

201-14820B

Robust Test Summaries for 1,3,4,6,7,8-Hexahydro-4,6,6,7,8,8-hexamethylcyclopenta- γ -2-benzopyran
(HHCB) CAS# 1222-05-5

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Robust Summary for HHCB

The evaluation of the quality of the following data uses a systematic approach described by Klimisch [Klimisch *et al.*, 1996]. Based on criteria relating to international testing standards for categorizing data reliability, four reliability categories have been established. The following categories are:

- Reliability code 1. Reliable without restrictions
- Reliability code 2. Reliable with restrictions
- Reliability code 3. Not reliable
- Reliability code 4. Not assignable

1 Chemical and Physical Properties

1.1 Melting Point

Substance Name	HHCB
CAS No.	1222-05-5
Method/guideline	Sample was cooled to -30° C and gradually warmed
GLP	No
Melting Point	-10 to 0 degrees C
Data Qualities Reliabilities	Reliability 2. Reliable with restriction
Remarks for Data Reliability	The substance is a mixture of isomers and is not expected to have a precise melting point.
References	IFF, 2001

1.2 Boiling Point

Substance Name	HHCB
CAS No.	1222-05-5
Method/guideline	Calculated using Syracuse Research Corporation SAR-based software
GLP	No
Year	2000
Boiling Point	162° C @ 760 mm
Data Qualities Reliabilities	Reliability 4. Not assignable
Remarks for Data Reliability	Data calculated by recognized SAR program with input of log Kow, VP and water solubility.
References	William Meylan and Philip Howard, 2000. EPI Suite v 3.10, Syracuse Research Corporation

Substance Name	HHCB
CAS No.	1222-05-5
Method/guideline	Measured during distillation
GLP	No
Year	2001
Boiling Point	160° C @ 4 mm Hg
Data Qualities Reliabilities	Reliability 2. Reliable with restriction
Remarks for Data Reliability	The measured boiling point was recorded in the distillation of HHCB in the manufacturing plant.
References	IFF, 2001

1.3 Vapor Pressure

Substance Name	HHCB
CAS No.	1222-05-5
Method/guideline	OECD 104
Vapor Pressure	0.0727 Pa
Temperature	25° C
Data Qualities Reliabilities	Reliability 1. Reliable without restrictions
Remarks for Data Reliability	Study conducted according to an OECD protocol under GLP and data are published in a peer-reviewed journal
References	Balk F and Ford RA, 1999a. Environmental risk assessment for the polycyclic musks AHTN and HHCB in the EU. I. Fate and Exposure Assessment. Toxicology Letters, 111, 57-79.

1.4 n-Octanol/Water Partition Coefficient

Substance Name	HHCB
CAS No.	1222-05-5
Method/guideline	OECD 117
GLP	Yes
Year	1999
Log Pow	5.9
Temperature	25° C
Remarks for Test Conditions	Conducted under GLP
Remarks for Results	Result is an average of 5.8 and 6.0, the values for the 2 principal isomers
Data Qualities Reliabilities	Reliability 1. Reliable without restrictions
Remarks for Data Reliability	Study conducted according to an OECD protocol under GLP and data are published in a peer-reviewed journal.
References	Balk F and Ford RA, 1999a. Environmental risk assessment for the polycyclic musks AHTN and HHCB in the EU. I. Fate and Exposure Assessment. Toxicology Letters, 111, 57-79.

1.5 Water Solubility

Substance Name	HHCB
CAS No.	1222-05-5
Method/Guideline	OECD 105
GLP	Yes
Year	1996
Value (mg/L) at Temperature	1.75 @ 25° C at a pH of 7
Remarks for Test Conditions	
Remarks for Results	Water solubility was 1.99 mg/L at a pH of 5 and 1.69 mg/L at a pH of 9.
Data Qualities Reliabilities	Reliability 1. Reliable without restrictions
Remarks for Data Reliability	Study conducted according to an OECD protocol under GLP and data are published in a peer-reviewed journal
References	Balk F and Ford RA, 1999a. Environmental risk assessment for the polycyclic musks AHTN and HHCB in the EU. I. Fate and Exposure Assessment. Toxicology Letters, 111, 57-79.

1.6 Relative Density

Substance Name	HHCB
CAS No.	1222-05-5
Method/Guideline	OECD 109
GLP	No
Year	2001
Value (mg/L) at Temperature	0.99 – 1.015 g/cm ³ @ 20° C
Remarks for Test Conditions	Measured with an oscillating densitometer.
Remarks for Results	
Data Qualities Reliabilities	Reliability 2. Reliable with restrictions
Remarks for Data Reliability	
References	IFF, 2001

1.7 Flashpoint

Substance Name	HHCB
CAS No.	1222-05-5
Method/Guideline	Pensky Martens Method (closed cup)
GLP	No
Year	2001
Value (mg/L) at Temperature	>100° C
Remarks for Test Conditions	
Remarks for Results	
Data Qualities Reliabilities	Reliability 2. Reliable with restrictions
Remarks for Data Reliability	
References	IFF, 2001

2 Environmental Fate and Pathways

2.1 Photodegradation

Substance Name	HHCB
CAS No.	1222-05-5
Method/guideline	
Test Type	Irradiation
Half-life t1/2	3.7 hours
Remarks for Test Conditions	Black irradiation lamps of $\lambda > 300$ nm at 25° C and 740 mmHg.
Remarks for Results	
Data Qualities Reliabilities	Reliability 1. Data are reliable without restriction
Remarks for Data Reliability	Data obtained under laboratory conditions using methyl vinyl ketone as a reference substance.
References	Aschman SM, Arey J, Atkinson R and Simonich SL, 2001. Atmospheric lifetimes and fates of selected fragrance materials and volatile model compounds. Environmental Science and Technology, 359180, 3595-3600.

2.2 Biodegradation

Substance Name	HHCB
CAS No.	1222-05-5
Remarks for Substance	HHCB undiluted (Purity 99%)
Method	OECD 301B
Test Type	Ready Biodegradability
GLP	Yes
Year	1994
Contact Time	28 days
Innoculum	Sewage effluent 1drop/L
Remarks for Test Conditions	Modified Sturm, CO ₂ Evolution, Sodium benzoate as reference substance.
Degradation % After Time	0%

Time required for 10% degradation	
Remarks Results	HHCB is not mineralized in the ready biodegradability test.
Conclusion Remarks	Further tests have shown that HHCB is inherently biodegradable.
Data Qualities Reliabilities	Reliability 1. Data are reliable without restriction
Remarks for Data Reliability	Data generated using approved OECD protocol under GLP and also published in a peer-reviewed journal.
Reference	Balk F and Ford RA, 1999a. Environmental risk assessment for the polycyclic musks AHTN and HHCB in the EU. I. Fate and Exposure Assessment. Toxicology Letters, 111, 57-79.

Substance Name	HHCB
CAS No.	1222-05-5
Remarks for Substance	Commercial sample (HHCB in diluent isopropyl myristate)
Method	Modified OECD 301B
Test Type	Ready Biodegradability
GLP	Yes
Year	1993
Contact Time	28 days
Innoculum	Sewage effluent from SCAS after 8 weeks adaptation, 1drop/L
Remarks for Test Conditions	Sealed vessel Total Inorganic Carbon (TIC) test, Benzyl alcohol as reference substance.
Degradation % After Time	0% (corrected for isopropyl myristate)
Time required for 10% degradation	
Remarks Results	HHCB is not mineralized in the ready biodegradability test.
Conclusion Remarks	Further tests have shown that HHCB is inherently biodegradable.
Data Qualities Reliabilities	Reliability 1. Data are reliable without restriction
Remarks for Data Reliability	Data generated using approved OECD protocol under GLP and also published in a peer-reviewed journal.
Reference	Balk F and Ford RA, 1999a. Environmental risk assessment for the polycyclic musks AHTN and HHCB in the EU. I. Fate and Exposure Assessment. Toxicology Letters, 111, 57-79.

Substance Name	HHCB
CAS No.	1222-05-5
Remarks for Substance	Commercial sample (HHCB in diluent)
Method	
Test Type	Primary Degradation
GLP	No
Year	
Contact Time	
Remarks for Test Conditions	64 samples from different soil types were screened for the presence of naturally occurring micro-organisms. Pure cultures of fungi (Aureobasidium pullulans and Phanerochaete chrysosporium) were incubated with HHCB. Ethyl acetate extracts of the cultures were analysed by GC MS.
Conclusion Remarks	HHCB was demonstrated to degrade to more polar metabolites with the lactone and the hydroxycarboxylic acid as likely intermediates.
Data Qualities Reliabilities	Reliability 2. Data are reliable with restrictions
Remarks for Data Reliability	Data published in a peer-reviewed journal.
Reference	Balk F and Ford RA, 1999a. Environmental risk assessment for the polycyclic musks AHTN and HHCB in the EU. I. Fate and Exposure Assessment. Toxicology Letters, 111, 57-79.

Substance Name	HHCB
CAS No.	1222-05-5
Remarks for Substance	Radiolabeled HHCB
Method	
Test Type	Soil Microcosm primary degradation
GLP	
Year	1998
Contact Time	1 year
Remarks for Test Conditions	Samples were taken from 1) oak forest 2) agricultural field, 3) sediment of the Delaware river in central New Jersey and 4)

sludge amended soil from a farm. Sealed flasks with soil spiked with 10 ug HHCB/g soil were incubated at laboratory temperatures for 1 year. Closed systems were used, with periodic flushing of headspace for oxygen replenishment and effluent gas was drawn through a train of scintillation fluids to capture volatiles and CO₂. After the incubation period, the flasks were extracted with solvent and analysed for HHCB.

Conclusion Remarks	An average of 14% of HHCB remained in the soil after one year demonstrating a half-life value of 4 months for HHCB in soils.
Data Qualities Reliabilities	Reliability 2. Data are reliable with restriction
Remarks for Data Reliability	Data published in a peer-reviewed journal.
Reference	Balk F and Ford RA, 1999a. Environmental risk assessment for the polycyclic musks AHTN and HHCB in the EU. I. Fate and Exposure Assessment. Toxicology Letters, 111, 57-79.

Substance Name	HHCB
CAS No.	1222-05-5
Remarks for Substance	Radiolabeled HHCB
Method	
Test Type	Biotransformation
GLP	
Year	2000
Contact Time	
Remarks for Test Conditions	To understand the fate of HHCB in the environment, biotransformation was examined under realistic conditions in activated sludge and river water. Radiolabeled HHCB was dosed to freshly collected activated sludge (25 ug/L) and river water (1 ug/L). The disappearance of parent and the formation of metabolites were monitored over time.
Conclusion Remarks	The half-lives for parent HHCB were 21 hours in activated sludge and 33 hours in river water. HHCB is biotransformed in activated sludge and river water to polar metabolites that are predicted to be less bioaccumulative and less toxic than the parent compound. Therefore, concentrations of HHCB measured in the environment are lower than predicted concentrations.

Data Qualities Reliabilities	Reliability 2. Data are reliable with restriction
Remarks for Data Reliability	Data published in a peer-reviewed journal.
Reference	<p>Balk F and Ford RA, 1999a. Environmental risk assessment for the polycyclic musks AHTN and HHCB in the EU. I. Fate and Exposure Assessment. Toxicology Letters, 111, 57-79.</p> <p>Langworthy DE, Itrich NR, Simonich SL, and Federle TW, 2000. Biotransformation of the Polycyclic Musk HHCB in activated sludge and river water. Presented at SETAC, May 2000, Brighton, U.K.</p>

2.3 Fugacity

Substance Name	HHCB
CAS No.	1222-05-5
Model Conditions	25° C, 100,000 lbs
Test Type	Environmental Equilibrium Partitioning Model
Method	Mackay
Model Used	EPT V 3.10 Level III
Input Parameters	MW, measured log Kow, measured water solubility and measured VP
Year	2003
Media	Air-Water Soil-Sediment-Partition Coefficient
Estimated Distribution and Media Concentration	
Model data and results	Air = 0.188%, Water = 5.58%, Soil = 38.6% and Sediment = 55.6%
Remarks	
Data Qualities Reliabilities	Reliability 4. Not assignable.
Remarks for Data Reliability	The data are obtained by a recognized fugacity calculation method. However, the method is an estimation.
References	USEPA, 2003 SRC EPIWIN Program

3 Ecotoxicity

3.1 Acute Toxicity to Fish

Substance Name	HHCB
CAS No.	1222-05-5
Remarks for Substance	Purity 99.15% isomeric mixture
Method/guideline	OECD 204
Test Type	21-day prolonged bluegill sunfish toxicity test
GLP	Yes
Year	1996
Species/Strain/Supplier	Lepomis macrochirus
Exposure Period	21 days
Analytical monitoring	Start, half-way through and at end.
Remarks for Test Conditions	Flow-through conditions. Nominal concentrations were 0.125 to 2.0 step size
Endpoint value	LC50 = 0.452, NOEC = 0.093, LC100=0.83, LOEC= 0.182
Unit	mg/L
Conclusion Remarks	Clinical signs included loss of equilibrium, irregular respiration and cessation of food intake. Results are expressed based on the mean measured concentrations of HHCB in the test which were 0.093, 0.182, 0.393, 0.830 and 1.566 mg/L.
Data Qualities Reliabilities	Reliability 1. Reliable without restriction
Remarks for Data Reliability	Study conducted according to an OECD protocol under GLP and the data are published in a peer-reviewed journal.
Reference	Balk F and Ford RA, 1999b. Environmental risk assessment for the polycyclic musks AHTN and HHCB in the EU. II. Effect Assessment and Risk Characterization. Toxicology Letters, 111, 81-94. Wuthrich, V. 1996a. HHCB: 21-day prolonged toxicity study in the bluegill sunfish under flow-through conditions. Report to RIFM., RCC Umweltchemie AG Project 380711.

3.2 Acute Toxicity to Aquatic Invertebrates

Substance Name	HHCB
CAS No.	1222-05-5
Remarks for Substance	Purity 99.15% isomeric mixture
Method/guideline	OECD 202
Test Type	Semi-static 21-day Daphnia
GLP	Yes
Year	1996
Species/Strain/Supplier	Daphnia magna
Analytical procedures	48-hour LC50 was calculated based on the 21-day test.
Test Details	Nominal concentrations ranged from 0.062 to 1.0 mg/l. Step size 2.
Remarks for Test Conditions	
EC50, EL50, LC0, at 24,48 hours	NOEC(rep) = 0.111, LOEC = 0.205, EC50 = 0.282 (48-hrs)
Unit	mg/L
Biological observations	
Remarks for Results	Results are based on the measured concentrations of HHCB in the test which were 0.049, 0.111, 0.205, 0.419, and 0.842 mg/L. 48-hour LC ₅₀ was calculated based on the 21-day test.
Data Qualities Reliabilities	Reliability 1. Reliability without restriction
Data Reliability Remarks	Study was conducted according to an OECD protocol under GLP and the data are published in a peer-reviewed journal.
Reference	Balk F and Ford RA, 1999b. Environmental risk assessment for the polycyclic musks AHTN and HHCB in the EU. II. Effect Assessment and Risk Characterization. Toxicology Letters, 111, 81-94. Wuthrich, V. 1996b. Influence of HHCB on the reproduction of Daphnia magna. Report to RIFM. RCC Umweltchemie AG Project 380687.

3.3 Acute Toxicity to Aquatic Plants

Substance Name	HHCB
CAS No.	1222-05-5
Method/guideline	OECD 201
Test Type	Algae Static growth inhibition test
Species/Strain/Supplier	Pseudokirchneriella subcapitata
Exposure Period	72 hours
Remarks for Test Conditions	Endpoint was growth rate as well as biomass. Start concentrations were 71-102% of nominal and end concentrations 54-85% of nominal. Mean measured concentrations were 0.042, 0.084, 0.201, 0.466 and 0.844 mg/L. Nominal concentrations ranged from 0.065 to 1.0 mg/L. Step size 2. Results are based on the mean measured concentrations.
Endpoint value	NOEC = 0.201, LOEC = 0.466, EC50 for biomass production = 0.72 mg/L, EC50 for growth = > 0.854 mg/L.
Conclusion Remarks	
Data Qualities Reliabilities	Reliability 1. Reliability without restriction
Remarks for Data Reliability	Study conducted according to an OECD protocol under GLP and the data are published in a peer-reviewed journal.
Reference	Balk F and Ford RA, 1999b. Environmental risk assessment for the polycyclic musks AHTN and HHCB in the EU. II. Effect Assessment and Risk Characterization. Toxicology Letters, 111 81-94. Van Dijk, A. 1997. Acute toxicity of HHCB to Pseudokirchneriella subcapitata. Report to RIFM, RCC Umweltchemie AG Project 380632.

4 Human Health Toxicity

4.1 Acute Toxicity

Substance Name	HHCB
CAS No.	1222-05-5
Method/guideline	
Test Type	Acute oral toxicity limit test
GLP	Pre-GLP
Year	1975
Species/strain	Rats, Wistar
Sex	Male and Female
# of animals per sex per dose	10
Vehicle	None
Route of Administration	Gavage
Remarks for Test Conditions	14-day observation period
Value LD50 or LC50 with confidence limits	> 3.25 g/kg
Number of deaths at each dose level	1/10 at 3.25 g/kg
Remarks for Results	The material as tested was a commercial sample and therefore, would have been an approximately 65% solution. Therefore the dose administered has been corrected from 5g/kg to 3.25 g/kg bw.
Data Qualities Reliabilities	Reliability code 2. Reliable with restrictions
Remarks for Data Reliability	Data collected prior to GLP by method comparable to present guidelines/standards.
References	Moreno, O.M. 1975. Galaxolide 50: acute oral toxicity in rats; dermal toxicity in rabbits. Project No. MB 75-770. MB Research Report to the Research Institute for Fragrance Materials, Inc. (RIFM). Ford RA, 1998. The human safety of the polycyclic musks, AHTN and HHCB in fragrances – A review, Dtsch, Lebens. Rdsch., 98(8), 268-275.

Substance Name	HHCB
CAS No.	1222-05-5
Method/guideline	
Test Type	Acute oral toxicity limit test
GLP	Pre-GLP
Year	1977
Species/strain	Rats
Sex	Female
# of animals per sex per dose	10
Vehicle	None
Route of Administration	Gavage
Remarks for Test Conditions	14-day observation period
Value LD50 or LC50 with confidence limits	> 3 g/kg
Number of deaths at each dose level	0 at highest dose
Remarks for Results	The material as tested was a commercial sample and therefore, would have been an approximately 65% solution.
Data Qualities Reliabilities	Reliability code 2. Reliable with restrictions
Remarks for Data Reliability	Data collected prior to GLP by method comparable to present guidelines/standards.
References	Ford RA, 1998. The human safety of the polycyclic musks, AHTN and HHCB in fragrances – A review, Dtsch, Lebens. Rdsch., 98(8), 268-275.

Substance Name	HHCB
CAS No.	1222-05-5
Method/guideline	
Test Type	Acute dermal toxicity limit test
GLP	Pre-GLP
Year	1975
Species/strain	New Zealand white rabbits
Sex	Not reported
# of animals per sex per dose	7
Vehicle	None
Route of Administration	Dermal
Remarks for Test Conditions	14-day observation period
Value LD50 or LC50 with confidence limits	> 3.25 g/kg
Number of deaths at each dose level	0/10 at 3.25 g/kg
Remarks for Results	The material as tested was a commercial sample and therefore, would have been an approximately 65% solution. Therefore, the dose administered has been corrected from 5g/kg to 3.25 g/kg bw. There were no deaths at that dose. Therefore, the LD50 can be listed as >3.25 g/kg bw.
Data Qualities Reliabilities	Reliability code 2. Reliable with restrictions
Remarks for Data Reliability	Data collected prior to GLP by method comparable to present guidelines/standards.
References	Moreno, O.M. 1975. Galaxolide 50: acute oral toxicity in rats; dermal toxicity in rabbits. Project No. MB 75-770. MB Research Report to the Research Institute for Fragrance Materials, Inc. (RIFM). Ford RA, 1998. The human safety of the polycyclic musks, AHTN and HHCB in fragrances – A review, Dtsch, Lebens. Rdsch., 98(8), 268-275.

Substance Name	HHCB
CAS No.	1222-05-5
Method/guideline	
Test Type	Acute dermal toxicity limit test
GLP	Pre-GLP
Year	1977
Species/strain	CRL Sprague-Dawley
Sex	Female
# of animals per sex per dose	5
Vehicle	Ethanol
Route of Administration	Dermal
Remarks for Test Conditions	7-day observation period
Value LD50 or LC50 with confidence limits	> 5 g/kg
Number of deaths at each dose level	0/5 at 5 g/kg
Remarks for Results	
Data Qualities Reliabilities	Reliability code 2. Reliable with restrictions
Remarks for Data Reliability	Data collected prior to GLP by method comparable to present guidelines/standards.
References	Ford RA, 1998. The human safety of the polycyclic musks, AHTN and HHCB in fragrances – A review, Dtsch, Lebens. Rdsch., 98(8), 268-275.

4.2 Genetic Toxicity

4.2.1 In vitro Genotoxicity

Substance Name	HHCB
CAS No.	1222-05-5
Remarks for Substance	Colorless viscous liquid sample supplied by IFF. Purity > 99% based on isomeric mixture.
Method/guideline	OECD 471
Test Type	Ames reverse mutation assay
System of Testing	
GLP	Yes
Year	1999
Species/Strain	Salmonella typhimurium TA98, TA100, TA1535, TA1537 and TA1538; Escherichia coli WP2 uvrA
Metabolic Activation	With and without S9 activation
Doses/Concentration	10, 33, 100, 333, 1000 or 5000 ug per plate
Statistical Methods	
Remarks for Test Conditions	All positive controls gave positive responses to the systems within acceptable ranges.
Results	No significant increase in the number of revertant colonies was observed with HHCB at doses of 10-5000 ug/plate.
Cytotoxic concentration	None
Genotoxic Effects	None
Conclusion Remarks	No mutagenic potential
Data Qualities Reliabilities	Reliability 1. Reliable without restrictions.
Remarks for Data Reliability	Data are by a standard method and have been published in a peer-reviewed journal.
References	Api, AM and San, RHC, 1999. Genotoxicity Tests with 6-Acetyl-1,1,2,4,4,7-hexamethyltetraline and 1,3,4,6,7,8-Hexahydro-4,6,6,7,8,8-hexamethylcyclopenta-gamma-benzopyran. Mutation Research, 446: 67-81.

Substance Name	HHCB
CAS No.	1222-05-5
Remarks for Substance	Material was obtained from a commercial source, Promochem, under the trade name Galaxolide. Galaxolide is marketed as a 65% solution in diethyl phthalate.
Method/guideline	Not reported
Test Type	Ames reverse mutation assay
System of Testing	
GLP	Not reported
Year	1998
Species/Strain	Salmonella typhimurium TA97, TA98, TA100 and TA102
Metabolic Activation	With and without S9 activation
Doses/Concentration	5-500 ug per plate (corrected concentrations range from 3.25 to 325 ug/plate based on testing of 65% Galaxolide)
Statistical Methods	
Remarks for Test Conditions	The doses were 5, 16.6, 50, 166.6 or 500 ug/plate (limit of solubility)
Results	No significant increase in the number of revertant colonies was observed with HHCB at doses of 5-500 ug/plate.
Cytotoxic concentration	None
Genotoxic Effects	None
Conclusion Remarks	No mutagenic potential
Data Qualities Reliabilities	Reliability 1. Reliable without restrictions.
Remarks for Data Reliability	Data are by a standard method and have been published in a peer-reviewed journal.
References	Mersch-Sundermann V, Kevekordes S and Jenter C, 1998a. Lack of mutagenicity of polycyclic musk fragrances in Salmonella typhimurium. Tox. In Vitro, 12: 389-393.

Substance Name	HHCB
CAS No.	1222-05-5
Remarks for Substance	Material was obtained from a commercial source, Promochem, under the trade name Galaxolide. Galaxolide is marketed as a 65% solution in diethyl phthalate.
Method/guideline	Not reported
Test Type	In vitro micronucleus
System of Testing	
GLP	No
Year	1997
Species/Strain	Human peripheral lymphocytes from healthy non-smoking donors
Metabolic Activation	With and without S9 activation
Doses/Concentration	0.05, 0.49, 4.85, 48.5, 97 micromolar. (corrected for 65% solution = 0.0325, 0.3185, 3.152, 31.52, 63.05, 126.1 uM)
Statistical Methods	
Remarks for Test Conditions	Positive controls significantly increased the frequency of micronuclei.
Results	No significant increase in the frequency of micronuclei
Cytotoxic concentration	194 micromolar
Genotoxic Effects	None
Conclusion Remarks	No mutagenic potential
Data Qualities Reliabilities	Reliability 1. Reliable without restrictions.
Remarks for Data Reliability	Data are by a standard method and have been published in a peer-reviewed journal.
References	Kevekordes S, Mersch-Sundermann V, Diez M and Dunkelberg H, 1997. In vitro genotoxicity of polycyclic musk fragrances in the micronucleus test. Mutation Research, 395 (2-3), 145-150.

Substance Name	HHCB
CAS No.	1222-05-5
Remarks for Substance	Material was obtained from a commercial source, Promochem, under the trade name Galaxolide. Galaxolide is marketed as a 65% solution in diethyl phthalate.
Method/guideline	Not reported
Test Type	In vitro micronucleus
System of Testing	
GLP	No
Year	1997
Species/Strain	Human hepatoma cells
Metabolic Activation	With and without S9 activation
Doses/Concentration	0.1, 0.97, 9.7, 97, 194, 387 micromolar (corrected for 65% solution = 0.065, 0.6305, 6.305, 63.05, 126.1, 251.5 uM)
Statistical Methods	
Remarks for Test Conditions	Incubation period was 2 hours after which, the cells were harvested and scored for micronuclei.
Results	No significant increase in the frequency of micronuclei was seen with HHCB treatment.
Cytotoxic concentration	387 micromolar
Genotoxic Effects	None
Conclusion Remarks	No mutagenic potential
Data Qualities Reliabilities	Reliability 1. Reliable without restrictions.
Remarks for Data Reliability	Data are by a standard method and have been published in a peer-reviewed journal.
References	Kevekordes S, Mersch-Sundermann V, Diez M and Dunkelberg H, 1997. In vitro genotoxicity of polycyclic musk fragrances in the micronucleus test. Mutation Research, 395 (2-3), 145-150.

Substance Name	HHCB
CAS No.	1222-05-5
Remarks for Substance	Colorless viscous liquid sample supplied by IFF. Purity > 99% based on isomeric mixture.
Method/guideline	OECD 482
Test Type	In vitro unscheduled DNA synthesis
System of Testing	
GLP	Yes
Year	1999
Species/Strain	Primary rat hepatocytes from Sprague-Dawley rats
Metabolic Activation	With and without S9 activation
Doses/Concentration	0.15, 0.50, 1.5, 5, 15, 50 ug/ml
Statistical Methods	
Remarks for Test Conditions	Positive control induced a significant increase in the average net nuclear grain count over controls.
Results	No significant increase in UDS
Cytotoxic concentration	50 ug/ml
Genotoxic Effects	None
Conclusion Remarks	No genotoxic potential
Data Qualities Reliabilities	Reliability 1. Reliable without restrictions.
Remarks for Data Reliability	Data are by a standard method and have been published in a peer-reviewed journal.
References	Api, AM and San, RHC, 1999. Genotoxicity Tests with 6-Acetyl-1,1,2,4,4,7-hexamethyltetraline and 1,3,4,6,7,8-Hexahydro-4,6,6,7,8,8-hexamethylcyclopenta-gamma-benzopyran. Mutation Research, 446: 67-81.

Substance Name	HHCB
CAS No.	1222-05-5
Remarks for Substance	Material was obtained from a commercial source, Promochem, under the trade name Galaxolide. Galaxolide is marketed as a 65% solution in diethyl phthalate.
Method/guideline	Not reported but similar to OECD 479
Test Type	Sister-chromatid exchange (SCE)
System of Testing	
GLP	Not reported
Year	1998
Species/Strain	Human lymphocytes obtained from healthy non-smoking donors
Metabolic Activation	With and without S9 activation
Doses/Concentration	0.025, 0.25, 2.43, 24.25, 48.5 or 97 micromolar (corrected for 65% solution = 0.0162, 0.1625, 1.579, 15.76, 31.52, 63.05 uM)
Statistical Methods	
Remarks for Test Conditions	Treatment time was 2 hours. Positive controls showed a significant increase in SCEs.
Results	No significant increase in the number of sister chromatid exchanges was observed with HHCB at the doses tested compared to non-treated lymphocytes.
Cytotoxic concentration	97 micromole
Genotoxic Effects	None
Conclusion Remarks	No mutagenic potential
Data Qualities Reliabilities	Reliability 1. Reliable without restrictions.
Remarks for Data Reliability	Data are by a standard method and have been published in a peer-reviewed journal.
References	Kevekordes S, Mersch-Sundermann V, Diez M, Bolten C and Dunkelberg H, 1998. Genotoxicity of polycyclic musk fragrances in the Sister-Chromatid Exchange test. Anticancer Research, 18: 449-452.

Substance Name	HHCB
CAS No.	1222-05-5
Remarks for Substance	Colorless viscous liquid sample supplied by IFF. Purity > 99% based on isomeric mixture.
Method/guideline	OECD 473
Test Type	Chromosome aberration with multiple harvest times
System of Testing	
GLP	Yes
Year	1999
Species/Strain	Chinese hamster ovary cells
Metabolic Activation	With and without S9 activation
Doses/Concentration	Wo/activation for 4/20, 20/20, 44/44 hr exposure/harvest (e/h) times at 5, 10, 20 microgram/ml; w/activation for 4/20 hr e/h with 9, 17, 34 microgram/ml and for 4/44 hr e/h with 23, 28, 30 microgram/ml
Statistical Methods	
Remarks for Test Conditions	Cells were assessed for structural chromosome aberrations at the 20 and 44-hr harvest time. Numerical chromosome aberrations were also assessed at the 44-hr harvest time.
Results	No significant increase in structural or numerical chromosome aberrations
Cytotoxic concentration	20 ug/ml w/o activation; 30 ug/ml with activation
Genotoxic Effects	None
Conclusion Remarks	No genotoxic potential
Data Qualities Reliabilities	Reliability 1. Reliable without restrictions.
Remarks for Data Reliability	Data are by a standard method and have been published in a peer-reviewed journal.
References	Api, AM and San, RHC, 1999. Genotoxicity Tests with 6-Acetyl-1,1,2,4,4,7-hexamethyltetraline and 1,3,4,6,7,8-Hexahydro-4,6,6,7,8,8-hexamethylcyclopenta-gamma-benzopyran. Mutation Research, 446: 67-81.

Substance Name	HHCB
CAS No.	1222-05-5
Remarks for Substance	Material was obtained from a commercial source, Promochem, under the trade name Galaxolide. Galaxolide is marketed as a 65% solution in diethyl phthalate.
Method/guideline	Not reported
Test Type	In vitro
System of Testing	SOS induction using E. coli PQ37
GLP	No
Year	1998
Species/Strain	Human hepatoma cells
Metabolic Activation	With and without S9 activation
Doses/Concentration	0.39, 0.78, 1.56, 3.125, 6.25, 12.5, 25 or 50 ug (corrected for 65% solution = 0.25, 0.507, 1.014, 2.03, 4.06, 8.12, 16.25, and 32.5 ug)
Statistical Methods	
Remarks for Test Conditions	Incubation period was 2 hours after which enzyme activities of beta-galactosidase and alkaline phosphatase was measured.
Results	Both positive controls significantly increased in inducing factors (IF) but no inducing potency nor toxicity was seen with HHCB at any dose.
Cytotoxic concentration	
Genotoxic Effects	None
Conclusion Remarks	No mutagenic potential
Data Qualities Reliabilities	Reliability 1. Reliable without restrictions.
Remarks for Data Reliability	Data are by a standard method and have been published in a peer-reviewed journal.
References	Mersch-Sundermann V, Kevelordes, S., and Jenter, C. 1998b. Testing of SOS induction of Artificial Polycyclic Musk Fragrances in E. coli PQ37. Toxicology Letters, 95: 147-154.

4.2.2 In vivo Genotoxicity

Substance Name	HHCB
CAS No.	1222-05-5
Remarks for Substance	Colorless viscous liquid sample > 99% purity based on isomeric mixture.
Method/guideline	OECD 474
Test Type	In Vivo mouse micronucleus cytogenetic assay
GLP	Yes
Year	1999
Species/Strain	ICR mice
Sex	Male and Female
Route of Administration	Intraperitoneal
Doses/Concentration	376, 750 or 1500 mg/kg
Exposure Period	Bone marrow cells were harvested and evaluated 24, 48 and 72 hours after dosing.
Remarks for Test Conditions	Negative control was corn oil and positive control was cyclophosphamide
Appropriate statistical evaluations?	
Effect on mitotic index or PCE/NCE ratio by dose level and sex	Moderate reductions (up to 25%) in the ratio of PCE to total erythrocytes were observed in groups on 1500 mg/kg bw after 48 and 72 hours indicating toxicity and bioavailability to the bone marrow.
Genotoxic effects	None
NOEL (C)/ LOEL (C)	
Remarks for Results	
Conclusion Remarks	No significant increase in micronucleated PCE in HHCB-treated groups relative to the respective vehicle control group was observed in male or female mice at 24, 48 or 72 hours after dose administration.
Data Qualities Reliabilities	Reliability 1. Reliable without restrictions.
Remarks for Data Reliability	Data are by a standard method and have been published in a peer reviewed journal.
References	Api, AM and San, RHC, 1999. Genotoxicity Tests with 6-Acetyl-1,1,2,4,4,7-hexamethyltetraline and 1,3,4,6,7,8-Hexahydro-

4,6,6,7,8,8-hexamethylcyclopenta-gamma-benzopyran.
Mutation Research, 446: 67-81.

4.3 Repeat dose Toxicity

Substance Name	HHCB
CAS No.	1222-05-5
Remarks for Substance	Colorless viscous liquid sample > 99% purity based on isomeric mixture.
Method/guideline	OECD 408
GLP	Yes
Year	1999
Species/strain	CrI:CD(SD)Br
Sex	Male and Female
Route of Administration	Diet
Doses/concentration Levels	5, 15, 50 or 150 mg/kg per day; 15M and 15F per dose
Exposure Period	13 weeks
Frequency of Treatment	Daily
Control Group	Diet only
Post Exposure	4 weeks post exposure observance for selected rats from control and high dose groups
Remarks for Test Conditions	HHCB was added to the diet to the desired concentration. The mean achieved daily intakes were 5.4, 15.7, 51.8 and 155.8 mg HHCB/kg bw for males and 5.1, 15.6, 51.9 and 154.6 mg HHCB/kg bw for females.
NOAEL (NOEL)	150 mg/kg
Toxic Response/effects by Dose Level	LOAEL based on 2 week range finding study = 347 mg/kg (increased liver weights seen at this dose)
Statistical Evaluation	
Conclusion Remarks	
Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Remarks for Data Reliability	Study exceeded requirements of OECD 408 and are published in a peer reviewed journal.
References	Api, AM and Ford, RA, 1999. Evaluation of the Oral Subchronic Toxicity of HHCB (1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethylcyclopenta-gamma-2-benzopyran) in the Rat.

Toxicology Letters, 111: 143-149.

4.4 Reproductive Toxicity

Substance Name	HHCB
CAS No.	1222-05-5
Remarks for Substance	Viscous neat material, purity > 95% supplied by IFF.
Method/guideline	International Conference on Harmonisation (ICH) Guideline on detection of Toxicity to Reproduction for Medicinal Products, endorsed by the ICH Steering Committee Step 4 of the ICH process. 24 June, 1993. Section 4.1.2
Test Type	Peri-and post-natal development
GLP	Yes
Year	1996
Species/Strain	CrI:CD(SD)Br
Sex	Female
Route of Administration	Oral gavage
Duration of Test	19 weeks
Doses/Concentration	2, 6 and 20 mg/kg/day. F1 offspring only exposed <i>in utero</i> or through mother's milk from birth to weaning.
Control Group and Treatment	Untreated
Frequency of Treatment	Daily
Remarks for Test Conditions	F0 were evaluated for behavioral effects and for reproductive effects resulting in an F2 generation
NOAEL(NOEL)	There is no NOAEL established in the study.
Appropriate statistical evaluations	
Parental data and F1 as Appropriate	No effects on F0 females. No effects of F1 males or females.
Remarks for Results	Exposure to test material by gavage to dams had no effects at any dose and exposure to F1 offspring through mother's milk had no effects on behavior or reproductive performance. F2 pups were without adverse effects. 20 mg/kg bw/day cannot be considered as the NOAEL for the purpose of risk characterization since it is the dose received by the dams and the study was designed to detect adverse effects on the pups.
Data Reliabilities Qualities	Reliability code 1. Reliable without restrictions.
Remarks for Data Reliability	Study conducted according to a recognized guideline and under GLP.
References	Ford, RA and Bottomley, A, 1997. A Method for Evaluation of the Potential Toxicity to the Neonate from Exposure to

Xenobiotics via Mother's Milk – Application to Three Fragrance materials. The Toxicologist 36, No.1, Part 2:367.

Jones, K., Bottomley A.M. and Gopinath, C. (1996) HHCB: Effects on peri- and post natal development including maternal function in the rat (Gavage administration). Report to RIFM. September, 1996

4.5 Developmental/Teratogenicity Toxicity

Substance Name	HHCB
CAS No.	1222-05-5
Remarks for Substance	Viscous neat material, purity > 95% supplied by IFF.
Test Type	Developmental toxicity
GLP	Yes
Year	1999
Species/strain	Rat/Crl:CD(SD)Br VAF/Plus (Sprague-Dawley)
Sex	Female
Route of Administration	Oral gavage in corn oil
Duration of Test	3 weeks
Doses/concentration Levels	50, 150 and 500 mg/kg/day
Exposure Period	Days 7 through 17 of pregnancy
Frequency of Treatment	Daily
Control Group and Treatment	Corn oil only
Remarks for Test Conditions	The study was conducted in accordance with ICH Harmonized Tripartite Guideline Stages C and D.
NOAEL(NOEL) maternal toxicity	50 mg/kg/day
LOAEL(LOEL) maternal toxicity	150 mg/kg/day
NOAEL (NOEL) developmental toxicity	150 mg/kg/day
LOAEL developmental Toxicity	500 mg/kg/day
Actual dose received by dose level and sex	
Maternal data with dose level	Reduction in maternal bodyweight gain and feed consumption at two highest doses.

Fetal Data with Dose Level	Decreased mean bodyweights with axial skeleton (vertebral/rib) variations increased in high dose group only.
Appropriate statistical evaluations	
Remarks for Results	Material was not more toxic to the conceptus than to the dam.
Conclusion Remarks	
Data Qualities Reliabilities	Reliability code 1. Reliable without restrictions.
Remarks for Data Reliability	Study conducted according to a recognized guideline under GLP and is published in a peer-reviewed journal.
References	Christian MS, Hoberman AM, Diener, RM, Parker RM and Api, AM (1999). Developmental toxicity study of four fragrances in rats. Toxicology Letters, 111: 169-174.
