNU- and UV-induced mutagenesis.

The effect on MNU-induced mutagenesis were examined by measuring revertant cells formed after incubation of E.coli K-12 tester strains in the presence of Vanillin.
For the effect on UV-induced mutagenesis, revertant cells were measured after postincubation of the UV-preirradiated E. coli K-12 AB1157 cells in a growth medium containing various concentrations of Vanillin.

GLP: Yes ( ) No ( ) ? (x)
Test substance: Vanillin from Wako Pure Chemicals Industries Ltd., Osaka Japan.
Purity: no data
Remarks:
Reference: Takahashi et al, 1990.

(m)
Type: DNA damage and repair assay
System for testing: Escherichia coli K-12 (several strains), plasmids.
Concentration: 600 ug/l (UV-survival)
500 ug/l (Plasmid recombination)
Metabolic activation: With ( ); Without (x); With and without ( ); No data ( )
Results: UV-irradiated uvrA umuC cells showed higher survival when plated on medium containing vanillin rather than medium without vanillin.
Antimutagenic effects of Vanillin on UV killing of umuC mutant strains of E.coli.
A significantly higher frequency of plasmid recombination was observed when vanillin was present in the culture medium.
The study suggest that the antimutagenic effect of vanillin is a result of enhancement of recA-dependent, error free, pathway of post replication repair.
Cytotoxicity conc.: With metabolic activation:
Without metabolic activation:
Precipitation conc.:
Genotoxic effects: With metabolic activation: + ( ) ? ( ) - ( )
Without metabolic activation: + ( ) ? ( ) - ( )
Method: Antimutagenic effect of vanillin on UV-irradiated E.coli.
Antimutagenic activity (UV-survival), post replication repair, plasmid recombination.
GLP: Yes ( ) No ( ) ? (x)
Test substance: Vanillin from Tokyo Kasei Kogyo, Tokyo Japan.
Purity: no data
Remarks:
Reference: Ohta et al, 1988.

(n)
Type: Escherichia coli reverse mutation assay
System for testing: Escherichia coli WP2s (uvrA, trpE)
Concentration: 0, 20 and 30 umol/plate (30 uM = 4560 ug)
Metabolic activation: With ( ); Without (x); With and without ( ); No data ( )
Results: Bio-antimutagenic effects of Vanillin on mutagenesis induced by 4-Nitroquinoline 1-oxide, at 2 ug/ml for 15 minutes: Strong suppressive activities.
Cytotoxicity conc.: With metabolic activation:
Without metabolic activation: > 30 umol/plate
Precipitation conc.:

Genotoxic effects: With metabolic activation: + ( ) ? ( ) - ( )
Without metabolic activation: + ( ) ? ( ) - (x)

Method: E. coli trp^+ reverse mutation assay.
Antimutagenic effects

GLP: Yes ( ) No ( ) ? (x)

Test substance: Vanillin from Tokyo Kasei Kogyo, Tokyo.
Purity: no data

Remarks:

Reference: Watanabe et al, 1988.

(o)

Type: Escherichia coli reverse mutation assay

System for testing: Escherichia coli PQ37

Concentration: 15, 50, 150, 500 and 1500 ug

Metabolic activation: With ( ); Without (x); With and without ( ); No data ( )

Results: Vanillin itself did not cause SOS induction.
Simultaneous treatment with UV irradiation, or with 4-Nitroquinoline-oxide or with N-methyl-N'-nitro-N-nitroso-guanidine increased the mutagenicity.

Cytotoxicity conc.: With metabolic activation:
Without metabolic activation:

Precipitation conc.:

Genotoxic effects: With metabolic activation: + ( ) ? ( ) - ( )
Without metabolic activation: + ( ) ? ( ) - ( )

Method: SOS chromotest

GLP: Yes ( ) No ( ) ? (x)

Test substance: Vanillin from Kisida Chemical Co., Japan.
Purity: no data

Remarks:

Reference: Sato et al, 1991.

(p)

Type: Escherichia coli reverse mutation assay

System for testing: Escherichia coli PQ37

Concentration: No data reported

Metabolic activation: With ( ); Without (x); With and without ( ); No data ( )

Results: Ambigous.
Vanillin nitrosated with Sodium nitrite showed strong genotoxicity: SOS-inducing factor = 0,21/nmol, calculated on the basis of 2 to 4 duplicate determinations of SOS-inducing factor at various concentrations (not reported).

Cytotoxicity conc.: With metabolic activation:
Without metabolic activation:

Precipitation conc.:

Genotoxic effects: With metabolic activation: + ( ) ? ( ) - ( )
Without metabolic activation: + ( ) ? ( ) - ( )

Method: SOS chromotest

GLP: Yes ( ) No ( ) ? (x)

Test substance: Vanillin after nitrosation with Sodium nitrite.
Purity: no data

Remarks:

Reference: Oshima et al, 1989.

(q)

Type:	Mitotic recombination in Saccharomyces cerevisiae (Mitotic gene conversion assay)
System for testing:	Saccharomyces cerevisiae strain D7
Concentration:	10 mg/ml (65.8 mM/l)
Metabolic activation:	With ( ); Without (x); With and without ( ); No data ( )
Results:	Negative. No induction of gene conversion at acidic pH levels. No significantly difference in gene conversion frequency at pH7 and at pH10.
Cytotoxicity conc.:	With metabolic activation: Without metabolic activation:
Precipitation conc.:	
Genotoxic effects:	With metabolic activation: + ( ) ? ( ) - ( ) Without metabolic activation: + ( ) ? ( ) - ( )
Method:	Mitotic recombination in Saccharomyces cerevisiae (Mitotic gene conversion assay)
GLP:	Yes ( ) No ( ) ? (x)
Test substance:	Vanillin from Sigma Chemical Co., St. Louis, MO USA. Purity: no data
Remarks:	
Reference:	Rosin, 1984.

B. Non-bacterial in vitro test

(a)

Type:	Cytogenetic assay
System for testing:	Human lymphocytes
Concentration:	0, 1, 2 and 4 mmol/l (4mM = 612 mg/l)
Metabolic activation:	With ( ); Without (x); With and without ( ); No data ( )
Results:	Ambigous. 2 experiments were made with lymphocytes from 2 different donors. One experiment showed a negative result (no increase in SCE above that of the solvent control), while in the other a slight increase in the number of aberrations/a slight increase in the chromosome gap was seen with increasing concentration of Vanillin. However, only the highest concentration (4 mmol/l) showed a statistically significance effect with gaps included (p<0,01).
Cytotoxicity conc.:	With metabolic activation: Without metabolic activation: > 4 mmol/l At the concentration of 6 mmol vanillin it was not possible to obtain enough metaphases to do an evaluation
Precipitation conc.:	
Genotoxic effects:	With metabolic activation: + ( ) ? ( ) - ( ) Without metabolic activation: + ( ) ? ( ) - (x)
Method:	Similar to OECD TG 473. (Chromosome aberration)
GLP:	Yes ( ) No ( ) ? (x)
Test substance:	Vanillin; commercially available Purity: 99.6% (estimated by gas chromatography)
Remarks:	Such concentration is neighter physiologic nor encountered at exposure.

Gaps are not included in the evaluation of chromosome aberration tests according to OECD Guidelines for Testing of Chemicals.
.

Reference: Jansson, et al. 1987.

(b)

Type: Cytogenetic assay
System for testing: BALB/c mouse 3T3 fibroblasts
Concentration: 2 to 8 mmol/l (8mM = 1224 mg/l)
Metabolic activation: With ( ); Without (x); With and without ( ); No data ( )
Results: Relative cytotoxicity NR_{50} (midpoint cytotoxicity) = 8 mmol/l.
Cytotoxicity conc.: With metabolic activation:
Without metabolic activation: 8 mmol/l
Precipitation conc.:
Genotoxic effects: With metabolic activation: + ( ) ? ( ) - ( )
Without metabolic activation: + ( ) ? ( ) - ( )
Method: Neutral red (NR) assay (quantification of the number of viable, uninjured cells after their incubation with test agents, based on the uptake and lysosomal accumulation of the supravital dye NR).
GLP: Yes ( ) No ( ) ? (x)
Test substance: Vanillin from J.T. Baker, Danvers, MA, USA (dissolved in 95% ethanol).
Purity: no data
Remarks: Microscopic examination: Vanillin, at slight to moderately toxic concentration (2-6 mmol/l), induced multinucleation in the 3T3 fibroblasts, giving rise to two or multiple nuclei.
This concentration is neither physiologic nor related to exposure.
Reference: Babich et al, 1993.

(c)

Type: DNA damage and repair assay
System for testing: Chinese Hamster ovary (CHO K-1) cells
Concentration: 10, 33 and 100 umol/l (100uM = 15200 ug/l)
Metabolic activation: With ( ); Without (x); With and without ( ); No data ( )
Results: Negative.
Vanillin did not induce chromosome aberrations.
Chromosome aberrations induced by UV-light or X-rays were suppressed by the post-treatment with Vanillin.
UV- or X-ray irradiated surviving cells increased in the presence of Vanillin.
Cytotoxicity conc.: With metabolic activation:
Without metabolic activation:
Precipitation conc.:
Genotoxic effects: With metabolic activation: + ( ) ? ( ) - ( )
Without metabolic activation: + ( ) ? ( ) - (x)
Method: Chromosome aberrations induced by UV-light or X-rays
GLP: Yes ( ) No ( ) ? (x)
Test substance: Vanillin from Tokyo Kasei Kogyo, Tokyo, Japan (dissolved in dimethyl sulfoxide just before each treatment)
Purity: no data
Remarks:
Reference: Sasaki et al, 1990a.

(d)

Type:	Mammalian cell gene mutation assay
System for testing:	Chinese Hamster ovary (CHO) cells
Concentration:	250 to 1500 ug/ml (-S9 mix), 183 to 2440 ug/ml (+S9 mix)
Metabolic activation:	With ( ); Without ( ); With and without (x); No data ( )
Results:	Vanillin was concidered negative for inducing chromosomal aberrations in CHO cells under both non-activation and activation, except at the highest dose level analyzed (2440 ug/ml) with metabolic activation (see comments below).
Cytotoxicity conc.:	With metabolic activation: Rangefinding assay: Complete cytotoxicity at 5090 ug/ml, no cell cycling delay. 2440 ug/ml caused severe cytotoxicity.*No increase in chromosomal aberrations was observed at the closely spaced subsequent dose level of 1830 ug/ml and lower doses.
	Without metabolic activation: Rangefinding assay: Complete cytotoxicity at 1700 and 5090 ug/ml, severe cell cycle delay at 509 ug/ml.
Precipitation conc.:	
Genotoxic effects:	With metabolic activation: + ( ) ? ( ) - (x)
	Without metabolic activation: + ( ) ? ( ) - (x)
Method:	OECD TG 473 "Genetic Toxicology: In vitro Mammalian Cytogenetic Test".
GLP:	Yes (x) No ( ) ? ( )
Test substance:	As prescribed by 1.1-1.2. (Lot no. 8932101 from Rhône-Poulenc) Purity: 99.9%
Remarks:	*) Aberrations observed at 2440 ug/ml were mostly simple chromosome breaks localized mainly at a single site. The biological significance of this response at an extremely toxic dose level is highly disputable.
Reference:	Murli, 1991.

(e)

Type:	Mammalian cell gene mutation assay
System for testing:	Chinese Hamster fibroblast cell line (CHL, from a lung of a newborn female)
Concentration:	3 different doses up to 1,0 mg/ml for 24 hours and 48 hours
Metabolic activation:	With ( ); Without (x); With and without ( ); No data ( )
Results:	Negative.
Cytotoxicity conc.:	With metabolic activation:
	Without metabolic activation: >1,0 mg/ml
Precipitation conc.:	
Genotoxic effects:	With metabolic activation: + ( ) ? ( ) - ( )
	Without metabolic activation: + ( ) ? ( ) - (x)
Method:	Chromosomal aberration test.
GLP:	Yes ( ) No ( ) ? (x)
Test substance:	Vanillin from the Japan Food Additives Association, Tokyo. Purity: checked, no further data
Remarks:	
Reference:	Ishidate et al, 1984.

(f)

Type:	Mammalian cell gene mutation assay

System for testing: Chinese Hamster V79 cells
Concentration: 0, 10, 33 and 100 umol/l (100 uM = 15,200 ug/l)
Metabolic activation: With ( ); Without (x); With and without ( ); No data ( )
Results: Negative.
Cytotoxicity conc.: With metabolic activation:
Without metabolic activation: >100 umol/l
Precipitation conc.:
Genotoxic effects: With metabolic activation: + ( ) ? ( ) - ( )
Without metabolic activation: + ( ) ? ( ) - (x)
Method: 6-TG (thioguanine) -resistant mutation test
GLP: Yes ( ) No ( ) ? (x)
Test substance: Vanillin from Tokyo Kasei Kogyo, Tokyo, Japan.
Purity: no data
Remarks: The frequencies of 6-TG-resistant mutations induced by UV or X-rays were decreased by treatment with Vanillin during the expression time. These decreases were depentent on the concentrations of Vanillin. Vanillin was not mutagenic to V79 cells and obvious increases or decreases in the frequency of spontaneous mutations were not observed. Antimutagenic effects: The number of surviving cells after irradiation increased in the presence of Vanillin. The frequencies of 6-TG-resistant mutants induced by UV or X-rays were decreased after 5-, 6-, 7- and 8-day expression times. No prolongation of growth rate was observed. Hence, the observed decreased mutation frequency was not due to the cytotoxicity of Vanillin or a delay in mutation fixation.
Reference: Imanishi et al, 1990.

(g)
Type: Mammalian cell gene mutation assay
System for testing: Chinese Hamster B241 cells
Concentration: 0.005, 0.02 and 0.04 umol/l
Metabolic activation: With ( ); Without ( ); With and without (x); No data ( )
Results: Negative.
No significant increase in structural and numerical chromosome aberrations, compared to control cells untreated or treated with dimethyl sulfoxide alone.
Cytotoxicity conc.: With metabolic activation: > 0.04 umol/l
Without metabolic activation: > 0.04 umol/l
Precipitation conc.:
Genotoxic effects: With metabolic activation: + ( ) ? ( ) - (x)
Without metabolic activation: + ( ) ? ( ) - (x)
Method: With another cell line, similar to OECD TG 476.
Chromosome aberrations.
GLP: Yes ( ) No ( ) ? (x)
Test substance: Vanillin from Nakarai Pharmaceutical Co. Ltd., Japan.
Purity: 90-95%
Remarks: Metabolic activation: S9 mix.
Reference: Kasamaki et al, 1982, Kasamaki et al, 1985.

(h)
Type: Sister chromatid exchange assay
System for testing: Chinese Hamster ovary (CHO) cells
Concentration: 10, 33, 100 and 333 umol/l (333 uM = 50616 ug/l)

Metabolic activation: With ( ); Without (x); With and without ( ); No data ( )
Results: Negative.
Vanillin induced neither SCEs nor chromosome aberrations by itself. However, an obvious increase in frequencies of SCEs was observed when mytomycine C (MMC)-pretreated cells were cultured in the presence of Vanillin.
SCEs-enhancing effects of Vanillin were also observed when induced by:
- EMS (ethyl methanesulphonate)
- EMNG (N-ethyl-N'-nitro-N-nitrosoguanidine)
- ENU (N-ethyl-N-nitrosourea)
- MNU (N-methyl-N-nitrosourea)

On the other hand, MMS (methyl methanesulphonate) or MNNG (N-methyl-N'-nitro-N-nitrosoguanidine)-induced SCEs were not influenced at all by Vanillin.
Cytotoxicity conc.: With metabolic activation:
Without metabolic activation: >= 333 umol/l (for MMS- or MNNG-pretreated cells).
Precipitation conc.:
Genotoxic effects: With metabolic activation: + ( ) ? ( ) - ( )
Without metabolic activation: + ( ) ? ( ) - ( )
Method: In vitro Sister Chromatid Exchange Assay in Mammalian Cells.
GLP: Yes ( ) No ( ) ? (x)
Test substance: Other TS: from Tokyo Kasei Kogyo, Tokyo Japan.
Purity: no data
Remarks: SCEs-enhancing effects seemed to be depentent on the quality of lesions in DNA.
Reference: Sasaki et al, 1987a, Sasaki et al, 1987b.

(i)
Type: Sister chromatid exchange assay
System for testing: Human lymphocytes
Concentration: 0.75 and 1.0 mmol/l (1mM = 152 mg/l)
Metabolic activation: With ( ); Without (x); With and without ( ); No data ( )
Results: Vanillin was a potent inducer of SCE, significant at 0.75 and 1.0 mmol/l.
Cytotoxicity conc.: With metabolic activation:
Without metabolic activation:
Precipitation conc.:
Genotoxic effects: With metabolic activation: + ( ) ? ( ) - ( )
Without metabolic activation: + (x) ? ( ) - ( )
Method: Similar to OECD TG 479
GLP: Yes ( ) No ( ) ? (x)
Test substance: Vanillin; commercially available
Purity: 99.6% (estimated by gas chromatography)
Remarks: Nothing is said about the toxicity of Vanillin as such huge concentrations as 1 mM, although Vanillin was studied as a component of cigarette smoke and present as such at lower concentration.
Reference: Jansson et al, 1986.

(j)
Type: Sister chromatid exchange assay
System for testing: Human lymphocytes

Concentration:	0, 1and 2 mmol/l (2 mM = 306 mg/l)
Metabolic activation:	With ( ); Without (x); With and without ( ); No data ( )
Results:	Positive. Significant ($p<0.001$) at 1 and 2 mmol/l.
Cytotoxicity conc.:	With metabolic activation: Without metabolic activation:
Precipitation conc.:	
Genotoxic effects:	With metabolic activation: + ( ) ? ( ) - ( ) Without metabolic activation: + ( ) ? ( ) - ( )
Method:	Similar to OECD TG 479
GLP:	Yes ( ) No ( ) ? (x)
Test substance:	Vanillin; commercially available Purity: 99.6% (estimated by gas chromatography)).
Remarks:	This study repeats the results from (Jansson et al, 1986) (which tested a longer list of compounds) about the induction of SCE by Vanillin. Comments in article: The results are probably suggesting a low ability of Vanillin to induce chromosome aberrations in human lymphocytes.
Reference:	Jansson et al, 1987.

5.6 GENETIC TOXICITY IN VIVO

(a)

Type:	Micronucleus assay
Species/strain:	Mouse, OF1
Sex:	Female (x); Male ( ); Male/Female ( ); No data ( )
Route of administration:	Gavage (orally)
Exposure period:	6 and 30 hours (before sampling of bone-marrow)
Doses:	500 and 1000 mg/kg, twice each.
Results:	Negative. No statistically significant increase in the frequency of micronuclei in erythrocytes when compared to the control. Lowest dose producing toxicity: 2000 mg/kg (2 lethalities out of 3).
Effect on mitotic index or P/N ratio:	
Genotoxic effects:	+ ( ) ? ( ) - (x)
Method:	OECD TG 474 "Genetic Toxicology: Micronucleus Test".
GLP:	Yes ( ) No (x) ? ( )
Test substance:	As prescribed by 1.1-1.2 (from Rhône-Poulenc) Purity: no data. (Most probably > 99.6%)
Remarks:	10 mice per group.
Reference:	Marzin, 1979b.

(b)

Type:	Micronucleus assay
Species/strain:	Mouse, BDF1
Sex:	Female ( ); Male (x); Male/Female ( ); No data ( )
Route of administration:	Gavage (orally)
Exposure period:	*Post-treatment:* Vanillin was administered every 3 hour (0, 3, 6, 9, 12, 15, 18 and 21 h) after MMC injection. Bone marrow cells were sampled 24 hours after injection of MMC (mitomycin C).

	Time course study: Vanillin was given 7.5 and 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.
Doses:	*Post treatment:* 125, 175, 250, 350 and 500 mg/kg *Time course study*: 500 mg/kg
Results:	Negative *Post treatment:* Vanillin did not induce any micronucleated polychromatic erythrocytes (MN-PCEs). Post-treatment with Vanillin oral dose 500 mg/kg, 7.5 hours after i.p. injection of 2 mg/kg of MMC, caused about 50% decrease in the frequency of MN-PCEs. *Time course study:* The suppressing effect was not due to a delay in the formation of MN-PCEs by the cytotoxic action of Vanillin. Vanillin acts as an anticlastogenic factor in vivo.
Effect on mitotic index or P/N ratio:	
Genotoxic effects:	+ ( ) ? ( ) - (x)
Method:	Micronucleus assay according to method described by Schmid (1976).
GLP:	Yes ( ) No ( ) ? (x)
Test substance:	Vanillin from Tokyo Kasei Kogyo, Tokyo, Japan. Purity: no data
Remarks:	Mice: 7 weeks of age. 3 mice in each experimental group.
Reference:	Inouye et al, 1988.

(c)

Type:	Micronucleus assay
Species/strain:	Mouse ddY
Sex:	Female ( ); Male (x); Male/Female ( ); No data ( )
Route of administration:	Gavage (orally)
Exposure period:	*Post treatment:* Vanillin was administered every 3 hour (0, 3, 6, 9, 12, 15 and 18 h) after irradiation. Bone marrow cells were sampled 24 hours after irradiation. *Time-course study:* Vanillin was given immediately after the irradiation, and bone marrow cells were sampled periodically (8, 12, 16, 24, 30, 36, 48, 72 hours) after irradiation.
Doses:	*Post treatment:* 250, 313 and 500 mg/kg *Time-course study:* 500 mg/kg
Results:	Negative. Suppressing effects on X-ray-induced micronuclei *Post treatment:* The most pronounced reduction in the frequency of MNPCEs was observed when Vanillin was administered to mice just after irradiation (0 hours) (decrease: 55%). Significant suppression until 9 hours after irradiation. Testing at 0 hours: 250 mg/kg: 42% decrease in MNPCEs. 313 mg/kg: 42% decrease in MNPCEs. 500 mg/kg: 55% decrease in MNPCEs. *Time-course study:* The observed reduction of MNPCEs was not not a reflection of the toxic effect of vanillin to the bone marrow.
Effect on mitotic	

index or P/N ratio:	
Genotoxic effects:	+ ( ) ? ( ) - (x)
Method:	Mouse micronucleus test according to Schmid (1976).
GLP:	Yes ( ) No ( ) ? (x)
Test substance:	Vanillin from Tokyo Kasei Kogyo, Tokyo, Japan Purity: no data
Remarks:	Mice: 8 weeks of age. Irradiated with X-rays at 200 rad.
Reference:	Sasaki et al, 1990b.

(d)

Type:	Mouse spot test
Species/strain:	Mouse, male PW, female C57BL/10 (mated)
Sex:	Female (x); Male ( ); Male/Female ( ); No data ( )
Route of administration:	Gavage (orally)
Exposure period:	I.p. injection with ENU (ethylnitrosourea) on the 10th day of pregnancy; received 3 successive oral administrations of vanillin at 0, 4 and 24 hours after ENU injection.
Doses:	0, 125, 250, 350 and 500 mg/kg
Results:	Negative. Vanillin was shown to act as an antimutagen in vivo. Three successive oral administrations of Vanillin at 500 mg/kg, 0, 4 and 24 hours after ENU injection decreased by approx. 50% the number of mice with RS induced by ENU at 50 mg/kg. This suppression was observed at >= 250 mg/kg of Vanillin. Vanillin did not affect the number of mice with WMVS and pups per female, which indicates that Vanillin did not have any toxic effects on the embryo.
Effect on mitotic index or P/N ratio:	
Genotoxic effects:	+ ( ) ? ( ) - (x)
Method:	Antimutagenic effect in mouse spot test; in vivo method to detect somatic cell mutations. The appearance of recessive color spots (RS) and white midventral spots (WMVS) was examined in pups aged about 30 days.
GLP:	Yes ( ) No ( ) ? (x)
Test substance:	Vanillin from Tokyo Kasei Kogyo, Tokyo, Japan Purity: no data
Remarks:	8-16 week-old males mated with 8 week-old female. Male (PW) has a recessive homozygous loci for coat colours and morphological characters.
Reference:	Imanishi et al, 1990.

(e)

Type:	Ring-X loss test in Drosophila melanogaster spermatozoa
Species/strain:	Drosophila melanogaster Female (C1), male (C1) - mated
Sex:	Female (x); Male ( ); Male/Female ( ); No data ( )
Route of administration:	Oral feed
Exposure period:	48 hours
Doses:	0.1, 0.5, 1 and 2%
Results:	- Suppressive effect of Vanillin on ring-X loss that occurs spontaneously in spermatozoa of D. melanogaster: Inhibition of 36%, 38%, 56% and 59% at concentrations of 0.1%, 0.5%, 1% and 2%, respectively.

	- Significant suppressive effect of Vanillin on mitomycin C induced ring-X loss (when males treated with MMC were mated with the vanillin-treated females): Inhibition of 32% and 49% at concentrations of 0.5% and 1%, respectively. However, this decrease was observed only in the first 3 days after the interruption of the female's treatment with Vanillin. In the subsequent 3 days the frequencies of ring-X loss were not altered. On the other hand, vanillin at 0.1% did not show any effect on MMC-induced ring X-loss. - In contrast, Vanillin did not show any effect on chromosome loss provoked by Methyl methanesulphonate.
Effect on mitotic index or P/N ratio:	
Genotoxic effects:	+ ( ) ? ( ) - (x)
Method:	No data
GLP:	Yes ( ) No ( ) ? (x)
Test substance:	Vanillin from Vetec Quimica Fina Ltd., Brazil Purity: no data
Remarks:	Males (aged 42-48 hours): fed with MMC (mitomycin C) and MMS (methyl methanesulfonate) for 24 hours. Females (18.24 hours): fed with test solutions for 48 hours (new solution after 24 h). Mated.
Reference:	De Andrade et al, 1992.

5.7 CARCINOGENICITY

(a)

Species/strain:	Mouse, S-strain
Sex:	Female ( ); Male ( ); Male/Female ( ); No data (x)
Route of administration:	Dermal
Exposure period:	3 weeks
Frequecy of treatment:	Total 10 applications
Postexposure observ. period:	18 weeks
Doses:	Roughly 3000 mg/kg bw per application; total dose of 600 mg
Control group:	Yes (x); No ( ); No data ( ) Concurrent no treatment ( ); Concurrent vehicle (x); Historical ( )
Results:	No co-carcinogenic effect. No significant increase in local tumours. The incidence of tumours of the lung (the only organ examined in detail) was evidently unaffected by the treatment.
Method:	Co-carcinogenic assay. Application of solutions, recording of tumours, examination of mice for lung adenomas at post-mortem, and histological examination.
GLP:	Yes ( ) No (x) ? ( )
Test substance:	Vanillin from L. Light and Co. Ltd. Purity: no data
Remarks:	A skin painting study. 20 mice. Applications three times a week, total 10 applications.20 mice. A concentration of 20% in acetone, application of 0.3 ml (roughly 3000 mg/kg bw/application, total Vanillin dose of 600 mg/mouse).

	Subsequent 18 weekly applications of 0.3 ml croton oil. First croton oil application 25 days after first application of Vanillin.
Reference:	Salaman et al, 1956.

(b)

Species/strain:	Mouse, A/He
Sex:	Female ( ); Male ( ); Male/Female (x); No data ( )
Route of administration:	i.p. injection
Exposure period:	8 weeks
Frequecy of treatment:	3 times a week
Postexposure observ. period:	16 weeks
Doses:	150 and 750 mg/kg bw (Total doses (24 injections): 3600 and 18,000 mg/kg bw)
Control group:	Yes (x); No ( ); No data ( ) Concurrent no treatment (x); Concurrent vehicle (x); Historical (x)
Results:	Not carcinogenic. Vanillin did not significantly induce lung tumours in mice.
Method:	Strain A mouse lung tumour bioassay. Test for carcinogenicity by the pulmonary tumour response in strain A mice.
GLP:	Yes ( ) No (x) ? ( )
Test substance:	Vanillin from JT Baker, lot no. 2-0022. Purity: Ranging from 85-99%, majority 95-99% (several chemicals tested).
Remarks:	Strain A mice have high spontaneous tumour incidence. Mice: 6-8 weeks old, average weight 18-20 g. 15 male and 15 female per dose level. Vehicle: tricaprylin
Reference:	Stoner et al, 1973, Stoner, 1991.

(c)

Species/strain:	Rat, Osborne-Mendel
Sex:	Female ( ); Male ( ); Male/Female (x); No data ( )
Route of administration:	Oral feed
Exposure period:	2 years
Frequency of treatment:	Daily
Postexposure observ. period:	None
Dose:	5000, 10,000 and 20,000 ppm
Control group:	Yes (x); No ( ); No data ( ) Concurrent no treatment ( ); Concurrent vehicle (x); Historical ( )
Results:	No carcinogenic or toxic effects. No effect on growth or haematology. No macroscopic or microscopic changes in the tissues (incl. testes).
Method:	12 male and 12 female rats (test and control groups). Vanillin was mixed in the diet. Fresh diets were made and distributed weekly. The concentration was adjusted so that all rats received a constant volume of 1 ml of solution/kg daily. 3% (w/w) propylene glycol added to control or test diets as a binder to reduce evaporation of the flavour. The rat's weight, food intake and general condition were recorded every week. Haematological examinations were made at 3, 6, 12 and 22 months.

	These examinations included white cell counts, red cell counts, haemoglobins and haematocrits. At the termination of the experiments the rats were sacrificed and exsaguinated. The tissues of all the rats were examined macroscopically at the time of sacrifice. The viscera were removed and the liver, kidneys, spleen, heart and testes were weighed. These organs, the remaining abdominal and thoracic viscera, and one hind leg, for bone, bone marrow, and muscle, were preserved in 10% buffered formalin-saline solution for histopathological examination. For routine histopathology, sections were embedded in paraffin wax and stained with haematoxylin and eosin. Detailed microscopic examinations in the subacute studies were generally done on 6 or 8 rats, evenly devided by sex, from the high dose group and the control group.
GLP:	Yes ( ) No (x) ? ( )
Test substance:	Vanillin; commercially available.
Reference:	Hagan et al, 1967.

(d)

Species/strain:	Rat, Osborne-Mendel
Sex:	Female ( ); Male (x); Male/Female ( ); No data ( )
Route of administration:	Oral feed
Exposure period:	1 year
Frequency of treatment:	Daily
Postexposure observ. period:	None
Dose:	20,000 and 50,000 ppm
Control group:	Yes (x); No ( ); No data ( ) Concurrent no treatment ( ); Concurrent vehicle (x); Historical ( )
Results:	No carcinogenic or toxic effects. No effect on growth or haematology. No macroscopic or microscopic changes in the tissues (incl. testes).
Method:	5 male rats (test and control groups). Vanillin was mixed in the diet. Fresh diets were made and distributed weekly. The concentration was adjusted so that all rats received a constant volume of 1 ml of solution/kg daily. 3% (w/w) corn oil added to control or test diets as a binder to reduce evaporation of the flavouring. The rat's weight, food intake and general condition were recorded every week. Haematological examinations were made at termination of the subacute studies, and at 3, 6, 12 and 22 months in the chronic experiment. These examinations included white cell counts, red cell counts, haemoglobins and haematocrits. At the termination of the experiments the rats were sacrificed and exsaguinated. The tissues of all the rats were examined macroscopically at the time of sacrifice. The viscera were removed and the liver, kidneys, spleen, heart and testes were weighed. These organs, the remaining abdominal and thoracic viscera, and one hind leg, for bone, bone marrow, and muscle, were preserved in 10% buffered formalin-saline solution for histopathological examination.

	For routine histopathology, sections were embedded in paraffin wax and stained with haematoxylin and eosin. Detailed microscopic examinations in the subacute studies were generally done on 6 or 8 rats, evenly devided by sex, from the high dose group and the control group.
GLP:	Yes ( ) No (x) ? ( )
Test substance:	Vanillin; commercially available.
Reference:	Hagan et al, 1967.

(e)

Species/strain:	Rat, Fischer 344
Sex:	Female ( ); Male (x); Male/Female ( ); No data ( )
Route of administration:	Oral feed
Exposure period:	4 weeks
Frequecy of treatment:	Daily
Postexposure observ. period:	None
Doses:	2.0%
Control group:	Yes (x); No ( ); No data ( ) Concurrent no treatment (x); Concurrent vehicle (x); Historical ( )
Results:	No significant increase in the thickness of the forestomach mucosa in the prefundic or mid regions. In the glandular stomach, thickness and labeling indices were significantly increased compared to the diet without vanillin and $NaNO_2$. No significant increase in the thickness or labeling indices in the esophagus.
Method:	Short term stomach cell proliferation study. The combined effects of Vanillin and $NaNO_2$ on cell proliferation in the upper digestive tract were examined. Groups of 5 rats were given Vanillin or basal diet either alone or in combination with 0.3% $NaNO_2$ for 4 weeks, and then killed. Mucosal thickness and proliferative indices in upper digestive tract (forestomach, glandular stomach and esophagus) were measured.
GLP:	Yes ( ) No ( ) ? (x)
Test substance:	Vanillin from Tokyo Kasei Kogyo, Tokyo, Japan. Purity: > 97.0%
Remarks:	Rats: 5 weeks old. Vehicle: $NaNO_2$. $NaNo_2$ can stimulate phenolic compound-induced cell proliferation in the upper digestive tract, particularly in the forestomach epithelium.
Reference:	Kawabe et al, 1994.

(f)

Species/strain:	Mouse, SKH/HR1
Sex:	Female (x); Male ( ); Male/Female ( ); No data ( )
Route of administration:	Oral feed
Exposure period:	40 weeks
Frequecy of treatment:	Constant
Postexposure observ. period:	None
Doses:	0.5% in diet
Control group:	Yes (x); No ( ); No data ( )

	Concurrent no treatment (x); Concurrent vehicle ( ); Historical ( )
Results:	No significant effect on weight. Vanillin was not toxic to liver, due to no significant effect on liver weight (week 40). 1) Vanillin did not reduce tumour latency (median tumour latency time: 21.5 weeks, 20.3 in control) but significantly reduced tumour multiplicity (week 21)(48%, $p<0.05$). 2) Vanillin was without effect.
Method:	Antiphotocarcinogenic and photoprotective properties. 1) The animals received constant, daily (5 days a week) sub-erythemic levels of UVB radiation from Westinghouse BZS-WLG lamps. Radiation was discontinued when 25 sunburn units had been delivered at week 11. Animals were evaluated weekly for tumor latency and multiplicity. Hepatomegaly was used as an indication to liver toxicity. 2) Epidermal ornithine decarboxylase (ODC) assay: Groups of 3 mice with diets for 2 weeks, exposed to 0.45 J/cm^2 of UVB, and episermal extract assayed for ODC activity 28 hours post-irradiation.
GLP:	Yes ( ) No ( ) ? (x)
Test substance:	Vanillin from Aldrich Chemical Company, Milwaukee, WI, USA Purity: no data
Remarks:	Hairless mice, 18.5 to 20 weeks old.
Reference:	Black et al, 1986.

5.8 TOXICITY TO REPRODUCTION

Type:	Fertility ( ); One generation study ( ); Two generation study ( ); Other ( )
Species/strain:	
Sex:	Female ( ); Male ( ); Male/Female ( ); No data ( )
Route of administration:	
Exposure period:	
Frequecy of treatment:	
Postexposure observ. period:	
Premating exposure period:	Male: Female:
Duration of the test:	
Doses:	
Control group:	Yes ( ); No ( ); No data ( ) Concurrent no treatment ( ); Concurrent vehicle ( ); Historical ( )
NOEL Parental:	
NOEL F1 Offspring:	
NOEL F2 Offspring:	
Results:	No data
General parental tox.:	
Toxicity to offspring:	
Method:	
GLP:	Yes ( ) No ( ) ? ( )
Test substance:	
Remarks:	
Reference:	

5.9 DEVELOPMENTAL TOXICITY /TERATOGENICITY

(a)

Species/strain:	Mouse; male PW, female C57BL/10 (mated)
Sex:	Female (x); Male ( ); Male/Female ( ); No data ( )
Route of administration:	Gavage (orally)
Duration of the test:	From 10th day of pregnancy untill pups aged about 30 days
Exposure period:	I.p. injection with ENU (ethylnitrosourea) on the 10th day of pregnancy; received 3 successive oral administrations at 0, 4 and 24 hours after ENU injection.
Frequecy of treatment:	3 administrations in 24 hours
Doses:	0, 125, 250, 350 and 500 mg/kg
Control group:	Yes (x); No ( ); No data ( ) Concurrent no treatment (x); Concurrent vehicle ( ); Historical ( ) One control group were treated with solvents for ENU and Vanillin, and one with solvents for ENU only.
NOEL Maternal Toxicity:	> 1500 mg/kg bw/day
NOEL Teratogenicity:	> 1500 mg/kg bw/ day
Results:	Negative. Vanillin was shown to act as an antimutagen in vivo. Three successive oral administrations of Vanillin at 500 mg/kg, 0, 4 and 24 hours after ENU injection decreased by approx. 50% the number of mice with RS induced by ENU at 50 mg/kg. This suppression was observed at >= 250 mg/kg of Vanillin. Vanillin did not affect the number of mice with WMVS and pups per female, which indicates that Vanillin did not have any toxic effects on the embryo.
Maternal general tox.:	
Pregnancy/litter data:	
Foetal data:	
Method:	Antimutagenic effect in mouse spot test; in vivo method to detect somatic cell mutations. The appearance of recessive color spots (RS) and white midventral spots (WMVS) was examined in pups aged about 30 days.
GLP:	Yes ( ) No ( ) ? (x)
Test substance:	Vanillin from Tokyo Kasei Kogyo, Tokyo Japan Purity: no data
Remarks:	8-16 week-old males mated with 8 week-old female. Male (PW) has a recessive homozygous loci for coat colours and morphological characters.
Reference:	Imanishi et al, 1990.

(b)

Species/strain:	Mouse, ICR
Sex:	Female (x); Male ( ); Male/Female ( ); No data ( )
Route of administration:	i.p.
Duration of the test:	18 days
Exposure period:	Day 11 of gestation
Frequecy of treatment:	Single i.p. injection
Doses:	50 mg/kg
Control group:	Yes (x); No ( ); No data ( ) Concurrent no treatment ( ); Concurrent vehicle (x); Historical ( )

1) Saline only
2) Saline + MNNG (40 mg/kg)
3) Saline + MNNG (60 mg/kg)

NOEL Maternal Toxicity:
NOEL Teratogenicity: >= 50 mg/kg bw/day
Results: 1) *The effect of Vanillin on fetal development in mice - control with saline only*:
Effects of Vanillin was comparable to that of saline control with respect to the number of live fetuses, fetal body weight, fetal mortality and incidence of external and skeletal abnormalities.

Maternal general tox.:
Pregnancy/litter data: No. of litters: 3
Foetal data: Mean no. of live fetuses: 13.7 +/- 1.8
Fetal body weight: 1.42 +/- 0,06 g
Fetal mortality: 9.6 +/- 4.8 %
External abnormalities: 0 %
Skeletal abnormalities: Malformations: 0 %
Variations: 25.7 +/- 17.4 %

2) *The influence of Vanillin on MNNG-induced fetal defects (40 mg/kg MNNG):*
The incidence of external malformations in MNNG+Vanillin group was comparable to that in MNNG+saline group.

Maternal general tox.:
Pregnancy/litter data: No. of litters: 5
Foetal data: Mean no. of live fetuses: 13.6 +/- 0.6
Fetal body weight: 1.21 +/- 0.04 g
Fetal mortality: 10.2 +/- 2.6 %
External abnormalities: 30.4 +/- 10.2 %
Brain: 0 %
Face: 4.0 +/- 2.7 %
Forelimb: 27.5 +/- 8.4 %
Hindlimb: 27.7 +/- 10.1 %
Others: 1.3 +/- 1.3 %

3) *The influence of Vanillin on MNNG-induced fetal defects (60 mg/kg MNNG):*
The incidence of external malformations in MNNG+Vanillin group was comparable to that in MNNG+saline group. The incidence of microcephaly and facial defects, hshowed a tendency to decrease with Vanillin.
Fore- and hindlimbs:
Significant decrease of syndactyly, decrease in oligodactyly noted, significant decrease of the incidence of cleft palate.
Bractylyly was higher in the fore- and hindlimbs in MNNG (60 mg/kg)+Vanillin compared to MNNG (60 mg/kg)+saline group.

Maternal general tox.:
Pregnancy/litter data: No. of litters: 5
Foetal data: Mean no. of live fetuses: 12.4 +/- 0.5
Fetal body weight: 1.06 +/- 0.06 g
Fetal mortality: 11.5 +/- 1.8 %
External abnormalities: 67.5 +/- 11.2 %
Brain: 1.8 +/- 1.8 %

Face: 25.6 +/- 6.1 %
Forelimb: 56.2 +/- 10.2 %
Hindlimb: 67.5 +/- 11.2 %
Others: 26.4 +/- 9.9 %

Method: Examination of whether vanillin, which have mutation suppressing effect, can modify the teratogenicity in mice caused by N-methyl-N'-nitro-N-nitrosoguanidine (MNNG).
2) and 3) MNNG was administered on day 11 of gestation, Vanillin was administered one hour later.
Killing of dams and examination of fetuses at day 18 of gestation.
The number of implants, resorptions, dead fetuses and live fetuses were counted.
Live fetuses were weighed and examined for external malformations under a dissecting microscope, and then cleared and stained by means of Dawson's technique (Dawson, 1926) for skeletal examination.
Furthermore, examination of the phalanges stained cartilagious and ossified components in the fore- and hindlimbs (modified method of Burdi, 1965).

GLP: Yes ( ) No ( ) ? (x)

Test substance: Vanillin from Tokyo Kasei Kogyo, Tokyo Japan
Purity: no data.

Remarks: Females (9-13 weeks old) mated with males. Day 0 of gestation.

Reference: Morita et al, 1988.

(c)

Species/strain: Single-Comb White Leghorn chickens(chicken embryo)

Sex: Female ( ); Male ( ); Male/Female ( ); No data (x)

Route of administration: Injection into yolk and through air cell

Duration of the test: Hatching

Exposure period: Preincubation 0 hour and at 96 hours

Frequecy of treatment: Single injection

Doses: 5 dose levels up to 10 mg/egg

Control group: Yes (x); No ( ); No data ( )
Concurrent no treatment (x); Concurrent vehicle (x); Historical ( )

NOEL Maternal Toxicity: n.a.

NOEL Teratogenicity: > 10 mg/egg

Results: Vanillin showed no teratogenic response.
(Other: LD_{50} = 0.82 mg/egg by injection through air cell at 0 hour.)

Maternal general tox.: n.a.

Pregnancy/litter data:

Foetal data:

Method: Study of teratogenicity of Vanillin in the developing chicken embryo.
Calculation of the percentage mortality at each dose level.
Calculation of the total number of birds having one or more abnormalities.
Calculation of the total number of birds having a structural abnormalty of the head, viscera, limbs or body skeleton.

GLP: Yes ( ) No (x) ? ( )

Test substance: Vanillin from Ruger Chemical Co, Irvington, NJ, USA
Purity: food grade quality, no further data.

Remarks: Study of toxicity and teratogenicity of 80 food additive chemicals, including Vanillin, in the developing chicken embryo.
Four test conditions were used:

- injection via the air cell
- injection via the yolk
- pre-incubation (0 hours)
- 96 hours of incubation time

For each condition at least 100 chicken embryos per dose level (at least 5) were treated.

Reference: Verrett et al, 1980.

5.10 OTHER RELEVANT INFORMATION

A. Specific toxicities

(a)

Type: Immunotoxicity

Results: No significant difference between group treated with Vanillin and untreated control group.

Remarks: Female CD-1 mice (30 mice/group).
Dosed intragastrically at 3 concentrations: 250, 500 and 1000 mg/kg Vanillin/day and vehicle control (1% methylcellulose) for 5 consecutive days.
Control untreated group, positive control group: host resistance to Listeria monocytogenes.
Anti-sheep red blood cell (SRBC) plaque-forming cell (PFC) assay: specific cellular activity, and total speen activity.

Reference: Gaworski et al, 1994.

(b)

Type: Immunotoxicity

Results: Vanillin directly suppressed the in vitro anti-sheep red blood cell (SRBC) antibody response at nontoxic doses.

Remarks: Twenty chemicals were examined for immunomodulatory effects using the Mishell-Dutton in vitro antibody-producing assay.
Vanillin (200 ug/culture) was found to be one of five chemicals tested that directly suppressed the in vitro anti-SRBC antibody response at nontoxic doses.
All of the five positive chemicals were reported to interrupt an early phase of the immune response.
They had no effect on the actual release of anti-SRBC antibody.

Reference: Kutz et al, 1980.

(c)

Type: Immunotoxicity

Results: Vanillin had an immunostimulatory effect.

Remarks:

Reference: Archer et al. 1978.

(d)

Type: Immunotoxicity

Results: 1) Vanillin was classified as a weak modulator macrophage function.

Remarks: 1) Examination of the immunomodulatory effects of 25 chemicals using alteration of peritoneal macrophage function as the study endpoint.

Vanillin and 4 other test substances were found to decrease the percentage of mouse peritoneal macrophage capable of ingesting yeast, although not below 50% of control.
LC_{50} = 2050 ug/ml (the chemical concentration which reduced microphage viability to 50% after 20 hours exposure).
4 indices: ingestion index, phatocytic capacity, killing index, adherence index).
Vanillin was classified as a weak modulator, due to causing significant modulation of an index at noncytotoxic doses, but the EC_{50} = 1/2 LC_{50}, and thus the modulatory index >= 0.50.
Effective doses: 1, 10, 100, 300 and 1000 ug/ml.
2) Screening for immunomodulators: effects of xenobiotics on macrophage chemiluminiscence in vitro.
Peak and total macrophage chemiluminiscence after 20 hours chemical exposure: EC_{50} >= 1000 ug/ml (EC_{50} = 50% effective concentration).
Concentration range: 0.1 - 1000 ug/ml.
Test substance: >= 98% pure, from ICN Pharmaceuticals, Plainview, NY, USA.

Reference: 1) Tam et al, 1984.
2) Tam et al, 1990.

(e)

Type: Cytotoxicity

Results:

Remarks: Toxicity values (a 10 point scale from 0 to 9) in 4 in vitro short term tests:
- Inhibition of cell growth in sarcoma BP8 ascitic cells.
- Inhibition of the oxidative metabolism in hamster brown fat cells.
- Membrane damage of human diploid embryonic lung fibroblasts.
- Inhibition of ciliary activity using chicken embryo trachea.

Vanillin scores were 3, 2, 0, 0, respectively.

Reference: Curvall et al, 1984.

(f)

Type: Other: Risk Assessment

Results:

Remarks: In both Ames test and chromosome aberration tests, Vanillin showed no mutagenic activity (Ishidate et al, 1984).
Rats fed with 50,000 ppm Vanillin for 1 year or 10,000 ppm for 2 years showed no deleterious effect (Hagan et al, 1967), pointing out Vanillin as a rather harmless food component or additive.

Reference: Feron et al, 1991.

(g)

Type: Other: Antioxidant and pro-oxidant activity

Results: Effects on free radicals, brain peroxidation and degradation of benzoate, deoxyribose, aminoacids and DNA.

Remarks: Test substance: from Wako Pure Chemicals Industries Ltd., Osaka Japan.

Reference: Liu et al, 1993.

(h)

Type: Other: Effects on ciliary activity of the embryo chicken trachea in vitro.

Results: Vanillin was found to be inactive on the ciliary activity within 60 minutes.

Remarks: Testing principles:

The ciliary activity was studied at 37^{o}C by means of inverted microscopy (magnification x 250).
One tracheal ring from chicken tracheal organ cultures (16-17 days old chicken embryos), was placed in a perpex testing chamber (Vol. 3.1 ml) containing the medium admixed with an ethanol or DMSO solution of each compound, at the same concentration; 5 mmol/l.
The ciliary activity was displayed continuously on a TV-monitor during the entire exposure time, maximized to 60 minutes.
Replicate tests were performed to at least 3 occasions, each experiment involving rings from different tracheal preparations.
Both solvents were nontoxic to the cilia at the concentrations used in the experiment.
Test substance: all the compounds were checked for purity by TLC, GC and NMR.

Reference: Petterson et al, 1982.

B. Toxicodynamics, toxicokinetics

(a)

Type: Metabolism

Results:

Remarks: Vanillin is metabolized by various mammalian species to a number of urinary products, primarily vanillic acid in both free and conjugated forms. Other metabolites are conjugated vanillin, conjugated vanillyl alcohol and catechol.
I.p. injection of a single 100 mg/kg dose of vanillin in propylene glycol/water solution to Sprague-Dawley rats resulted in the urinary excretion of free and conjugated vanillic acids (17 and 24% of dose), free and conjugated cathechol (trace and 4%) and conjugates of both vanillyl alcohol (10%) and vanillin (6.5%) within a 24 hour period.

Reference: Wong et al, 1966.

(b)

Type: Metabolism

Results: Formaldehyde formation for Vanillin was only as a trace (= 70 to 200 pmol/mg microsomal protein/minute) with rat nasal microsomal enzymes, and 70 pmol/mg microsomal protein/minute with liver microsomal enzymes.

Remarks: Screening assays to identify compounds which might be metabolized to formaldehyde in the nasal cavity.
Test substance: from Sigma Chemical Co., St. Louis, MO USA.

Reference: Dahl et al, 1983.

(c)

Type: Metabolism

Results:

Remarks: A single oral administration to male albino rats of 100 mg/kg bw resulted in the urinary excretion of most of its metabolites within 24

hours, mainly as glucuronide and/or sulphate conjugates, although the acids formed were also excreted free and as their glycine conjugates.
In 48 hours, 94% of the dose had been excreted in the following proportions:
- 47% Vanillic acid
- 19% Vanillyl alcohol
- 10% Vanilloylglycine
- 8% Catechol
- 7% Vanillin
- 2% 4-methylcatechol
- 0.6% 4-methylguaiacol
- 0.5% guaiacol.

No metabolites were found in the urine collected 48-96 hours after dosing.

Reference: Strand et al, 1975.

(d)

Type: Metabolism

Results:

Remarks: The conversion of Vanillin to Vanillic acid was demonstrated in human and rat liver cells in culture.

Reference: BIBRA Toxicity Profile, 1990.

(e)

Type: Metabolism

Results:

Remarks: An early study found only trace amounts of Vanillin in the urine of rabbits given 2 g/day orally (about 1 g/kg bw/day), the Vanillin being conjugated with sulphuric acid.
Most of the dose was oxidized to Vanillic acid, and this too was excreted as the ethereal sulphate conjugate.

Reference: BIBRA Toxicity Profile, 1990.

(f)

Type: Metabolism

Results:

Remarks: Eighty-three percent of an oral dose (1g/kg) of Vanillin administered to rabbits was accounted for after 24 hours in the urine.
Vanillic acid was observed free (64%) and conjugated (25%) either as a glucuronide or a sulfate.
Unchanged Vanillin (14%) was present in the urine, mostly as glucurovanillin, up to 6 hours after dosing, but its presence ceased abruptly at that time.

Reference: Sammons et al, 1941.

(g)

Type: Metabolism

Results:

Remarks: The vanillic acid content in the 24 hour urine of one human maintained on a plant free diet for 2 days dropped from ca 9 mg to 0.3 mg. At this time an oral dose of 100 mg vanillin resulted in the urinary excretion of 96 mg vanillic acid (ca 94% of the administered dose) in the next 24 hour period. No unchanged vanillin was observed in the urine.

Reference: Dirscherl et al, 1964.

(h)
Type: Metabolism
Results:
Remarks: In vitro incubation of 0.3 mg vanillin with rat (Sprague-Dawley; M) liver homogenates for 2 hours in a phosphate buffer at 37^{o}C, followed by addition of 0.2 ml of concentrated HCl resulted in the formation of vanillic acid with an 81% yield.
Reference: Dirscherl et al, 1966.

(i)
Type: Metabolism
Results:
Remarks: Anaerobic incubation of 5-10 mg vanillin with .... caecal extracts for 46 hours resulted in the formation of vanillic acid, 4-methylguaiacol, 4-methylcatechol, protocatechuic acid, and a 4th unidentified product. In addition, catechol and unchanged parent compound were detected in 1 of 3 experiments.
Reference: Scheline, 1972.

(j)
Type: Metabolism
Results:
Remarks: Guinea pigs (390 g) were administered daily doses (oral implied) of 15 mg vanillin (385 mg/kg) for 10 days. A total of 200 mg pure vanillic acid and very slight amounts of benzoic acid were isolated from the urine.
Reference: Bernhard et al, 1955.

(k)
Type: Metabolism
Results:
Remarks: Two human subjects, male and female, were placed on a plant free diet 72 hours prior to treatment and maintained on the diet for 24 hours following administration of vanillin. Treatment consisted of one subject orally ingesting 60 ml of vanilla extract within 5 minutes, and second ingesting 10 average servings of an artificially flavoured vanilla pudding within 12 hours. Urine analysis of samples collected for a 24 hour period during and following both treatments revealed trace levels of 3-methoxy-4-hydroxy-benzylamine (vanillylamine) as a result of both vanilla extract and pudding ingestion, respectively.
Reference: Perry et al, 1965.

5.11 EXPERIENCE WITH HUMAN EXPOSURE

(a)
Results: No primary irritation
Remarks: In closed-patch tests on human skin, vanillin caused no primary irritation when tested at concentrations of 20% on 29 normal subjects, of 2% on 30 normal subjects and of 0,4% in 35 subjects with dermatoses.

No further details known.

Reference: Opdyke, 1977.

(b)

Results: No sensitization

Remarks: Maximation tests were carried out on groups of 25 volunteers. The material was tested at concentrations of 2% and 5% in petrolatum and produced no sensitization reactions.

Reference: Opdyke, 1977.

(c)

Results: No sensitization or irritation

Remarks: Vanillin applied undiluted for 48 hours in the standard occluded aluminium patch test used by the North American Contact Dermatitis Research Group (NACDRG) did not produce any irritation or sensitization in a 62 year old subject with a perfume dermatitis.

Reference: Opdyke, 1977.

(d)

Results:

Remarks: Positive reactions to Vanillin were reported in eight out of 142 patients who were already senstized to Balsam of Peru.
In studies of sensitization to Balsam of Peru and its components, Vanillin (pure or 10% in vaseline) produced positive patch test reactions in 21 out of 164 patients sensitive to the balsam. Vanillin was concidered to be a secondary allergen, since sensitivity was found only in patients sensitive to Vanilla, isoeugenol and coniferyl benzoate. Cross-sensitization to other substituted benzaldehydes was particularly uncommon. Vanillin was found not to be responsible for most cases of sensitivity to natural Vanilla.

Reference: Opdyke, 1977.

(e)

Results:

Remarks: In well-conducted (double-blind) challenge tests, an asthmatic patient was given Vanillin at 1.5 hours intervals, two or three times, providing no reaction occured within 15 minutes of a challenge. There was some evidence that Vanillin reduced lung function at oral doses of 0.24 and 1 mg. Itching of the ears and throut was also described.

Reference: BIBRA Toxicity Profile, 1990.

(f)

Results:

Remarks: Chinese manufacturer of Vanillin - packaging section:
Persons tested:
- Persons who have/had dermatitis (working in the packaging section).
- Control group: Healthy workers from other units.
Test material removed 48 hours after application.
Results read 1 hour later.
Negative in both groups.
From the negative results of the above patch tests with Vanillin, it is concidered that the dermatitis occuring in the packaging section may be

	due to mechanical irritation of Vanillin dust stuck on the skin, rather than chemical irritation or sensitization. The itching subsiding after taking shower in most of the workers support the above postulation.
Reference:	Wang et al, 1987.
(g)	
Results:	
Remarks:	Bronchospasm was reported caused by Vanillin mixed with lactose in a controlled double-blind challenge test in a 52 year old asthmatic patient. However, the patient also reacted to the "placebo", lactose.
Reference:	Van Assendelft, 1984.

6. REFERENCES

ACF ChemieFarma NV, Netherlands. Product data sheet. Vanillin ex lignin. 0882/6.

Ash, M&I. 1995. Handbook of Food Additives. 783.

Archer, D.L. et al. 1978. *Use of an in vitro Antibody-Producing System for Recognizing Potentially Immunosuppressive Compounds.* Int. Archs. Allergy Appl. Immun. 56: 90-93.

Babich, H. et al. 1993. *Comparative cytotoxicities of selected minor dietary non-nutrients with chemopreventive properties.* Cancer Lett. (Shannon Irel.). 73 (2): 127-133.

Bernhard, K. et al. 1955. *The question of the formation of benzoic acid in the animal body*. Helv. Chim. Acta. 38: 1438-1444. (Citation: FEMA. 1985. Scientific Literature Review of Vanillin and Derivatives)

Bewley, R.J.F. et al. 1984. *Degradation of Vanillin in soil-clay mixtures treated with simulated acid rain*. Soil Sci. 137 (6): 415-418. (Citation: Euclid Data Sheet for Vanillin by Rhône-Poulenc Chimie, created 26.01.95, updated 01.06.95)

BIBRA Toxicity Profile. 1990. 1st ed.

Black, H.S. et al. 1986. *Evaluation of structurally-related phenols for anti-photocarcinogenic and photoprotective properties.* Photochem. Photobiol. 43 (4): 403-408.

Bodor, N. et al. 1989. *A new Method for the Estimation of Partition Coefficient*. J. Am. Chem. Sci. 111 (11): 3783-3786.

British Pharmacopeia. 1993. Vol. 1. 699.

Brooke, L.T. et al. 1984. *Acute Toxicities of Organic Chemicals to Fathead Minnows (Pimephales promelas).* Vol 1. Center for Lake Superior Environmental Studies, University of Wisconsin-Superior.

Buswell, J.A. et al. 1994. *Effect of lignin-related phenols and their methylated derivatives on growth of eight white-rot fungi*. World J. Microbiol. Biotechnol. 10 (2): 169-174. (Citation: Euclid Data Sheet for Vanillin by Rhône-Poulenc Chimie, created 26.01.95, updated 01.06.95)

California Regional Water Quality Control Board. 1983. Analysis Report from EAL Corporation 02.02.1983, EAL W O No 45651-5300.

Caujolle et al. 1953. *Sur la toxicitè comparèe de la vanilline et du dialdèhyde hydroxy-4 mèthoxy-5 isophtalique.* C. R. Hebdo Seanc. Acad. Sci. Paris. 236: 2549. (Citation: Euclid Data Sheet for Vanillin by Rhône-Poulenc Chimie, created 26.01.95, updated 01.06.95)

Caujolle et al. 1954. *Sur la toxicitè comparèe de l'orthovanilline et de l'èthylorthovanilline.* C. R. Hebdo Seanc. Acad. Sci. Paris. 238: 2576. (Citation: Euclid Data Sheet for Vanillin by Rhône-Poulenc Chimie, created 26.01.95, updated 01.06.95)

Caujolle et al. 1956. *Sur la toxicitè des acides et dièthylamides de la sèrie vanillique.* C. R. Hebdo Seanc. Acad. Sci. Paris. 243: 609.

Clayton, G.D. et al. 1993. Patty's Industrial Hygiene and Toxicology. 4th ed. Vol. IIA: 450-451.

Cronin, M.T.D. et al. 1991. *QSAR studies of comparative toxicity in aquatic organisms.* Sci. Total Environ. 109/110, 431-439.

Curvall, M. et al. 1984. *An evaluation of the utility of four in vitro short term tests for predicting the cytotoxicity of individual compounds derived from tobacco smoke.* Cell. Biol. Toxicol. 1 (1): 173-193. (Citation: Euclid Data Sheet for Vanillin by Rhône-Poulenc Chimie, created 26.01.95, updated 01.06.95)

Dahl, A.R. et al. 1983. *Formaldehyde Production Promoted by Rat Nasal Cytochrome P-450-Depentent Monooxygenases with Nasal Decongestants, Essences, Solvents, Air Pollutants, Nicotine, and Cocaine as Substrates.* Toxicol. Appl. Pharmacol. 67 (2): 200-205. (Citation: Euclid Data Sheet for Vanillin by Rhône-Poulenc Chimie, created 26.01.95, updated 01.06.95)

Dahl, K. 1996. *Six compartment Fugacity Level I Calculation*. Fugacity Level I, II and III Programs for OECD Workshop, Version , Jan 1992. Unpublished report. (Borragaard EuroVanillin, Norway).

De Andrade, H.H.R. et al. 1992. *Suppressing effect of vanillin on chromosome aberrations that occur spontaneously or are induced by mitomycin C in the germ cell line of Drosophila melanogaster.* Mutat. Res. 279 (4): 281-287.

Dedonder, A. et al. 1971. *The effects of phenolics and related compounds on the growth and the respiration of Chlorella vulgaris.* Z. Pfl. Physiol. 65: 70-80.

De Flora, S. et al. 1994. *Inhibition of the "spontaneous" mutagenicity in Salmonella typhimurium TA102 and TA104.* Mutat. Res. 307 (1): 157-167.

Deichman, W. et al. 1940. *On the Toxicity of Vanillin and Ethyl Vanillin for Rabbits and Rats*. J. Am. Pharm. Ass. Sci. Ed. 29: 425-428.

Deutsches Arzneibuch. 1994. 10th ed. Vol. 4.

Di Biase et al. 1979. *Direct Synthesis of -Unsaturated Nitriles from Acetonitrile and Carbonyl Compounds: Survey, Crown Effects, and Experimental Conditions.* J. Org. Chem. 44 (25): 4640-4646. (Citation: Euclid Data Sheet for Vanillin by Rhône-Poulenc Chimie, created 26.01.95, updated 01.06.95)

Directive 91/322/EEC about establishment of a guide for exposure limit values.

Direktoratet for Arbeidstilsynet (The Directorate for Labour Inspection, Norway). 1995. Administrative normer for forurensning i arbeidsatmosfære. Page 18.

Dirscherl, W. et al. 1964. *Vanillic acid in human urine, its isolation, determination and origin*. Hoppe-Seyler's Z. Physiol. Chem. 336: 81-90. (Citation: FEMA. 1985. Scientific Literature Review of Vanillin and Derivatives)

Dirscherl, W. et al. 1966. *Exogenous precursors of vanillic acid: vanillin, protocatechuic acid, caffeic acid, and ferulic acid. Homogenate experiments with rat and human livers*. Hoppe-Seyler's Z. Physiol. Chem. 346: 55-59. (Citation: FEMA. 1985. Scientific Literature Review of Vanillin and Derivatives)

EuroVanillin KS. 1993. Borregaard Ind. Ltd. Laboratory Report dated 11.08.93. Norway. Unpublished data.

EuroVanillin KS. 1995a. Borregaard Ind. Ltd. Laboratory Report no. 259280396. Norway. Unpublished data.

EuroVanillin KS. 1995b. Borregaard Ind. Ltd. Laboratory Report no. 486250895. Norway. Unpublished data.

EuroVanillin KS. 1995c. Norway. Safety Data Sheet According to 93/112/EEC, dated 22.05.95.

FEMA. 1985. Scientific Literature Review of Vanillin and Derivatives.

Feron, V.J. et al. 1991. *Aldehydes: occurence, carcinogenic potential, mechanism of action and risk assessment.* Mutat. Res. 259 (3-4): 363-385.

Florin, I. et al. 1980. *Screening of tobacco smoke constituents for mutagenicity using Ame's test.* Toxicology. 18: 219-232.

Food Chemicals Codex. 1994. 3rd ed. 418-419.

Furia, T.E. et al. 1975. Fenaroli's Handbook of Flavour Ingredients. 2nd ed. 2: 560.

Frazer. 1967. FAO/OMS Report Ser. Vol 44A: 78. (Citation: Euclid Data Sheet for Vanillin by Rhône-Poulenc Chimie, created 26.01.95, updated 01.06.95)

Fujii, T. et al. 1972. *Studies on Compounded Perfumes for Toilet Goods On the Non-irritative Compounded Perfumes for Soaps*. Yukaguku. 29: 904.

Gad, S.C. et al. 1986. *Development and Validation of an Alternative Dermal Sensitization Test: Mouse Ear Swelling Test (MEST).* Toxicol. Appl. Pharmacol. 84 (1): 93-114.

Gad, S.C. 1988. *A Scheme for the Prediction and Ranking of Relative Potencies of Dermal Sensitizers Based on Data from Several Systems.* J. Appl. Toxicol. 8 (5): 361-368.

Gardner, J.R. 1987. *Acute oral toxicity to rats of Vanillin USP*. Huntingdon Research Centre Ltd. England. Report no. 861395D/BOD 14/AC. Unpublished report (EuroVanillin KS, Norway).

Garshol, S. 1996. Estimation done by Borregaard Synthesis. "OECD rapporten". Telefax from Sidsel Garshol Borregaard Synthesis to Borregaard EuroVanillin 15.07.96.

Gaworski, C.L. et al. 1994. *An Immunotoxicity Assessment of Food Flavouring Ingredients.* Food Chem. Toxicol. 32 (5): 409-415. (Citation: Euclid Data Sheet for Vanillin by Rhône-Poulenc Chimie, created 26.01.95, updated 01.06.95)

Greif, N. 1967. *Cutaneous safety of fragrance material as measured by the Maximization Test.* Am. Perfumer Cosm. 82: 54-57.

Hagan, E.C. et al. 1967. *Food flavourings and compounds of related structure. II. Subacute and chronic toxicity*. Food Cosmet. Toxicol. 5 (2): 141-157

Hake C.L. et al. 1963. Industrial Hygiene and Toxicology edited by F.A. Patty. 2nd ed, vol 2: 1695-1698. (Interscience Publishers, New York, summarized by Opdyke (1977)).

Hartenstein, R. 1982. *Effect of aromatic compounds,, humic acids and lignins on growth of the earthworm Eisenia Foetida.* Soil Biol. Biochem. 14 (6): 595-599.

Hausen, B.M. et al. 1992. *Propolis allergy (IV). Studies with further sensitizers from propolis and constituents common to propolis, poplar buds and balsam of Peru*. Contact Dermatitis. 26 (1): 34-44.

Hawley, G.G. 1971. The Condensed Chemical Dictionary. 8th ed. 921.

Hazleton Laboratory. 1955. Monsanto Chemical Company unpublished study no. m-86. (Citation: Euclid Data Sheet for Vanillin by Rhône-Poulenc Chimie, created 26.01.95, updated 01.06.95)

Health and Safety Executive. 1996 EH40/96 Occupational exposure limits 1996.

Healy, J.B. et al. 1979. *Anaerobic Biodegradation of Eleven Aromatic Compounds to Methane.* Appl. Environ. Microbiol. 38 (1): 84-89.

Holmes, H. et al. 1976. Suffield Tech. Note 365 Defense Res., Ralston, Canada. (Citation: Euclid Data Sheet for Vanillin by Rhône-Poulenc Chimie, created 26.01.95, updated 01.06.95)

HSDB (Hazardous Substances Data Bank) No. 1027, updated 27.10.1994. (Citation: Euclid Data Sheet for Vanillin by Rhône-Poulenc Chimie, created 26.01.95, updated 01.06.95)

Imanishi, H. et al. 1990. *Suppression of 6-TG-resistant mutations in V79 cells and recessive spot formations in mice by Vanillin*. Mutat. Res. 243 (2): 151-158.

Inoue, K. et al. 1981. *Quinones and Related Compounds in Higher Plants. Part 16. Naphthoquinones from Radermachera sinica Hemsl. (Bignoniaceae).* J. Chem. Soc. Perkin Trans. 1: 2764-2770. (Citation: Euclid Data Sheet for Vanillin by Rhône-Poulenc Chimie, created 26.01.95, updated 01.06.95)

Inouye, T. et al. 1988. *Suppression of mitomycin C-induced micronuclei in mouse bone marrow cells by post-treatment with vanillin.* Mutat. Res. 202 (1): 93-95.

Ishidate, M. Jr. et al. 1984. *Primary Mutagenicity Screening of Food Additives Currently Used in Japan.* Food Chem. Toxicol. 22 (8): 623-636.

Ishihara, M. et al. 1986. Skin Res. 28: 230. (Citation: Euclid Data Sheet for Vanillin by Rhône-Poulenc Chimie, created 26.01.95, updated 01.06.95)

Jansson, T. et al. 1986. *In vitro studies of biological effects of cigarette smoke condensate. II. Induction of sister-chromatid exchanges in human lymphocytes by weakly acidic, semivolatile constituents.* Mutat. Res. 169 (3): 129-139.

Jansson, T. et al. 1987. *Effects of Vanillin on sister-chromatid exchanges and chromosome aberrations in human lymphocytes.* Mutat. Res. 190 (3): 221-224.

Jenner, P.M. et al. 1964. *Food Flavourings and Compounds of Related Structure. I. Acute Oral Toxicity*. Food Cosmet. Toxicol. 2: 327-343.

Jones, E. et al. 1986. *Ames metabolic activation test to assess the potential mutagenic effect of Vanillin, USP*. Huntingdon Research Center Ltd. England. Report no. BOD 15A/861683. Unpublished report (EuroVanillin KS, Norway).

Kasamaki, A. et al. 1982. *Genotoxicity of flavouring agents.* Mutat. Res. 105 (6): 387-392.

Kasamaki, A. et al. 1985. *Transforming potency of flavoring agents in chinese hamster cells.* J. Toxicol. Sci. 10 (3): 177-185.

Kawabe, M. et al. 1994. *Effects of Combined Treatment with Phenolic Compounds and Sodium Nitrite on Two-stage Carcinogenesis and Cell Proliferation in the Rat Stomach*. Jpn. J. Cancer Res. 85 (1): 17-25.

Kålqvist, T. 1996a. *Aerobic biodegradation of Vanillin measured as BOD_5/COD*. NIVA (Norwegian Institute for Water Research), Norway. Report no. G001/1c. Unpublished report (EuroVanillin KS, Norway).

Kålqvist, T. 1996b. *Effect of Vanillin on the reproduction of Daphnia magna*. NIVA (Norwegian Institute for Water Research), Norway. Report no. G001/1a. Unpublished report (EuroVanillin KS, Norway).

Kirchhof et al. 1934. Chim. Farm. Promysl. 2: 13. (Citation: Euclid Data Sheet for Vanillin by Rhône-Poulenc Chimie, created 26.01.95, updated 01.06.95)

Kirk-Othmer. 1983. Encyclopedia of Chemical Technology. 3rd ed. 23: 704-717.

Klecak, G. et al. 1977. *Screening of fragrance materials for allergenicity in the guinea pig. I. Comparison of four testing methods.* J. Soc. Cosmet. Chem. 28: 53-64.

Koch, H.P. et al. 1993. *The Yeast Test: An Alternative Method for the Testing of Acute Toxicity of Drug Substances and Environmental Chemicals.* Methods Find Exp. Clin. Pharmacol. 15 (3): 141-152.

Korenman, Y.I. et al. 1975. *Extraction of vanillins from aqueous solutions.* Zh. Prikl. Khim. 48(1): 195-198. (Citation: Euclid Data Sheet for Vanillin by Rhône-Poulenc Chimie, created 26.01.95, updated 01.06.95)

Kutz, S.A. et al. 1980. *Evaluation of Chemicals for Immunomodulatory Effects Using an in Vitro Antibody-Producing Assay*. Environ. Res. 22 (2): 368-376.

Lawlor, T.E. 1991. *Mutagenicity test on Vanillin in the Salmonella/mammalian-microsome reverse mutation assay (Ames test)*. Hazleton Laboratories America, Inc. Report no. 12389-0-401. Unpublished report (Rhône-Poulenc, USA).

Leo, A.J. 1978. Report on the calculation of octanol/water Log P values for structures in EPA Files. (Citation: Euclid Data Sheet for Vanillin by Rhône-Poulenc Chimie, created 26.01.95, updated 01.06.95)

Lewis, R.J. 1992. Sax's Dangerous Properties of Industrial Materials. 8th ed. 3482.

Lheritier, M. 1991. *Test to evaluate the acute toxicity following a single cutaneous application (limit test) in the rat*. Hazleton France. Report no. 109364. Unpublished report (Rhône-Poulenc, France).

Lheritier, M. 1992. *Test to evaluate the acute toxicity following a single oral administration (LD50) in the rat*. Hazleton France. Report no. 201371. Unpublished report (Rhône-Poulenc, France).

Lide, D.R. 1993-1994. CRC Handbook of Chemistry and Physics. 74th ed. 3516.

Lide, D.R. 1994-1995. CRC Handbook of Chemistry and Physics. 75th ed. 3-18.

List of MAK and BAT values 1995. Page 134.

Liu, J. et al. 1993. *Antioxidant and pro-oxidant activities of p-hydroxybenzyl alcohol and vanillin: Effects on free radicals, brain peroxidation and degradation of benzoate, deoxyribose, amino acids and DNA.* Neuropharmacology. 32 (7): 659-669. (Citation: Euclid Data Sheet for Vanillin by Rhône-Poulenc Chimie, created 26.01.95, updated 01.06.95)

LOGP and Related Parameters Computerized Database. 1987. Pomona College Medicinal Chemistry Project, Pomona College, Clarement, CA. Technical Database Services (TDS) Inc. (Citation: CEARS (Chemical Evaluation Search and Retrival System). Record no. 00156)

Mange, C.E. et al. 1924. *Solubilities of Vanillin.* Ind. Eng. Chem. 16 (12): 1258-1260. (Citation: Euclid Data Sheet for Vanillin by Rhône-Poulenc Chimie, created 26.01.95, updated 01.06.95)

Martinez, N.L. et al. 1993. *The biological purification of waste products from olive oil extraction.* Bioresource Technol. 43 (3): 215-219.

Marzin, D. 1979a. Rhône-Poulenc unpublished / DREBS (Department Recherche et Essais Biologiques Stallergènes) study R-905017. (Citation: Euclid Data Sheet for Vanillin by Rhône-Poulenc Chimie, created 26.01.95, updated 01.06.95)

Marzin, D. 1979b. *Recherche d'une action mutagène par le test du micronucleus chez la souris du produit: Vanilline*. DREBS (Department Recherche et Essais Biologiques Stallergènes). Report no. 906010. Unpublished report (Rhône-Poulenc, France).

Mattson, V.R. et al. 1976. *Acute toxicity of selected organic compounds to fathead minnows*. Ecol. Res. Ser. US Environ. Prot. Agency. No EPA-600/3-76-097.

Mercier, O. 1992. *Test to evaluate the sensitizing potential in the guinea pig (Guinea pig maximization test)*. Hazleton France. Report no. 201334. Unpublished report (Rhône-Poulenc, France).

Morita, Y. et al. 1988. *Effects of antimutagens on the teratogenicity of N-methyl-N'-nitro-N-nitroso-guanidine in mice*. Congenital Anomalies. 28 (3): 157-167.

Mortelmans, K. et al. 1986. Environ. Mutagen. 8 (supplt 7): 1-119.

Murli, H. 1991. *Mutagenicity test on Vanillin in an in vitro cytogenic assay measuring chromosomal aberration frequencies in Chinese Hamster ovary (CHO) cells*. Hazleton Laboratories America Inc. Report no. 12389-0-437. Unpublished report (Rhône Poulenc).

Nagabhushan, M. et al. 1985. *Mutagenicity of Chili Extract and Capsaicin in Short-Term Tests.* Environ. Mutagen. 7 (6): 881-888.

Neuhauser, E.F. et al, 1978. *Soil invertebrates and the degradation of Vanillin, cinnamic acid and lignins*. Soil Biol. Biochem. 10 (5): 431-435. (Citation: Euclid Data Sheet for Vanillin by Rhône-Poulenc Chimie, created 26.01.95, updated 01.06.95)

Nordisk Miljøapport (Nordic Environmental Report). 1990. *Miljöfarliga kemiska ämnen och produkter*. Miljøapport 1990:10. 14.

Numata, A. et al. 1992. *Occurence of epijuvabione-type sesquiterpenoids in Abies Sachalinensis.* Phytochemistry. 31 (11): 3773-3780. (Citation: Euclid Data Sheet for Vanillin by Rhône-Poulenc Chimie, created 26.01.95, updated 01.06.95)

Ohta, T. et al. 1986. *Inhibitory effects of flavourings on mutagenesis induced by chemicals in bacteria.* Food Chem. Toxicol. 24 (1): 51-54.

Ohta, T. et al. 1988. *Post replication repair and recombination in uvrA umuC strains of Escherichia coli are enhanced by vanillin, an antimutagenic compound*. Mutat. Res. 201 (1): 107-112.

Opdyke, D.L.J. 1977. *Monographs on fragrance raw materials. Vanillin*. Food Cosmet. Toxicol. 15 (6): 633-638.

Oshima, H. et al. 1989. *Formation of direct-acting genotoxic substances in nitrosated smoked fish and meat products: Identification of simple phenolic precursors and phenyldiazonium ions as reactive products*. Food Chem. Toxicol. 27 (3): 193-203.

Osol, A. et al. 1975. Remington Pharmaceutical Sciences. 15th ed. Easton, Pennsylvania: Mack Publishing Co. 1236.

Palmer, C.M. et al. 1955. *Preliminary Screening for Potential Algicides*. Ohio J. Sci. 55 (1): 1-8.

Parke, D.V. 1968. The Biochemistry of foreign compounds. Oxford: Pergamon Press. 141.

Perry, Th.S. et al. 1965. *Exogenous urinary amines of plant origin*. Clin. Chim. Acta. 11 (1): 24-34. (Citation: FEMA. 1985. Scientific Literature Review of Vanillin and Derivatives)

Pettersen, M. 1996. *Free-Flow Project MP-007*. Internal report Borregaard EuroVanillin, Norway.

Petterson, B. et al. 1982. *Effects of tobacco smoke compounds on the ciliary activity of the embryo chicken trachea in vitro.* Toxicology. 23 (1): 41-55. (Citation: Euclid Data Sheet for Vanillin by Rhône-Poulenc Chimie, created 26.01.95, updated 01.06.95)

Pool, B.L. et al. 1982. *Mutagenicity testing in the Salmonella Typhimurium assay of phenolic compounds and phenolic fractions obtained from smokehouse smoke condensates.* Food Chem. Toxicol. 20 (4): 383-391. (Citation: Euclid Data Sheet for Vanillin by Rhône-Poulenc Chimie, created 26.01.95, updated 01.06.95)

Project Euromarketing. 1993. EuroVanillin, Norway. Unpublished report. 20.

Ramberg, L. 1996. *Støvmålinger i Pakkeriet, Vanillinfabrikken*. Internal memo Borregaard, Norway, dated 23.07.96.

Reynolds, T. 1978. *Comparative Effects of Aromatic Compounds on Inhibition of Lettuce Fruit Germination.* Ann. Botany. 42: 419-428.

Rhône-Poulenc. 1983 Unpublished data. (Citation: Euclid Data Sheet for Vanillin by Rhône-Poulenc Chimie, created 26.01.95, updated 01.06.95)

Rhône-Poulenc. 1988. Unpublished study. (Citation: Euclid Data Sheet for Vanillin by Rhône-Poulenc Chimie, created 26.01.95, updated 01.06.95)

Rhône-Poulenc. 1993. Material Safety Data Sheet. 14.07.93. Cranbury, N.J.

Rhône-Poulenc. 1994. Material Safety Data Sheet. 19.12.94. Cranbury, N.J. (Citation: Euclid Data Sheet for Vanillin by Rhône-Poulenc Chimie, created 26.01.95, updated 01.06.95)

Rhône-Poulenc Chimie. 1988. Safety Data Sheet Feb.88, 2nd ed. Division Specialites Chimiques. Cedex no. 29, 92097 PARIS La Defence.

Riffle, M.S. et al. 1990. *Devil's-claw (Proboscidea louisianica), essential oil and its components: Potential Allelochemical Agents on Cotton and Wheat.* J. Chem. Ecol. 16 (6): 1927-1940.

Robinson, R.A. et al. 1955. *The ionization constants of vanillin and two of its isomers.* Trans Faraday Soc. 51: 1398-1402. (Citation: Euclid Data Sheet for Vanillin by Rhône-Poulenc Chimie, created 26.01.95, updated 01.06.95)

Rosin, M.P. 1984. *The influence of pH on the convertogenic activity of plant phenolics.* Mutat. Res. 135 (2): 109-113.

Sagabiel, J.C. et al. 1993. *Studies on the occurence and distribution of wood smoke marker compounds in foggy atmospheres.* Environ. Toxicol. Chem. 12 (5): 813-822.

Salaman, M.H. et al. 1956. *Further tests for tumour-initiating activity: N,N-Di-(2-chloroethyl)-p-aminophenylbutyric acid (CB1348) as an initiator of skin tumour formation in the mouse.* Br. J. Cancer. 10: 363-378.

Sammons H.G. et al. 1941. *Studies in detoxication. 12. The metabolism of Vanillin and Vanillic acid in the rabbit. The identification of glucurovanillin and glucurovanillic acid*. Biochem. J. 35: 1175-1189. (Citation: FEMA. 1985. Scientific Literature Review of Vanillin and Derivatives)

Sasaki, Y.F. et al. 1987a. *Effects of Vanillin on sister-chromatid exchanges and chromosome aberrations induced by mitomycin C in cultured Chinese hamster ovary cells*. Mutat. Res. 191: 193-200.

Sasaki, Y.F. et al. 1987b. *Effects of antimutagenic flavourings on SCEs induced by chemical mutagens in cultured Chinese hamster cells.* Mutat. Res. 189 (3): 313-318.

Sasaki, Y.F. et al. 1990a. *Suppressing effect of antimutagenic flavourings on chromosome aberrations induced by UV-light or X-rays in cultured Chinese hamster cells.* Mutat. Res. 229: 1-10.

Sasaki, Y.F. et al. 1990b. *Suppressing effects of vanillin, cinnamaldehyde, and anisaldehyde on chromosome aberrations induced by X-rays in mice.* Mutat. Res. 243 (4): 299-302.

Sato, T. et al. 1991. *Evaluation of the SOS Chromotest for the Detection of Antimutagens.* Environ. Mol. Mutagen. 17 (4): 258-263.

Scheline, R.R. 1972. *Metabolism of some aromatic aldehydes and alcohols by the rat intestinal microflora*. Xenobiotica. 2 (3): 227-236. (Citation: FEMA. 1985. Scientific Literature Review of Vanillin and Derivatives)

Semb, S.I. et al. 1996. *Determination of the Rate of Hydrolysis: Vanillin*. NIVA (Norwegian Institute for Water Research), Norway. Report no. G001/1b. Unpublished report (EuroVanillin KS, Norway).

Sierra-Alvarez, R. et al. 1991. *The Methanogenic Toxicity of Wastewater Lignins and Lignin Related Compounds.* J. Chem. Technol. Biotechnol. 50 (4): 443-455.

Sigma-Aldrich. 1988. Library Chemical Safety Data. 2nd ed. (Citation: Euclid Data Sheet for Vanillin by Rhône-Poulenc Chimie, created 26.01.95, updated 01.06.95)

Stoner, G.D. et al. 1973. *Test for carcinogenicity of food additives and chemotherapeutic agents by the pulmonary response in strain A mice*. Cancer Res. 33 (12): 3069-3085.

Stoner, G.D. 1991. *Lung Tumors in Strain A Mice as a Bioassay for Carcinogenicity of Environmental Chemicals.* Exp. Lung Res. 17 (2): 405-423.

Strand, L.P. et al. 1975. *The Metabolism of Vanillin and Isovanillin in the Rat*. Xenobiotica. 5 (1): 49-63. (Citation: Euclid Data Sheet for Vanillin by Rhône-Poulenc Chimie, created 26.01.95, updated 01.06.95)

Strzelczyc, E. et al. 1972. *Utilization of Aromatic Compounds byBenthic Microorganisms of an Euthrophic Lake.* Acta Microbiol. Pol. Ser. B. 4 (4), 191-200.

Susuki, M. et al. 1978. *Study of thermal regeneration of spent activated carbons: Thermogravimetric measurement of various single component organics loaded on activated carbons.* Chem. Eng. Sci. 33: 271-273. (Citation: Euclid Data Sheet for Vanillin by Rhône-Poulenc Chimie, created 26.01.95, updated 01.06.95)

Takahashi, K. et al. 1990. *Effects of vanillin and o-vanillin on induction of DNA-repair networks: modulation of mutagenesis in Escherichia coli.* Mutat. Res. 230 (2): 127-134.

Tam, P.E. et al. 1984. *Evaluation of Immunomodulatory Chemicals: Alteration of Macrophage Function in Vitro*. Toxicol. Appl. Pharmacol. 76 (1) 183-194.

Tam, P.E. et al. 1990. *Screening for Immunomodulators: Effects of Xenobiotics on Macrophage Chemiluminescence in Vitro.* Fundam. Appl. Toxicol. 14 (3): 542-553.

The Merck Index. 11th ed, 1989. Monograph 9839. Vanillin.

The United States Pharmacopeia 23. The National Formulary 18. 1995. 2122, 2316.

Tieman, N. 1877. Chem. Ber. 10: 211. (Citation: Euclid Data Sheet for Vanillin by Rhône-Poulenc Chimie, created 26.01.95, updated 01.06.95)

Van Assendelft, A.H.W. 1984. *Bronchospasm induced by vanillin and lactose*. Eur. J. Respir. Dis. 65: 468-472.

Verrett, M.J. et al. 1980. *Toxicity and Teratogenicity of Food Additive Chemicals in Developing Chicken Embryo.* Toxicol. Appl. Pharmacol. 56 (2): 265-273.

Verschueren, K. 1983. Handbook of Environmental Data on Organic Chemicals, 2nd ed.

Vïkel, W. 1995. *Estimation of the degradation of Vanillin by Photo-oxidation in air (Model calculation according to Atkinson)*. RCC (Registration and Consulting Company Ltd.), Switzerland. RCC Project 606396. Unpublished report (EuroVanillin KS, Norway).

Wang, Xia-Sheng et al. 1987. *Occupational contact dermatitis in manufacture of Vanillin*. Chinese Medical J. 100 (3): 250-254.

Watanabe, K. et al. 1988. *Antimutagenic Effects of Benzaldehyde and Its Derivatives on Mutagenesis Induced by 4-Nitroquinoline 1-Oxide in Escherichia coli*. Agric. Biol. Chem. 52 (4): 1041-1045.

Weast, R.C. et al. 1989. Handbook of Data on Organic Compounds. 2nd ed. CRC Press. (Citation: Euclid Data Sheet for Vanillin by Rhône-Poulenc Chimie, created 26.01.95, updated 01.06.95)

Wong, K.P. et al. 1966. *Metabolism of Vanillin and related substances in the rat*. Can. J. Biochem. 44 (5): 635-644. (Citation: FEMA. 1985. Scientific Literature Review of Vanillin and Derivatives)

Younger Laboratories Inc. 1976. Report no. Y-76-261. Unpublished report (Monsanto Chemical Company, USA). (Citation: Euclid Data Sheet for Vanillin by Rhône-Poulenc Chimie, created 26.01.95, updated 01.06.95)

EXTRACT FROM IRPTC LEGAL FILE

File: 17.01 LEGAL **rn : 401061**

systematic name:Benzaldehyde,4-hydroxy-3-methoxy-
common name :vanillin
reported name :VANILLIN
cas no :121-33-5 rtecs no :YW5775000
area : CZE type : REG

subject	specification	descriptor
FOOD		MPC

LIMIT OF ADDITIVE PRESENT DUE TO PRODUCTION, PACKING, TRANSPORT AND STORAGE OF FOOD PRODUCTS: 1.0G/KG.
entry date: DEC 1991 effective date: 1JUL1986

title: DIRECTIVE NO. 50/1978 ON FOREIGN SUBSTANCES IN FOODSTUFFS
original : HPMZC*, HYGIENICKE PREDPISY MINISTERSTVA ZDRAVOTNICTVI CSR(HYGIENIC REGULATIONS OF MINISTRY OF HEALTH OF CSR), 43 , , , 1978
amendment: HPMZC*, HYGIENICKE PREDPISY MINISTERSTVA ZDRAVOTNICTVI CSR(HYGIENIC REGULATIONS OF MINISTRY OF HEALTH OF CSR), 61 , , , 1986

****

File: 17.01 LEGAL **rn : 1122371**

systematic name:Benzaldehyde,4-hydroxy-3-methoxy-
common name :vanillin
reported name :VANILLIN
cas no :121-33-5 rtecs no :YW5775000
area : RUS type : REG

subject	specification	descriptor
AIR	OCC	MAC CLASS

CLV : 1.5 MG/M3 (VAPOUR, AEROSOL) HAZARD CLASS: III
entry date: MAY 1990 effective date: 01JAN1989

amendment: GOSTS*, GOSUDARSTVENNYI STANDART SSSR(STATE STANDARD OF USSR), 12.1.005 , , , 1988

****

File: 17.01 LEGAL **rn : 1504568**

systematic name:Benzaldehyde,4-hydroxy-3-methoxy-
common name :vanillin
reported name :VANILLIN
cas no :121-33-5 rtecs no :YW5775000
area : F/W type : REC

subject	specification	descriptor
FOOD	ADDIT	ADI
FOOD	ADDIT	MXL
USE	ADDIT	PRMT

USE: FLAVOUR; ADI: 0-10 MG/KG BW; MXL: JAMS AND JELLIES, CREAM: LIMITED BY GOOD MANUFACTURING PRACTICE; CHOCOLATE, COCOA POWDERS AND DRY COCOA-SUGAR MIXTURES, COMPOSITE AND FILLED CHOCOLATE, COCOA MASS AND COCOA PRESS CAKE: IN SMALL AMOUNTS TO BALANCE THE FLAVOUR; CANNED BABY FOODS, PROCESSED CEREAL-BASED FOODS FOR INFANTS AND CHILDREN: 70 MG/KG OF READY-TO-EAT PRODUCT
entry date: MAY 1991 effective date: 1983

amendment: FAOCA*, CODEX ALIMENTARIUS, XIV , , 262 , 1983