SCREENING-LEVEL HAZARD CHARACTERIZATION OF HIGH PRODUCTION VOLUME CHEMICALS

SPONSORED CHEMICAL

1,3,4,6,7,8-Hexahydro-4,6,6,7,8,8,-hexamethylcyclopenta[g]-2-benzopyran (HHCB)
(CAS No. 1222-05-5)
[9th CI Name: Cyclopenta[g]-2-benzopyran, 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8- hexamethyl-]

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INTERIM

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The High Production Volume (HPV) Challenge Program[1] is a voluntary initiative aimed at developing and making publicly available screening-level health and environmental effects information on chemicals manufactured in or imported into the United States in quantities greater than one million pounds per year. In the Challenge Program, producers and importers of HPV chemicals voluntarily sponsor chemicals; sponsorship entails the identification and initial assessment of the adequacy of existing toxicity data/information, conducting new testing if adequate data do not exist, and making both new and existing data and information available to the public. Each complete data submission contains data on 18 internationally agreed to "SIDS" (Screening Information Data Set[1,2]) endpoints that are screening-level indicators of potential hazards (toxicity) for humans or the environment.

The Environmental Protection Agency's Office of Pollution Prevention and Toxics (OPPT) is evaluating the data submitted in the HPV Challenge Program on approximately 1400 sponsored chemicals. OPPT is using a hazard-based screening process to prioritize review of the submissions. The hazard-based screening process consists of two tiers described below briefly and in more detail on the Hazard Characterization website[3].

Tier 1 is a computerized sorting process whereby key elements of a submitted data set are compared to established criteria to "bin" chemicals/categories for OPPT review. This is an automated process performed on the data as submitted by the sponsor. It does not include evaluation of the quality or completeness of the data.

In Tier 2, a screening-level hazard characterization is developed by EPA that consists of an objective evaluation of the quality and completeness of the data set provided in the Challenge Program submissions. The evaluation is performed according to established EPA guidance[2,4] and is based primarily on hazard data provided by sponsors. EPA may also include additional or updated hazard information of which EPA, sponsors or other parties have become aware. The hazard characterization may also identify data gaps that will become the basis for a subsequent data needs assessment where deemed necessary. Under the HPV Challenge Program, chemicals that have similar chemical structures, properties and biological activities may be grouped together and their data shared across the resulting category. This approach often significantly reduces the need for conducting tests for all endpoints for all category members. As part of Tier 2, evaluation of chemical category rationale and composition and data extrapolation(s) among category members is performed in accord with established EPA[2] and OECD[5] guidance.

The screening-level hazard characterizations that emerge from Tier 2 are important contributors to OPPT's existing chemicals review process. These hazard characterizations are technical documents intended to support subsequent decisions and actions by OPPT. Accordingly, the documents are not written with the goal of informing the general public. However, they do provide a vehicle for public access to a concise assessment of the raw technical data on HPV chemicals and provide information previously not readily available to the public. The public, including sponsors, may offer comments on the hazard characterization documents.

The screening-level hazard characterizations, as the name indicates, do not evaluate the potential risks of a chemical or a chemical category, but will serve as a starting point for such reviews. In 2007, EPA received data on uses of and exposures to high-volume TSCA existing chemicals, submitted in accordance with the requirements of the Inventory Update Reporting (IUR) rule. For the chemicals in the HPV Challenge Program, EPA will review the IUR data to evaluate exposure potential. The resulting exposure information will then be combined with the screening-level hazard characterizations to develop screening-level risk characterizations[4,6]. The screening-level risk characterizations will inform EPA on the need for further work on individual chemicals or categories. Efforts are currently underway to consider how best to utilize these screening-level risk characterizations as part of a risk-based decision-making process on HPV chemicals which applies the results of the successful U.S. High Production Volume Challenge Program and the IUR to support judgments concerning the need, if any, for further action.

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[1] U.S. EPA. High Production Volume (HPV) Challenge Program; http://www.epa.gov/chemrtk/index.htm.
[2] U.S. EPA. HPV Challenge Program – Information Sources; http://www.epa.gov/chemrtk/pubs/general/guidocs.htm.
[3] U.S. EPA. HPV Chemicals Hazard Characterization website (http://www.epa.gov/hpvis/abouthc.html).
[4] U.S. EPA. Risk Assessment Guidelines; http://cfpub.epa.gov/ncea/raf/rafguid.cfm.
[5] OECD. Guidance on the Development and Use of Chemical Categories; http://www.oecd.org/dataoecd/60/47/1947509.pdf.
[6] U.S. EPA. Risk Characterization Program; http://www.epa.gov/osa/spc/2riskchr.htm.

SCREENING-LEVEL HAZARD CHARACTERIZATION
1,3,4,6,7,8-Hexahydro-4,6,6,7,8,8,-hexamethylcyclopenta[g]-2-benzopyran (HHCB)
(CAS No. 1222-05-5)

Introduction

The sponsor, International Flavors and Fragrances, submitted a Test Plan and Robust Summaries to EPA for 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8,-hexamethylcyclopenta[g]-2-benzopyran (HHCB) (CAS No. 1222-05-5, 9th CI name: cyclopenta [g]-2-benzopyran, 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethyl) on October 30, 2003. EPA posted the submission on the ChemRTK HPV Challenge website on December 15, 2003 (http://www.epa.gov/oppt/chemrtk/pubs/summaries/cyclopen/c14820tc.htm). EPA comments on the original submission were posted to the website on April 19, 2004. Public comments were also received and posted to the website. The sponsor submitted updated/revised documents on June 14, 2004, which were posted to the ChemRTK website on August 27, 2004.

This screening level hazard characterization is based primarily on the review of the test plan and robust summaries of studies submitted by the sponsor(s) under the HPV Challenge Program. In preparing the hazard characterization, EPA considered its own comments and public comments on the original submission as well as the sponsor's responses to comments and revisions made to the submission. A summary table of SIDS endpoint data with the structure(s) of the sponsored chemical(s) is included in the appendix. The screening-level hazard characterization for environmental and human health effects is based largely on SIDS endpoints and is described according to established EPA or OECD effect level definitions and hazard assessment practices.

The acute toxicity and some of the genetic toxicity studies submitted were conducted using commercial mixtures containing 65% HHCB with either diethyl phthalate or isopropyl myristate. In the robust summaries for several of the studies on the commercial preparations, doses and effect levels were converted to the equivalent dose of the pure compound. In order to provide a consistent basis for comparing effect levels and characterizing hazard, EPA performed similar conversions for the few studies where the conversion was not reported in the robust summary.

Summary-Conclusion

The log K_{ow} of HHCB indicates that its potential to bioaccumulate is expected to be high. HHCB is not readily biodegradable, indicating that it has the potential to persist in the environment.

The evaluation of available toxicity data for fish, aquatic invertebrates and aquatic plants indicates that the potential acute hazard of HHCB to aquatic organisms is high.

Acute oral toxicity of HHCB to rats and acute dermal toxicity to rabbits and rats is low. Following dietary exposure of HHCB to rats for 13 weeks, no adverse effects were reported. No effects on reproduction in parental animals or in F1 or F2 offspring were seen following exposure of HHCB in a reproductive toxicity study. In a developmental toxicity study in rats, reduced maternal body weight gain, decreased mean fetal body weight and increased incidence of either variations or malformations in pups were reported. HHCB did not induce gene mutations or chromosomal aberrations.

The potential health hazard of HHCB is moderate based on developmental toxicity.

No data gaps were identified under the HPV Challenge Program.

1. Physical-Chemical Properties and Environmental Fate

A summary of physical-chemical and environmental fate data submitted is provided in the Appendix. For the purpose of the screening-level hazard characterization, the review and summary of these data were limited to the octanol-water partition coefficient and biodegradation endpoints as indicators of bioaccumulation and persistence, respectively.

Octanol-Water Partition Coefficient

Log K_{ow}: 5.9 (measured)

Biodegradation

In a Modified Sturm test using sewage effluent as inoculum, 0% of the HHCB had degraded after 28 days.
HHCB is not readily biodegradable.

Conclusion: The log K_{ow} of HHCB indicates that its potential to bioaccumulate is expected to be high. HHCB is not readily biodegradable, indicating that it has the potential to persist in the environment.

2. Environmental Effects – Aquatic Toxicity

Acute Toxicity to Fish

The 21-day chronic study in bluegill sunfish (*Lepomis macrochirus*) described below fulfills this endpoint.

Acute Toxicity to Aquatic Invertebrates

In the 21-day chronic study in *Daphnia magna* described below, a 48-hour EC_{50} was also calculated.
48-h EC_{50} = 0.282 mg/L

Toxicity to Aquatic Plants

Green algae (*Pseudokirchneriella subcapitata*) were exposed to HHCB at measured concentrations of 0.042, 0.084, 0.201, 0.466 and 0.854 mg/L under static conditions for 72 hours. Marked inhibition of growth and growth rate were observed at 0.466 and 0.854 mg/L.
72-h EC_{50} (growth) = 0.854 mg/L
72-h EC_{50} (biomass) = 0.72 mg/L

Chronic Toxicity to Fish

Bluegill sunfish (*Lepomis macrochirus*) were exposed to HHCB at 0, 0.093, 0.182, 0.393, 0.830 or 1.566 mg/L under flow-through conditions for 21 days. At 0.182 and 0.393mg/L, 10% mortality was observed and effects on growth were observed at 0.393 mg/L. All fish were dead after 6 days of exposure to 1.566 mg/L and after 14 days of exposure to 0.830 mg/L. Concentration- and time-dependent signs of toxicity at $\geq$ 0.182 mg/L included loss of equilibrium and righting reflex, irregular respiration and bottom and tail dominant swimming.
21-d LC_{50} = 0.452 mg/L
21-d NOEC = 0.093 mg/L

Chronic Toxicity to Aquatic Invertebrates

Daphnia magna were exposed to HHCB at measured concentrations of 0.049, 0.111, 0.205, 0.419 and 0.842 mg/L under semi-static conditions for 21 days. Mortality (100%) was observed at 0.842 and 0.419 and 0.842 mg/L after 6 and 17 days, respectively. Effects on reproduction were evaluated during the 21 day exposure period.
21-d NOEC (reproduction) = 0.111 mg/L
21-d LOEC (reproduction) = 0.205 mg/L

Conclusion: The evaluation of available toxicity data for fish, aquatic invertebrates and aquatic plants indicates that the potential hazard of HHCB to aquatic organisms is high.

3. Human Health Effects

Acute Oral Toxicity

(1) Wistar rats (10/sex) were administered commercial grade HHCB (65% HHCB in either diethyl phthalate or isopropyl myristate) via gavage at 5000 mg/kg-bw and observed for 14 days. The corrected dose of HHCB was 3250 mg/kg-bw. One death occurred at this dose.
LD_{50} > 3250 mg/kg-bw

(2) Rats (10 females/dose; strain not specified) were administered commercial sample (65% HHCB in either diethyl phthalate or isopropyl myristate) via gavage at 3000 mg/kg-bw and observed for 14 days. It is not clear whether the reported dose reflected dose of the mixture or of HHCB. Therefore, a conservative estimate of the LD_{50} is considered to be 65% of the test concentration. No mortality was observed during the study.
LD_{50} > 1950 mg/kg-bw

Acute Dermal Toxicity

(1) Rabbits (7/dose, sex not reported) were administered commercial grade HHCB (65% HHCB in either diethyl phthalate or isopropyl myristate) dermally at 5000 mg/kg-bw and observed for 14 days. The corrected dose of HHCB was 3250 mg/kg-bw. The number and sex of animals were not clearly reported. No deaths occurred during the study.
LD_{50} > 3250 mg/kg-bw

(2) Female rats (5/dose) were administered commercial grade HHCB (65% HHCB in either diethyl phthalate or isopropyl myristate) in ethanol dermally at 5000 mg/kg-bw and observed for 7 days. The corrected dose of HHCB was 3250 mg/kg-bw. No mortality was observed during the study.
LD_{50} > 3250 mg/kg-bw

Repeated-Dose Toxicity

Sprague-Dawley rats (15/sex/dose) were administered HHCB via the diet at 0, 5, 15, 50 or 150 mg/kg-bw/day for 13 weeks. Test concentrations were determined from a range finding study in which a LOAEL of 300 mg/kg-bw/day (based on hepatic effects) was determined. Mean estimated test substance intakes were 5.4, 15.7, 51.8 or 155.8 mg/kg-bw/day for males and 5.1, 15.6, 51.9 or 154.6 mg/kg-bw/day for females. There were no mortalities, adverse clinical signs or treatment-related effects on body weight, hematology or ophthalmologic evaluation. Slightly lower mean plasma triglyceride levels were observed at week13 in males at 50 and 150 mg/kg-bw/day. Slightly lower plasma glucose concentrations were noted at week 7 in males and females given 15, 50 and 150 mg/kg-bw/day and at week 13 in males given 50 and 150 mg/kg-bw/day; these effects were not seen at the end of the 4-week recovery period. There were no treatment-related differences in absolute organ weights or organ weight relative to body weight after 13 weeks of treatment. The robust summary reported that "histopathological examination of the ovaries and vaginas showed an association between the distension and the proestrus stage of the life cycle"; however, this statement is not clarified any further in the robust summary. Histopathological examination revealed no treatment-related effects on male or female reproductive organs or any other tissues/organs at any dose.
NOAEL = 150 mg/kg-bw/day (based on no effects at the highest dose tested)

Reproductive Toxicity

Mated female Crl:CD(SD)Br rats (animals/sex/dose not specified) were administered HHCB via gavage at 0, 2, 6 or 20 mg/kg-bw/day beginning on gestation day 14. The F1 offspring were exposed in utero and throughout lactation. At the end of the pre-weaning period, 24 male and 24 female pups per dose were retained for further study. On day 22 post-partum, excess pups and parents were sacrificed and examined for abnormalities. When offspring were 84 days of age, males and females were mated and produced litters. After day 21 post-partum, all F2 pups and F1 dams were sacrificed and examined internally and externally for abnormalities. No adverse effects on behavior or reproduction were observed at any dose in parental animals or in F1 or F2 pups.
NOAEL (systemic and reproductive toxicity) = 20 mg/kg-bw/day (based on no effects at the highest dose tested)

Developmental Toxicity

Pregnant female Sprague-Dawley rats (25/dose) were administered HHCB via gavage at 0, 50, 150 or 500 mg/kg-bw/day on gestation days 7 − 17. All rats were sacrificed on day 20 and subjected to gross necropsy. No deaths, abortions or premature deliveries occurred during the study. At 500 mg/kg-bw/day, dams exhibited excess salivation, urine stained abdominal fur, red or brown substance on forepaws and alopecia. Reductions in maternal bodyweight gain and food consumption were seen at 150 and 500 mg/kg-bw/day. At 500 mg/kg-bw/day, decreased mean fetal body weights and increases in fetal malformations or variations were observed. The fetal effects were discounted by the study authors, but the robust summary did not provide sufficient details or a clear rationale for doing so.
LOAEL (maternal toxicity) = 150 mg/kg-bw/day (based on decreased body weight, food consumption and clinical signs of toxicity)
NOAEL (maternal toxicity) = 50 mg/kg-bw/day
LOAEL (developmental toxicity) = 500 mg/kg-bw/day (based on decreased fetal body weight and increased malformations or variations)
NOAEL (developmental toxicity) = 150 mg/kg-bw/day

Genetic Toxicity – Gene Mutation

In vitro
(1) *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538 and *Escherichia coli* WP2 uvrA were exposed to HHCB at concentrations of 10, 33, 100, 333, 1000 or 5000 µg/plate in the presence and absence of metabolic activation. All positive controls gave acceptable responses. Cytotoxicity was not observed. No significant increase in the number of revertant colonies was observed at any concentration.
HHCB was not mutagenic in this assay.

(2) *Salmonella typhimurium* strains TA97, TA98, TA100 and TA102 were exposed to a commercial sample that contained 65% HHCB in diethyl phthalate at concentrations of 5, 16.6, 50, 166.6 or 500 µg/plate in the presence and absence of metabolic activation. The corrected concentrations of HHCB ranged from 3.25 to 325 µg/plate. Cytoxicity was not observed in this assay. No significant increase in the number of revertant colonies was observed at any concentration.
HHCB was not mutagenic in this assay.

Genetic Toxicity – Chromosomal Aberrations

In vitro
(1) Human peripheral lymphocytes were exposed to a commercial sample that contained 65% HHCB in diethyl phthalate at 0.05, 0.49, 4.85, 48.5 or 97 µM in the presence and absence of metabolic activation. The corrected concentrations of HHCB were 0.0325, 0.3185, 3.152, 31.52, 63.05 or 126.1 µM. The positive controls responses were appropriate. The cytotoxic concentration was 194 µM (as reported in the robust summary—possibly taken from the range-finding study).
HHCB did not induce micronuclei formation in this assay.

(2) Human hepatoma cells were exposed to a commercial sample that contained 65% HHCB in diethyl phthalate at 0.1, 0.97, 9.7, 97, 194 or 387 µM in the presence and absence of metabolic activation. The corrected concentrations

of HHCB were 0.065, 0.6305, 6.305, 63.05, 126.1 or 251.5 µM. Cells were incubated for 2 hours and then harvested and scored for micronuclei. The cytotoxic concentration was 387 µM.
HHCB did not induce micronuclei in this assay.

(3) Chinese hamster ovary cells were exposed to HHCB at 5, 10 or 20 µg/mL for 4, 20 or 44/hours (and harvested at 20, 20 or 44 hours, respectively) in the absence of metabolic activation and 9, 17 or 34 µg/mL for 4 hours (harvested at 20 hours) and 23, 28 or 30 µg/mL for 4 hours (harvested at 44 hours) in the presence of metabolic activation. The positive control showed significant increases in the percentage of cells with chromosome aberrations. The cytotoxic concentration was 20 µg/mL without activation and 30 µg/mL with activation. No significant increases in structural or numerical chromosome aberrations were observed.
HHCB did not induce chromosomal aberrations in this assay.

In vivo
Male and female ICR mice were administered HHCB via intraperitoneal injection at doses of 376, 750 or 1500 mg/kg-bw. Positive and negative controls were used; however, responses were not reported. Moderate reductions (up to 25%) in the ratio of polychromatic erythrocytes (PCEs) to total erythrocytes were observed at 1500 mg/kg-bw after 48 and 72 hours, indicating toxicity and bioavailability to the bone marrow. No increases in micronucleated PCE were observed in any of the HHCB-treated groups relative to the vehicle control group when assessed 24, 48 or 72 hours after dose administration.
HHCB did not induce micronuclei in this assay.

Genetic Toxicity – Other

In vitro
(1) In an unscheduled DNA synthesis assay, primary rat hepatocytes were exposed to HHCB at 0.15, 0.50, 1.5, 5, 15 or 50 µg/mL. Positive controls responded appropriately. The cytotoxic concentration was 50µg/mL.
HHCB did not induce unscheduled DNA synthesis in this assay.

(2) Human lymphocytes were exposed to a commercial sample that contained 65% HHCB in diethyl phthalate at 0.025, 0.25, 2.43, 24.25, 48.5 or 97 µM in the presence and absence of metabolic activation for 2 hours. The corrected concentrations of HHCB were 0.0162, 0.1625, 1.579, 15.76, 31.52 or 63.05 µM. The positive controls showed a significant increase in sister chromatid exchanges (SCEs). The cytotoxic concentration was 97 µM.
HHCB did not induce sister chromatid exchanges in this assay.

Conclusion: Acute oral toxicity of HHCB to rats and acute dermal toxicity to rabbits and rats is low. Following dietary exposure of HHCB to rats for 13 weeks, no adverse effects were reported. No effects on reproduction in parental animals or in F1 or F2 offspring were seen following exposure of HHCB in a reproductive toxicity study. In a developmental toxicity study in rats, reduced maternal body weight gain, decreased mean fetal body weight and increased incidence of either variations or malformations in pups were reported. HHCB did not induce gene mutations or chromosomal aberrations.

The potential health hazard of HHCB is moderate based on developmental toxicity.

4. Hazard Characterization

The log K_{ow} of HHCB indicates that its potential to bioaccumulate is expected to be high. HHCB is not readily biodegradable, indicating that it is expected to persist in the environment.

The evaluation of available toxicity data for fish, aquatic invertebrates and aquatic plants indicates that the potential hazard of HHCB to aquatic organisms is high.

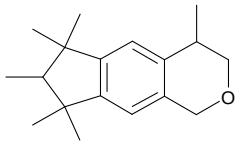
Acute oral toxicity of HHCB to rats and acute dermal toxicity to rabbits and rats is low. Following dietary exposure of HHCB to rats for 13 weeks, no adverse effects were reported. No effects on reproduction in parental animals or in F1 or F2 offspring were seen following exposure of HHCB in a reproductive toxicity study. In a developmental toxicity study in rats, reduced maternal body weight gain, decreased mean fetal body weight and increased incidence of either variations or malformations in pups were reported. HHCB did not induce gene mutations or chromosomal aberrations.

The potential health hazard of HHCB is moderate based on developmental toxicity.

5. Data Gaps

No data gaps were identified under the HPV Challenge Program.

APPENDIX

Summary Table of the Screening Information Data Set as Submitted under the U.S. HPV Challenge Program	
Endpoints	**SPONSORED CHEMICAL 1,3,4,6,7,8-Hexahydro-4,6,6,7,8,8,-hexamethylcyclopenta[g]-2-benzopyran (HHCB) (1222-05-5)**
Structure	
Summary of Physical-Chemical Properties and Environmental Fate Data	
Melting Point (°C)	-10 – 0
Boiling Point (°C)	160 (at 5.3 hPa)
Vapor Pressure (hPa at 25°C)	7.27×10^{-4}
Log K_{ow}	5.9
Water Solubility (mg/L at 25°C)	1.75
Direct Photodegradation	Half-life under black irradiation lamps = 3.7 h
Stability in Water (Hydrolysis) ($t_{1/2}$)	Not expected to undergo hydrolysis based on structure
Fugacity (Level III Model) **Air (%)** **Water (%)** **Soil (%)** **Sediment (%)**	 0.188 5.58 38.6 55.65
Biodegradation at 28 days (%)	0 Not readily biodegradable
Summary of Environmental Effects – Aquatic Toxicity Data	
Fish 96-h LC_{50} (mg/L)	Endpoint fulfilled with chronic fish toxicity test.
Aquatic Invertebrates 48-h EC_{50} (mg/L)	0.282
Aquatic Plants 72-h EC_{50} (mg/L) **(growth)** **(biomass)**	 0.854 0.720
Chronic Toxicity to Fish 21-day LC_{50} (mg/L)	0.452 NOEC = 0.093
Chronic Toxicity to Invertebrates 21-day NOEC/LOEC	NOEC = 0.111 LOEC = 0.205

Summary Table of the Screening Information Data Set as Submitted under the U.S. HPV Challenge Program	
Endpoints	**SPONSORED CHEMICAL 1,3,4,6,7,8-Hexahydro-4,6,6,7,8,8,-hexamethylcyclopenta[g]-2-benzopyran (HHCB) (1222-05-5)**
Summary of Human Health Data	
Acute Oral Toxicity **LD_{50} (mg/kg-bw)**	> 3000
Acute Dermal Toxicity **LD_{50} (mg/kg-bw)**	> 3250
Repeated-Dose Toxicity **NOAEL/LOAEL** **Oral (mg/kg-bw/day)**	NOAEL = 150 (highest dose tested)
Reproductive Toxicity **NOAEL/LOAEL** **Oral (mg/kg-bw/day)** **Systemic and Reproductive Toxicity**	NOAEL = 20 (highest dose tested)
Developmental Toxicity **NOAEL/LOAL** **Oral (mg/kg-bw/day)** **Maternal Toxicity** **Developmental Toxicity**	 NOAEL = 50 LOAEL = 150 NOAEL = 150 LOAEL = 500
Genetic Toxicity – Gene Mutation ***In vitro***	Negative
Genetic Toxicity – Chromosomal Aberrations ***In vitro***	Negative
Genetic Toxicity – Chromosomal Aberrations ***In vivo***	Negative
Genetic Toxicity – Other ***In vitro*** **Unscheduled DNA Synthesis in Mammalian Cells**	Negative