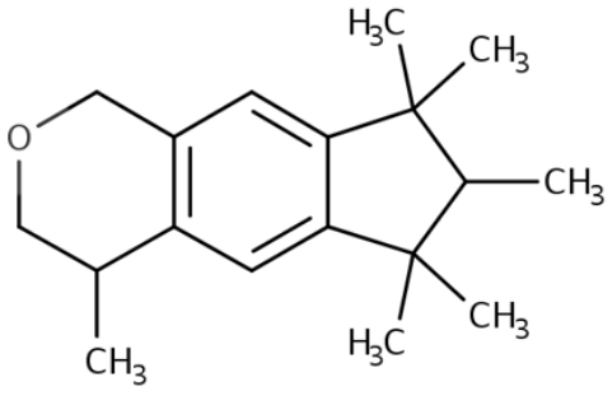
United States
Environmental Protection Agency

March 2026
Office of Chemical Safety and
Pollution Prevention

Draft Data Quality Evaluation Information for Human Health Hazard Animal Toxicology for 1,3,4,6,7,8-Hexahydro-4,6,6,7,8,8-hexamethylcyclopenta[γ]-2-benzopyran (HHCB)

Systematic Review Support Document for the Draft Risk Evaluation

CASRN: 1222-05-5



March 2026

This supplemental file contains information regarding the data quality evaluation conducted for references that met the PECO screening criteria described in the *Draft Human Health and Environmental Hazard Assessment for 1,3,4,6,7,8-Hexahydro-4,6,6,7,8,8-hexamethylcyclopenta [γ]-2-benzopyran (HHCB)*. EPA conducted data quality evaluation based on author-reported descriptions and results; additional analyses (*e.g.*, statistical analyses performed during data integration into the risk evaluation) potentially conducted by EPA are not contained in this supplemental file. For the data quality evaluation, EPA used the TSCA systematic review process described in the *Draft Systematic Review Protocol Supporting TSCA Risk Evaluations for Chemical Substances* (also referred to as '2021 Draft Systematic Review Protocol'). Any updated steps in the systematic review process since the publication of the 2021 Draft Systematic Review Protocol are described in the *Draft Systematic Review Protocol for 1,3,4,6,7,8-Hexahydro-4,6,6,7,8,8-hexamethylcyclopenta [γ]-2-benzopyran (HHCB)*.

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Mixture: HHCB and DEP

Short-term (>1-30 days)

Mixture: Galaxolide racemic mixture Cas 1222-05-5

Subchronic (>30-91 days)

Mixture: HHCB

Reproductive/Developmental

Study Citation:	IFF, (2016). Galaxolide undiluted. Evaluation of acute dermal toxicity in rats. (sanitized).
Health Outcome(s):	Mortality; Neurological/Behavioral; Nutritional/Metabolic; Endocrine (Endocrine); Lung/Respiratory; Ocular/Sensory; Cardiovascular; Reproductive/Developmental; Gastrointestinal; Immune/Hematological; Hepatic/Liver; Renal/Kidney;
Reported Health Effect(s):	Mortality: Mortality; Neurological/Behavioral: Neurological-related clinical signs (spontaneous activity, Preyer's reflex, convulsions, tremors, body temperature, muscle tone, salivation, pupil appearance, righting reflex).; Nutritional/Metabolic: Body weights; Endocrine (Endocrine): Gross necropsy of the adrenals and pancreas; Lung/Respiratory: Clinical signs: Respiratory rate; gross necropsy of lungs and trachea; Ocular/Sensory: Clinical signs: lacrimation, palpebral opening; Cardiovascular: Gross necropsy of the heart; Reproductive/Developmental: Gross necropsy of testicles, ovaries, and uterus; Gastrointestinal: Gross necropsy of esophagus, stomach, duodenum, jejunum, ileon, caecum, colon, and rectum.; Immune/Hematological: Gross necropsy of the spleen and thymus; Hepatic/Liver: Gross necropsy of the liver; Renal/Kidney: Gross necropsy of the kidneys and urinary bladder;
Duration:	Acute (less than or equal to 24 hr) Acute- 24 hours
Chemical:	HHCB- Parent compound
HERO ID:	8785033

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	All Outcomes: Test substance was identified as Galaxolide undiluted. This is a common tradename for the chemical 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8,-hexamethylcyclopenta[g]benzopyran or HHCB.
	Metric 2:	Test Substance Source	High	All Outcomes: Based on the information provided, the test substance appears to have been supplied by the sponsor, although the word "source" was not explicitly stated. The Batch number was provided. Certificates of analysis were provided in the appendix, although some information was redacted.
	Metric 3:	Test Substance Purity	Low	All Outcomes: The purity of the test substance was not reported.
Domain 2: Test Design				
	Metric 4:	Negative and Vehicle Controls	N/A	All Outcomes: A negative control group is not required for acute lethality study.
	Metric 5:	Positive Controls	N/A	All Outcomes: A positive control group is not required for this study type.
	Metric 6:	Randomized Allocation of Animals	Low	All Outcomes: The study did not report how animals were allocated to study groups.
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and Storage of Test Substance	High	All Outcomes: The test substance was stored at room temperature in glass flasks. Undiluted test substance was administered.
	Metric 8:	Consistency of Exposure Administration	High	Mortality: The volume applied to skin was 2.09 mL/kg body weight.; Neurological/Behavioral: The volume applied to skin was 2.09 mL/kg body weight.; Nutritional/Metabolic: Volume applied to skin was 2.09 mL/kg body weight.; Endocrine (Endocrine): Volume applied to skin was 2.09 mL/kg body weight.; Lung/Respiratory: Volume applied to skin was 2.09 mL/kg body weight.; Ocular/Sensory: Volume applied to skin was 2.09 mL/kg body weight.; Cardiovascular: Volume applied to skin was 2.09 mL/kg body weight.; Reproductive/Developmental: Volume applied to skin was 2.09 mL/kg body weight.; Gastrointestinal: Volume applied to skin was 2.09 mL/kg body weight.; Immune/Hematological: Volume applied to skin was 2.09 mL/kg body weight.; Hepatic/Liver: Volume applied to skin was 2.09 mL/kg body weight.; Renal/Kidney: Volume applied to skin was 2.09 mL/kg body weight.

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Study Citation:	IFF, (2016). Galaxolide undiluted. Evaluation of acute dermal toxicity in rats. (sanitized).
Health Outcome(s):	Mortality; Neurological/Behavioral; Nutritional/Metabolic; Endocrine (Endocrine); Lung/Respiratory; Ocular/Sensory; Cardiovascular; Reproductive/Developmental; Gastrointestinal; Immune/Hematological; Hepatic/Liver; Renal/Kidney;
Reported Health Effect(s):	Mortality: Mortality; Neurological/Behavioral: Neurological-related clinical signs (spontaneous activity, Preyer's reflex, convulsions, tremors, body temperature, muscle tone, salivation, pupil appearance, righting reflex).; Nutritional/Metabolic: Body weights; Endocrine (Endocrine): Gross necropsy of the adrenals and pancreas; Lung/Respiratory: Clinical signs: Respiratory rate; gross necropsy of lungs and trachea; Ocular/Sensory: Clinical signs: lacrimation, palpebral opening; Cardiovascular: Gross necropsy of the heart; Reproductive/Developmental: Gross necropsy of testicles, ovaries, and uterus; Gastrointestinal: Gross necropsy of esophagus, stomach, duodenum, jejunum, ileon, caecum, colon, and rectum.; Immune/Hematological: Gross necropsy of the spleen and thymus; Hepatic/Liver: Gross necropsy of the liver; Renal/Kidney: Gross necropsy of the kidneys and urinary bladder;
Duration:	Acute (less than or equal to 24 hr) Acute- 24 hours
Chemical:	HHCB- Parent compound
HERO ID:	8785033

Domain	Metric		Rating	Comments
	Metric 9:	Reporting of Doses/Concentrations	High	Mortality: The dose was clearly reported as 2000 mg/kg bw.; Neurological/Behavioral: Dose were clearly reported as 2000 mg/kg bw.; Nutritional/Metabolic: Dose were clearly reported as 2000 mg/kg bw.; Endocrine (Endocrine): Dose were clearly reported as 2000 mg/kg bw.; Lung/Respiratory: Dose were clearly reported as 2000 mg/kg bw.; Ocular/Sensory: Dose were clearly reported as 2000 mg/kg bw.; Cardiovascular: Dose were clearly reported as 2000 mg/kg bw.; Reproductive/Developmental: Dose were clearly reported as 2000 mg/kg bw.; Gastrointestinal: Dose were clearly reported as 2000 mg/kg bw.; Immune/Hematological: Dose were clearly reported as 2000 mg/kg bw.; Hepatic/Liver: Dose were clearly reported as 2000 mg/kg bw.; Renal/Kidney: Dose were clearly reported as 2000 mg/kg bw.
	Metric 10:	Exposure Frequency and Duration	High	All Outcomes: The exposure frequency and duration were appropriate for the study's aim (24 hours).
	Metric 11:	Number of Exposure Groups and Dose/Concentration Spacing	High	All Outcomes: A single dose group of 2000 mg/kg bw was studied. This was akin to a limit dose and therefore acceptable.
	Metric 12:	Exposure Route and Method	High	All Outcomes: The exposure route and method were appropriate an in agreement with OECD guidelines.
Domain 4: Test Animals				
	Metric 13:	Test Animal Characteristics	Low	All Outcomes: The sex, age, starting body weight, strain and species were reported. The source of the animals was redacted in the copy used for evaluation.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	All Outcomes: Temperature, humidity, light/dark cycle, number of animals/cage and diet/water availability were reported.
	Metric 15:	Number of Animals per Group	Medium	All Outcomes: 5 males and 5 females were studied. This agrees with OECD guidelines.
Domain 5: Outcome Assessment				
	Metric 16:	Outcome Assessment Methodology	High	All Outcomes: The outcome methodology was sensitive and appropriate for the outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	High	All Outcomes: Outcome assessment was consistent across all animals.
	Metric 18:	Sampling Adequacy	High	All Outcomes: There was adequate sampling for outcomes of interest (5/sex).

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Study Citation:	IFF, (2016). Galaxolide undiluted. Evaluation of acute dermal toxicity in rats. (sanitized).
Health Outcome(s):	Mortality; Neurological/Behavioral; Nutritional/Metabolic; Endocrine (Endocrine); Lung/Respiratory; Ocular/Sensory; Cardiovascular; Reproductive/Developmental; Gastrointestinal; Immune/Hematological; Hepatic/Liver; Renal/Kidney;
Reported Health Effect(s):	Mortality: Mortality; Neurological/Behavioral: Neurological-related clinical signs (spontaneous activity, Preyer's reflex, convulsions, tremors, body temperature, muscle tone, salivation, pupil appearance, righting reflex).; Nutritional/Metabolic: Body weights; Endocrine (Endocrine): Gross necropsy of the adrenals and pancreas; Lung/Respiratory: Clinical signs: Respiratory rate; gross necropsy of lungs and trachea; Ocular/Sensory: Clinical signs: lacrimation, palpebral opening; Cardiovascular: Gross necropsy of the heart; Reproductive/Developmental: Gross necropsy of testicles, ovaries, and uterus; Gastrointestinal: Gross necropsy of esophagus, stomach, duodenum, jejunum, ileon, caecum, colon, and rectum.; Immune/Hematological: Gross necropsy of the spleen and thymus; Hepatic/Liver: Gross necropsy of the liver; Renal/Kidney: Gross necropsy of the kidneys and urinary bladder;
Duration:	Acute (less than or equal to 24 hr) Acute- 24 hours
Chemical:	HHCB- Parent compound
HERO ID:	8785033

Domain	Metric		Rating	Comments
	Metric 19:	Blinding of Assessors	N/A	Mortality: Blinding is not required for the endpoint (mortality); Neurological/Behavioral: Blinding was not reported, but is not required for the endpoint of interest (clinical signs).; Nutritional/Metabolic: Blinding was not reported and is not relevant for a study type with a single group.; Endocrine (Endocrine): Blinding was not reported and is not relevant for a study type with a single group.; Lung/Respiratory: Blinding was not reported and is not relevant for a study type with a single group.; Ocular/Sensory: Blinding was not reported and is not relevant for a study type with a single group.; Cardiovascular: Blinding was not reported and is not relevant for a study type with a single group.; Reproductive/Developmental: Blinding was not reported and is not relevant for a study type with a single group.; Gastrointestinal: Blinding was not reported and is not relevant for a study type with a single group.; Immune/Hematological: Blinding was not reported and is not relevant for a study type with a single group.; Hepatic/Liver: Blinding was not reported and is not relevant for a study type with a single group.; Renal/Kidney: Blinding was not reported and is not relevant for a study type with a single group.
	Metric 20:	Negative Control Response	N/A	All Outcomes: Negative control was not required for acute lethality study.
Domain 6: Confounding / Variable Control				
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	All Outcomes: Although the study did not report all information to determine confounding, reported information did not identify differences. Study reports no changes in mortality, body weight, clinical signs. Food intake was not reported.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	All Outcomes: There was no information either to support or dismiss the suggestion that there were differences among groups in animal attrition or health outcomes unrelated to exposure (e.g., infection) that could influence the outcome assessment.

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Study Citation:	IFF, (2016). Galaxolide undiluted. Evaluation of acute dermal toxicity in rats. (sanitized).
Health Outcome(s):	Mortality; Neurological/Behavioral; Nutritional/Metabolic; Endocrine (Endocrine); Lung/Respiratory; Ocular/Sensory; Cardiovascular; Reproductive/Developmental; Gastrointestinal; Immune/Hematological; Hepatic/Liver; Renal/Kidney;
Reported Health Effect(s):	Mortality: Mortality; Neurological/Behavioral: Neurological-related clinical signs (spontaneous activity, Preyer's reflex, convulsions, tremors, body temperature, muscle tone, salivation, pupil appearance, righting reflex).; Nutritional/Metabolic: Body weights; Endocrine (Endocrine): Gross necropsy of the adrenals and pancreas; Lung/Respiratory: Clinical signs: Respiratory rate; gross necropsy of lungs and trachea; Ocular/Sensory: Clinical signs: lacrimation, palpebral opening; Cardiovascular: Gross necropsy of the heart; Reproductive/Developmental: Gross necropsy of testicles, ovaries, and uterus; Gastrointestinal: Gross necropsy of esophagus, stomach, duodenum, jejunum, ileon, caecum, colon, and rectum.; Immune/Hematological: Gross necropsy of the spleen and thymus; Hepatic/Liver: Gross necropsy of the liver; Renal/Kidney: Gross necropsy of the kidneys and urinary bladder;
Duration:	Acute (less than or equal to 24 hr) Acute- 24 hours
Chemical:	HHCB- Parent compound
HERO ID:	8785033

Domain	Metric		Rating	Comments
	Metric 23:	Data Presentation and Analysis	N/A	Mortality: Statistical analysis was not applicable since no mortality was observed.; Neurological/Behavioral: Statistical analysis was applicable since data were negative and/or there was not a control group to compare to.; Nutritional/Metabolic: Statistical analysis was applicable since data were negative and/or there was not a control group to compare to.; Endocrine (Endocrine): Statistical analysis was applicable since data were negative and/or there was not a control group to compare to.; Lung/Respiratory: Statistical analysis was applicable since data were negative and/or there was not a control group to compare to.; Ocular/Sensory: Statistical analysis was applicable since data were negative and/or there was not a control group to compare to.; Cardiovascular: Statistical analysis was applicable since data were negative and/or there was not a control group to compare to.; Reproductive/Developmental: Statistical analysis was applicable since data were negative and/or there was not a control group to compare to.; Gastrointestinal: Statistical analysis was applicable since data were negative and/or there was not a control group to compare to.; Immune/Hematological: Statistical analysis was applicable since data were negative and/or there was not a control group to compare to.; Hepatic/Liver: Statistical analysis was applicable since data were negative and/or there was not a control group to compare to.; Renal/Kidney: Statistical analysis was applicable since data were negative and/or there was not a control group to compare to.
	Metric 24:	Reporting of Data	High	All Outcomes: Data were fully reported.

Additional Comments: None

Overall Quality Determination High

Study Citation:	IFF, (2016). Galaxolide undiluted. Evaluation of acute dermal toxicity in rats. (sanitized).
Health Outcome(s):	Irritation
Reported Health Effect(s):	Irritation: Skin erythema at the site of application
Duration:	Acute (less than or equal to 24 hr) Acute- 24 hours
Chemical:	HHCB- Parent compound
HERO ID:	8785033

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Test substance was identified as Galaxolide undiluted. This is a common tradename for the chemical 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8,-hexamethyl-cyclopenta[g]benzopyran or HHCB.
	Metric 2:	Test Substance Source	High	Based on the information provided, the test substance appears to have been supplied by the sponsor, although the word ”source” was not explicitly stated. The Batch number was provided. Certificates of analysis were provided in the appendix, although some information was redacted.
	Metric 3:	Test Substance Purity	Low	The purity of the test substance was not reported.
Domain 2: Test Design				
	Metric 4:	Negative and Vehicle Controls	N/A	A negative control group is not required for acute lethality study.
	Metric 5:	Positive Controls	N/A	A positive control group is not required for this study type.
	Metric 6:	Randomized Allocation of Animals	Low	The study did not report how animals were allocated to study groups.
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and Storage of Test Substance	High	The test substance was stored at room temperature in glass flasks. Undiluted test substance was administered.
	Metric 8:	Consistency of Exposure Administration	High	The volume applied to skin was 2.09 mL/kg body weight.
	Metric 9:	Reporting of Doses/Concentrations	High	Dose were clearly reported as 2000 mg/kg bw.
	Metric 10:	Exposure Frequency and Duration	High	The exposure frequency and duration were appropriate for the study’s aim (24 hours).
	Metric 11:	Number of Exposure Groups and Dose/Concentration Spacing	High	A single dose group of 2000 mg/kg bw was studied. This was akin to a limit dose and therefore acceptable.
	Metric 12:	Exposure Route and Method	High	The exposure route and method were appropriate an in agreement with OECD guidelines.
Domain 4: Test Animals				
	Metric 13:	Test Animal Characteristics	Low	The sex, age, starting body weight, strain and species were reported. The source of the animals was redacted in the copy used for evaluation.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	Temperature, humidity, light/dark cycle, number of animals/cage and diet/water availability were reported.
	Metric 15:	Number of Animals per Group	Medium	5 males and 5 females were studied. This agrees with OECD guidelines.
Domain 5: Outcome Assessment				

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Study Citation:	IFF, (2016). Galaxolide undiluted. Evaluation of acute dermal toxicity in rats. (sanitized).
Health Outcome(s):	Irritation
Reported Health Effect(s):	Irritation: Skin erythema at the site of application
Duration:	Acute (less than or equal to 24 hr) Acute- 24 hours
Chemical:	HHCB- Parent compound
HERO ID:	8785033

Domain	Metric		Rating	Comments
	Metric 16:	Outcome Assessment Methodology	Medium	The outcome methodology was not clearly described. Skin was observed for irritation, but the frequency of observations was not specified, and the criteria for determining erythema were not reported. This is not expected to impact the study results significantly.
	Metric 17:	Consistency of Outcome Assessment	High	Outcome assessment was consistent across all animals.
	Metric 18:	Sampling Adequacy	High	There was adequate sampling for outcomes of interest (5/sex).
	Metric 19:	Blinding of Assessors	N/A	Blinding was not reported for skin irritation examinations; however, it is not relevant to a study type with a single group.
	Metric 20:	Negative Control Response	N/A	Negative control was not required for acute lethality study.
Domain 6: Confounding / Variable Control				
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	Although the study did not report all information to determine confounding, reported information did not identify differences. Study reports no changes in mortality, body weight, clinical signs. Food intake was not reported.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	There was no information either to support or dismiss the suggestion that there were differences among groups in animal attrition or health outcomes unrelated to exposure (e.g., infection) that could influence the outcome assessment.
	Metric 23:	Data Presentation and Analysis	N/A	Statistical analysis was applicable since data were negative and/or there was not a control group to compare to.
	Metric 24:	Reporting of Data	Medium	Data were fully reported, including a description of severity.

Additional Comments: None

Overall Quality Determination High

Study Citation:	IFF, (1975). Galaxolide 50 assay 1975.
Health Outcome(s):	Irritation
Reported Health Effect(s):	Irritation: Dermal irritation (abraded and intact skin)
Duration:	Acute (less than or equal to 24 hr) 24 hour dermal exposure
Chemical:	HHCB- Parent compound
HERO ID:	8785084

Domain		Metric	Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	Medium	Test substance was identified as Galaxolide 50 (trade name for HHCB). CASRN and structure were not provided. A formulation was tested (approximate concentrations in formulation were 17.5% galaxolide + 7.5% DEP + 75% alcohol SDA 3)
	Metric 2:	Test Substance Source	Low	The source of the test substance was not reported and the test substance identity was not analytically verified.
	Metric 3:	Test Substance Purity	Low	A formulation was tested (approximate concentrations in formulation were 17.5% galaxolide + 7.5% DEP + 75% alcohol SDA 3 (vehicle)). Purity and/or grade of galaxolide were not reported.
Domain 2: Test Design				
	Metric 4:	Negative and Vehicle Controls	N/A	N/A for skin irritation
	Metric 5:	Positive Controls	N/A	
	Metric 6:	Randomized Allocation of Animals	N/A	N/A. Three albino rabbits were tested. All three rabbits received the same treatment.
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and Storage of Test Substance	Low	Information on preparation and storage of test material was not reported.
	Metric 8:	Consistency of Exposure Administration	High	Details of exposure administration were reported and exposures were administered consistently.
	Metric 9:	Reporting of Doses/Concentrations	Medium	0.5 ml of test substance was applied. Approximate final test concentrations were reported to be 17.5% galaxolide, 7.5% DEP, 75.0% alcohol SDA3, but final concentration of galaxolide was not analytically verified.
	Metric 10:	Exposure Frequency and Duration	High	Test substances was applied to unabraded and intact skin for 24 hours.
	Metric 11:	Number of Exposure Groups and Dose/Concentration Spacing	N/A	A single concentration (17.5%) of galaxolide was tested. No irritancy was observed. However, the goal of the study was not to investigate dose-dependency.
	Metric 12:	Exposure Route and Method	High	0.5mL Test substance was applied to a 2x2 patch of clipped skin (abraded and intact skin exposed) and occluded for the duration of the 24-hour exposure period.
Domain 4: Test Animals				
	Metric 13:	Test Animal Characteristics	Low	Study authors report using health, normal, albino rabbits. The source, sex, and age of the test animals was not reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	Animal husbandry conditions were not reported.
	Metric 15:	Number of Animals per Group	Medium	Three albino rabbits were included.

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Study Citation:	IFF, (1975). Galaxolide 50 assay 1975.
Health Outcome(s):	Irritation
Reported Health Effect(s):	Irritation: Dermal irritation (abraded and intact skin)
Duration:	Acute (less than or equal to 24 hr) 24 hour dermal exposure
Chemical:	HHCB- Parent compound
HERO ID:	8785084

Domain	Metric		Rating	Comments
Domain 5: Outcome Assessment				
	Metric 16:	Outcome Assessment Methodology	High	Treated skin was evaluated according to the method of Draize, as described in "Appraisal of the Safety of Chemicals in Food, Drugs and Cosmetics"
	Metric 17:	Consistency of Outcome Assessment	High	Skin was evaluated according to the method of Draize at the end of a 24-hour exposure period and again 48-hours later.
	Metric 18:	Sampling Adequacy	High	
	Metric 19:	Blinding of Assessors	N/A	Blinding of assessors is not necessary for this study type.
	Metric 20:	Negative Control Response	N/A	Negative controls were not uses.
Domain 6: Confounding / Variable Control				
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	Study authors did not report all information to determine confounding, however, reported information did not identify differences among study groups.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	There was no information either to support or dismiss the suggestion that there were differences among groups in animal attrition or health outcomes unrelated to exposure that could influence the outcome assessment.
	Metric 23:	Data Presentation and Analysis	N/A	Statistical analysis was not necessary
	Metric 24:	Reporting of Data	High	Irritation response scores for erythema, eschar, edema formation were reported for all evaluated exposure times.

Additional Comments: None

Overall Quality Determination Medium

Study Citation:	IFF, (1973). Galaxolide 50 Assay 1973 (sanitized).
Health Outcome(s):	Ocular/Sensory
Reported Health Effect(s):	Ocular/Sensory: Eye irritation and lesions (conjunctival irritation)
Duration:	Acute (less than or equal to 24 hr) acute
Chemical:	HHCB- Parent compound
HERO ID:	8785111

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	Medium	Test substance was identified as Galaxolide 50
	Metric 2:	Test Substance Source	Low	Source was not reported (Note HHCB was discovered by IFF so assume they were source of test chemical for their study)
	Metric 3:	Test Substance Purity	Medium	Dose used is clear. 50% galaxolide-50 is equivalent to 35% galaxolide (i.e. 35% HHCB). This is because galaxolide-50 is 70% HHCB according to the study. So, 50% galaxolide-50 has a final concentration of 35% galaxolide (which again is HHCB).
Domain 2: Test Design				
	Metric 4:	Negative and Vehicle Controls	High	The study protocol allows use of animal as its own control (i.e., test substance instilled into the right eye and left eye served as the untreated control).
	Metric 5:	Positive Controls	N/A	Not necessary for this study type
	Metric 6:	Randomized Allocation of Animals	N/A	
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and Storage of Test Substance	Low	Information on preparation and storage was not reported and lack of details could substantially impact results
	Metric 8:	Consistency of Exposure Administration	Medium	Single treatment. Timing of when the test substance was instilled into the eye was not described.
	Metric 9:	Reporting of Doses/Concentrations	Low	Single treatment of test chemical in a mixture containing about 35%-50% galaxolide (both percentages are reported in the study report) in 0.1ml solution.
	Metric 10:	Exposure Frequency and Duration	Medium	The test substance was instilled into the eye as a single treatment.
	Metric 11:	Number of Exposure Groups and Dose/Concentration Spacing	N/A	
	Metric 12:	Exposure Route and Method	High	Test material was instilled into the eye.
Domain 4: Test Animals				
	Metric 13:	Test Animal Characteristics	Low	Animal strain and sex was not reported. Source of animals was not reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	Husbandry conditions were not sufficiently reported to evaluate if husbandry was adequate and whether differences occurred between control and exposed populations.
	Metric 15:	Number of Animals per Group	Medium	
Domain 5: Outcome Assessment				

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Study Citation:	IFF, (1973). Galaxolide 50 Assay 1973 (sanitized).
Health Outcome(s):	Ocular/Sensory
Reported Health Effect(s):	Ocular/Sensory: Eye irritation and lesions (conjunctival irritation)
Duration:	Acute (less than or equal to 24 hr) acute
Chemical:	HHCB- Parent compound
HERO ID:	8785111

Domain	Metric		Rating	Comments
	Metric 16:	Outcome Assessment Methodology	Medium	Eye irritation was scored using the Draize method. No further details regarding the outcome assessment methodology were provided.
	Metric 17:	Consistency of Outcome Assessment	Low	Details regarding the execution of the study protocol for outcome assessment were limited or not reported (e.g., timing of when treated vs. control eyes were evaluated was not provided)
	Metric 18:	Sampling Adequacy	High	Both the control and treated eyes were examined every 24 hours for four days and then again on the seventh day.
	Metric 19:	Blinding of Assessors	N/A	
	Metric 20:	Negative Control Response	Low	The biological response of the negative control groups were not reported
Domain 6: Confounding / Variable Control				
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	The study did not report all information to determine confounding.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	There was no information either to support or dismiss the suggestion that there were differences among groups in health outcomes unrelated to exposure that could influence the outcome assessment.
	Metric 23:	Data Presentation and Analysis	N/A	Qualitative outcome measured, no statistics required.
	Metric 24:	Reporting of Data	Low	Numerical scoring results for control eye were not reported but was described qualitatively.

Additional Comments: None

Overall Quality Determination Medium

Study Citation:	Api, A.M., Ford, R.A. (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(1-2):143-149.
Health Outcome(s):	Hepatic/Liver; Mortality; Nutritional/Metabolic;
Reported Health Effect(s):	Hepatic/Liver: No adverse effects were reported in the histopathological analysis of the liver.; Mortality: There were no treatment-related deaths.; Nutritional/Metabolic: Bodyweight gain and food consumption were increased in males, but not females, at all doses. However, there were no treatment-related effects on absolute bodyweight. Treatment-related decreases in glucose in males and females and triglycerides in males.;
Duration:	Short-term (>1-30 days) 2 weeks
Chemical:	HHCB- Parent compound
HERO ID:	5427830

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Hepatic/Liver: ; Mortality: ; Nutritional/Metabolic:
	Metric 2:	Test Substance Source	Low	All Outcomes: A source was not identified.
	Metric 3:	Test Substance Purity	Low	All Outcomes: Purity of test substance was not mentioned.
Domain 2: Test Design				
	Metric 4:	Negative and Vehicle Controls	High	Hepatic/Liver: ; Mortality: ; Nutritional/Metabolic:
	Metric 5:	Positive Controls	N/A	Hepatic/Liver: ; Mortality: ; Nutritional/Metabolic:
	Metric 6:	Randomized Allocation of Animals	Low	All Outcomes: Randomization not reported.
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and Storage of Test Substance	Low	All Outcomes: No mention of test preparation or storage.
	Metric 8:	Consistency of Exposure Administration	High	Hepatic/Liver: ; Mortality: ; Nutritional/Metabolic:
	Metric 9:	Reporting of Doses/Concentrations	High	Hepatic/Liver: ; Mortality: ; Nutritional/Metabolic:
	Metric 10:	Exposure Frequency and Duration	High	Hepatic/Liver: ; Mortality: ; Nutritional/Metabolic:
	Metric 11:	Number of Exposure Groups and Dose/Concentration Spacing	High	Hepatic/Liver: ; Mortality: ; Nutritional/Metabolic:
	Metric 12:	Exposure Route and Method	High	Hepatic/Liver: ; Mortality: ; Nutritional/Metabolic:
Domain 4: Test Animals				
	Metric 13:	Test Animal Characteristics	Medium	All Outcomes: Starting age of rats was not reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	All Outcomes: No mention of husbandry at all.
	Metric 15:	Number of Animals per Group	Low	All Outcomes: Not reported.
Domain 5: Outcome Assessment				
	Metric 16:	Outcome Assessment Methodology	High	Hepatic/Liver: ; Mortality: ; Nutritional/Metabolic:
	Metric 17:	Consistency of Outcome Assessment	High	Hepatic/Liver: ; Mortality: ; Nutritional/Metabolic:
	Metric 18:	Sampling Adequacy	High	Hepatic/Liver: ; Mortality: ; Nutritional/Metabolic:
	Metric 19:	Blinding of Assessors	N/A	Hepatic/Liver: ; Mortality: ; Nutritional/Metabolic:

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Study Citation:	Api, A.M., Ford, R.A. (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(1-2):143-149.
Health Outcome(s):	Hepatic/Liver; Mortality; Nutritional/Metabolic;
Reported Health Effect(s):	Hepatic/Liver: No adverse effects were reported in the histopathological analysis of the liver.; Mortality: There were no treatment-related deaths.; Nutritional/Metabolic: Bodyweight gain and food consumption were increased in males, but not females, at all doses. However, there were no treatment-related effects on absolute bodyweight. Treatment-related decreases in glucose in males and females and triglycerides in males.;
Duration:	Short-term (>1-30 days) 2 weeks
Chemical:	HHCB- Parent compound
HERO ID:	5427830

Domain	Metric		Rating	Comments
	Metric 20:	Negative Control Response	High	Hepatic/Liver: ; Mortality: ; Nutritional/Metabolic:
Domain 6: Confounding / Variable Control				
	Metric 21:	Confounding Variables in Test Design and Procedures	High	Hepatic/Liver: ; Mortality: ; Nutritional/Metabolic:
	Metric 22:	Health Outcomes Unrelated to Exposure	High	Hepatic/Liver: ; Mortality: ; Nutritional/Metabolic:
	Metric 23:	Data Presentation and Analysis	High	Hepatic/Liver: ; Mortality: ; Nutritional/Metabolic:
	Metric 24:	Reporting of Data	Low	All Outcomes: Insufficient data reporting across many outcomes.

Additional Comments: None

Overall Quality Determination Medium

Study Citation:	IFF, (1973). Galaxolide assay 1973 (sanitized).
Health Outcome(s):	Irritation (Dermal Irritation)
Reported Health Effect(s):	Irritation (Dermal Irritation): Dermal irritation that led to edema, erythema, and eschar formation.
Duration:	Short-term (>1-30 days) 24-72 hours
Chemical:	HHCB- Parent compound
HERO ID:	8785040

Domain		Metric	Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	Low	No characterization of test substance outside of name.
	Metric 2:	Test Substance Source	Low	No manufacturer identification and batch/lot number.
	Metric 3:	Test Substance Purity	Low	There was no analytical verification of purity. The test substance was just identified as galaxolide or HHCB.
Domain 2: Test Design				
	Metric 4:	Negative and Vehicle Controls	Low	The negative control was a different vehicle.
	Metric 5:	Positive Controls	N/A	
	Metric 6:	Randomized Allocation of Animals	Low	No mention of randomization of animals.
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and Storage of Test Substance	Low	Lack of details surrounding preparation of HHCB or why DEP was used, however, information in the literature have documented DEP as a diluent used in dermal irritation studies.
	Metric 8:	Consistency of Exposure Administration	High	
	Metric 9:	Reporting of Doses/Concentrations	Medium	Only nominal percentages were reported.
	Metric 10:	Exposure Frequency and Duration	High	
	Metric 11:	Number of Exposure Groups and Dose/Concentration Spacing	High	No justification was provided, but the dose spacing was reasonable and provided a graded response.
	Metric 12:	Exposure Route and Method	High	
Domain 4: Test Animals				
	Metric 13:	Test Animal Characteristics	Medium	There was no reporting of test animal characteristics (e.g., age, or starting body weight), however, this is unlikely to have a substantial impact on results
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	Husbandry conditions were not sufficiently reported to evaluate if husbandry was adequate.
	Metric 15:	Number of Animals per Group	Low	An insufficient number of animals were used, but the results clearly demonstrated the compound is a dermal irritant.
Domain 5: Outcome Assessment				
	Metric 16:	Outcome Assessment Methodology	High	
	Metric 17:	Consistency of Outcome Assessment	Medium	There is limited description of the outcome assessment.

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Study Citation:	IFF, (1973). Galaxolide assay 1973 (sanitized).
Health Outcome(s):	Irritation (Dermal Irritation)
Reported Health Effect(s):	Irritation (Dermal Irritation): Dermal irritation that led to edema, erythema, and eschar formation.
Duration:	Short-term (>1-30 days) 24-72 hours
Chemical:	HHCB- Parent compound
HERO ID:	8785040

Domain	Metric		Rating	Comments
	Metric 18:	Sampling Adequacy	High	
	Metric 19:	Blinding of Assessors	N/A	
	Metric 20:	Negative Control Response	High	
Domain 6: Confounding / Variable Control				
	Metric 21:	Confounding Variables in Test Design and Procedures	High	
	Metric 22:	Health Outcomes Unrelated to Exposure	High	
	Metric 23:	Data Presentation and Analysis	N/A	
	Metric 24:	Reporting of Data	Low	Severity scores were not described.

Additional Comments: None

Overall Quality Determination Medium

Study Citation:	IFF, (1963). HHCB eye irritation studies. Acute eye irritation study in rabbits. Galaxolide (sanitized).
Health Outcome(s):	Irritation
Reported Health Effect(s):	Irritation: Eye irritation study in rabbits
Duration:	Short-term (>1-30 days) 7 days
Chemical:	HHCB- Parent compound
HERO ID:	8785657

Domain		Metric	Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	
	Metric 2:	Test Substance Source	High	The source of the test substance was reported as a manufacturer
	Metric 3:	Test Substance Purity	Low	Purity and/or grade of test substance were not reported
Domain 2: Test Design				
	Metric 4:	Negative and Vehicle Controls	High	
	Metric 5:	Positive Controls	N/A	
	Metric 6:	Randomized Allocation of Animals	Low	The study did not report how animals were allocated to study group
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and Storage of Test Substance	High	
	Metric 8:	Consistency of Exposure Administration	Medium	Details of exposure administration are incompletely reported
	Metric 9:	Reporting of Doses/Concentrations	High	
	Metric 10:	Exposure Frequency and Duration	High	
	Metric 11:	Number of Exposure Groups and Dose/Concentration Spacing	Low	The number of exposure groups and dose/concentration spacing were not explicitly justified by study authors
	Metric 12:	Exposure Route and Method	High	
Domain 4: Test Animals				
	Metric 13:	Test Animal Characteristics	Low	The source and sex of the test animal was not reported
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	Husbandry conditions were not sufficiently reported
	Metric 15:	Number of Animals per Group	Medium	
Domain 5: Outcome Assessment				
	Metric 16:	Outcome Assessment Methodology	High	
	Metric 17:	Consistency of Outcome Assessment	Low	Details regarding the execution of the study protocol for outcome assessment were limited.
	Metric 18:	Sampling Adequacy	High	
	Metric 19:	Blinding of Assessors	N/A	
	Metric 20:	Negative Control Response	N/A	

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Study Citation:	IFF, (1963). HHCB eye irritation studies. Acute eye irritation study in rabbits. Galaxolide (sanitized).
Health Outcome(s):	Irritation
Reported Health Effect(s):	Irritation: Eye irritation study in rabbits
Duration:	Short-term (>1-30 days) 7 days
Chemical:	HHCB- Parent compound
HERO ID:	8785657

Domain	Metric		Rating	Comments
Domain 6: Confounding / Variable Control				
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	The study did not report all information to determine whether confounding bias may exist.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	There was no information either to support or dismiss the suggestion that there were differences among groups in animal attrition or health outcomes unrelated to exposure (e.g., infection) that could influence the outcome assessment.
	Metric 23:	Data Presentation and Analysis	N/A	
	Metric 24:	Reporting of Data	High	

Additional Comments: None

Overall Quality Determination Medium

Study Citation:	Li, M., Wang, P. (2023). Adverse effect of environmental androgenic compounds Galaxolide and Irgacure 369 on the male reproductive system. Reproductive Toxicology 122:108477.
Health Outcome(s):	Reproductive/Developmental
Reported Health Effect(s):	Reproductive/Developmental: Serum testosterone, LH, FSH, GnRH levels; organ weights (seminal vesicles, testes, prostate), histopathology (testicles, seminal vesicles, prostate, epididymis); sperm parameters (concentration, motility, morphology), oxidative stress,
Duration:	Short-term (>1-30 days) Short-term ip injection
Chemical:	HHCB- Parent compound
HERO ID:	12338848

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The test substance administered was Galaxolide, which was identified definitively by name and CAS number (1222-05-5) as HHCB.
	Metric 2:	Test Substance Source	High	The source of the test substance was reported. The test substance was obtained from a commercial source. The batch/lot number was not reported.
	Metric 3:	Test Substance Purity	Medium	The test substance purity (>75%) was reported; impurities were not reported.
Domain 2: Test Design				
	Metric 4:	Negative and Vehicle Controls	High	A vehicle control group that received a mixture of 95% corn oil and 5% DMSO by intraperitoneal injection was reported.
	Metric 5:	Positive Controls	N/A	A positive control is not required for this study type.
	Metric 6:	Randomized Allocation of Animals	Medium	Test animals were randomly allocated into test groups.
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and Storage of Test Substance	Low	The test substance was dissolved in DMSO, and the stock solution was diluted with corn oil. No details on the storage of the test substance were reported. Given that the test substance was administered for 30 days, details of test substance storage could substantially impact results.
	Metric 8:	Consistency of Exposure Administration	Medium	All test animals were dosed daily via intraperitoneal injection for 30 days. Injection volume is not reported, but this is unlikely to have a substantial impact on results.
	Metric 9:	Reporting of Doses/Concentrations	Medium	The nominal dose of HHCB administered was reported. The concentration in the test solution was not analytically verified.
	Metric 10:	Exposure Frequency and Duration	High	The exposure frequency and duration of exposure were clearly reported, and appropriate for this study type.
	Metric 11:	Number of Exposure Groups and Dose/Concentration Spacing	N/A	Only one concentration of the test substance was administered. The dose was selected based on a prior study by the study authors that demonstrated androgenic activity at the selected dose. The purpose of the study was not to identify a dose-response.
	Metric 12:	Exposure Route and Method	High	Test animals were administered the test substance by intraperitoneal injection daily for 30 consecutive days.
Domain 4: Test Animals				
	Metric 13:	Test Animal Characteristics	High	The study used male Sprague-Dawley rats. Source, age, and initial body weights were all reported.

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Study Citation:	Li, M., Wang, P. (2023). Adverse effect of environmental androgenic compounds Galaxolide and Irgacure 369 on the male reproductive system. Reproductive Toxicology 122:108477.
Health Outcome(s):	Reproductive/Developmental
Reported Health Effect(s):	Reproductive/Developmental: Serum testosterone, LH, FSH, GnRH levels; organ weights (seminal vesicles, testes, prostate), histopathology (testicles, seminal vesicles, prostate, epididymis); sperm parameters (concentration, motility, morphology), oxidative stress,
Duration:	Short-term (>1-30 days) Short-term ip injection
Chemical:	HHCB- Parent compound
HERO ID:	12338848

Domain	Metric		Rating	Comments
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Medium	Most husbandry conditions (temperature, humidity, light-dark cycle, diet, water availability) were reported. The number of animals per cage was not reported. Animals were kept under specific pathogen free (SPF) conditions.
	Metric 15:	Number of Animals per Group	Medium	The number of animals per group was adequate. The study used 10 animals per group, which is appropriate for a rodent short-term study.
Domain 5: Outcome Assessment				
	Metric 16:	Outcome Assessment Methodology	High	The outcome methodology was sensitive and appropriate for the outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	High	Outcome assessment was consistent across all animals. Histopathology was performed in a randomly selected half of the test animals in each group. RNAseq and gene expression were evaluated in testicle samples of 4 randomly selected animals per group.
	Metric 18:	Sampling Adequacy	High	The sampling for the reproductive outcomes was sufficient. All animals were accounted for.
	Metric 19:	Blinding of Assessors	N/A	Blinding is not required for these endpoints.
	Metric 20:	Negative Control Response	High	The biological response of the negative control group was adequate.
Domain 6: Confounding / Variable Control				
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	There is no information reported on the use of plasticizers or polycarbonate cages, and this is likely to have a substantial impact on the reproductive outcomes assessed.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	There was no information either to support or dismiss the suggestion that there were differences among groups in animal attrition or health outcomes unrelated to exposure (e.g., infection) that could influence the outcome assessment.
	Metric 23:	Data Presentation and Analysis	High	Statistical analysis was performed and appropriate.
	Metric 24:	Reporting of Data	High	Data were reported for all outcomes by exposure group. Absolute organ weights were reported for the testes and seminal vesicles, but not the prostate. Some, but not all, histopathological findings were reported quantitatively.

Additional Comments: None

Overall Quality Determination High

Study Citation:	Li, M., Wang, P. (2023). Adverse effect of environmental androgenic compounds Galaxolide and Irgacure 369 on the male reproductive system. Reproductive Toxicology 122:108477.
Health Outcome(s):	Nutritional/Metabolic
Reported Health Effect(s):	Nutritional/Metabolic: Body weight, body weight gain
Duration:	Short-term (>1-30 days) Short-term ip injection
Chemical:	HHCB- Parent compound
HERO ID:	12338848

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The test substance administered was Galaxolide, which was identified definitively by name and CAS number as HHCB.
	Metric 2:	Test Substance Source	High	The source of the test substance was reported. The test substance was obtained from a commercial source. The batch/lot number was not reported.
	Metric 3:	Test Substance Purity	Medium	The test substance purity (>75%) was reported; impurities were not reported.
Domain 2: Test Design				
	Metric 4:	Negative and Vehicle Controls	High	A vehicle control group that received a mixture of 95% corn oil and 5% DMSO by intraperitoneal injection was reported.
	Metric 5:	Positive Controls	N/A	A positive control is not required for this study type.
	Metric 6:	Randomized Allocation of Animals	Medium	Test animals were randomly allocated into test groups.
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and Storage of Test Substance	Low	The test substance was dissolved in DMSO, and the stock solution was diluted with corn oil. No details on the storage of the test substance were reported. Given that the test substance was administered for 30 days, details of test substance storage could substantially impact results.
	Metric 8:	Consistency of Exposure Administration	Medium	All test animals were dosed daily via intraperitoneal injection for 30 days. Injection volume is not reported, but this is unlikely to have a substantial impact on results.
	Metric 9:	Reporting of Doses/Concentrations	Medium	The nominal dose of HHCB administered was reported. The concentration in the test solution was not analytically verified.
	Metric 10:	Exposure Frequency and Duration	High	The exposure frequency and duration of exposure were clearly reported, and appropriate for this study type.
	Metric 11:	Number of Exposure Groups and Dose/Concentration Spacing	N/A	Only one concentration of the test substance was administered. The dose was selected based on a prior study by the study authors that demonstrated androgenic activity at the selected dose. The purpose of the study was not to identify a dose-response.
	Metric 12:	Exposure Route and Method	High	Test animals were administered the test substance by intraperitoneal injection daily for 30 consecutive days.
Domain 4: Test Animals				
	Metric 13:	Test Animal Characteristics	High	The study used male Sprague-Dawley rats. Source, age, and initial body weights were all reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Medium	Most husbandry conditions (temperature, humidity, light-dark cycle, diet, water availability) were reported. The number of animals per cage was not reported. Animals were kept under specific pathogen free (SPF) conditions.

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Study Citation:	Li, M., Wang, P. (2023). Adverse effect of environmental androgenic compounds Galaxolide and Irgacure 369 on the male reproductive system. Reproductive Toxicology 122:108477.
Health Outcome(s):	Nutritional/Metabolic
Reported Health Effect(s):	Nutritional/Metabolic: Body weight, body weight gain
Duration:	Short-term (>1-30 days) Short-term ip injection
Chemical:	HHCB- Parent compound
HERO ID:	12338848

Domain	Metric		Rating	Comments
	Metric 15:	Number of Animals per Group	Medium	The number of animals per group was adequate. The study used 10 animals per group, which is appropriate for a rodent short-term study.
Domain 5: Outcome Assessment				
	Metric 16:	Outcome Assessment Methodology	High	The outcome methodology was sensitive and appropriate for the outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	High	Test animal body weights were examined consistently across groups.
	Metric 18:	Sampling Adequacy	High	All animals used in the study were weighed before dosing and at the conclusion of the study period. All animals were accounted for.
	Metric 19:	Blinding of Assessors	N/A	Blinding is not required for this endpoint.
	Metric 20:	Negative Control Response	High	The biological response of the negative control group was adequate.
Domain 6: Confounding / Variable Control				
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	There was no information either to support or dismiss the suggestion that confounding bias may exist among study groups that could influence the outcome assessment.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	There was no information either to support or dismiss the suggestion that there were differences among groups in animal attrition or health outcomes unrelated to exposure (e.g., infection) that could influence the outcome assessment.
	Metric 23:	Data Presentation and Analysis	High	Statistical analysis was performed and appropriate.
	Metric 24:	Reporting of Data	High	Initial body weight and body weight gain were reported for each test group. Data were presented as means with variance.

Additional Comments: None

Overall Quality Determination High

Study Citation:	Li, M., Wang, P. (2023). Adverse effect of environmental androgenic compounds Galaxolide and Irgacure 369 on the male reproductive system. Reproductive Toxicology 122:108477.
Health Outcome(s):	Hepatic/Liver; Renal/Kidney;
Reported Health Effect(s):	Hepatic/Liver: Liver weight; Renal/Kidney: Kidney weight;
Duration:	Short-term (>1-30 days) Short-term ip injection
Chemical:	HHCB- Parent compound
HERO ID:	12338848

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	All Outcomes: The test substance administered was Galaxolide, which was identified definitively by name and CAS number (1222-05-5) as HHCB.
	Metric 2:	Test Substance Source	High	All Outcomes: The source of the test substance was reported. The test substance was obtained from a commercial source. The batch/lot number was not reported.
	Metric 3:	Test Substance Purity	Medium	All Outcomes: The test substance purity (>75%) was reported; impurities were not reported.
Domain 2: Test Design				
	Metric 4:	Negative and Vehicle Controls	High	All Outcomes: A vehicle control group that received a mixture of 95% corn oil and 5% DMSO by intraperitoneal injection was reported.
	Metric 5:	Positive Controls	N/A	All Outcomes: A positive control is not required for this study type.
	Metric 6:	Randomized Allocation of Animals	Medium	All Outcomes: Test animals were randomly allocated into test groups.
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and Storage of Test Substance	Low	All Outcomes: The test substance was dissolved in DMSO, and the stock solution was diluted with corn oil. No details on the storage of the test substance were reported. Given that the test substance was administered for 30 days, details of test substance storage could substantially impact results.
	Metric 8:	Consistency of Exposure Administration	Medium	All Outcomes: All test animals were dosed daily via intraperitoneal injection for 30 days. Injection volume is not reported, but this is unlikely to have a substantial impact on results.
	Metric 9:	Reporting of Doses/Concentrations	Medium	All Outcomes: The nominal dose of HHCB administered was reported. The concentration in the test solution was not analytically verified.
	Metric 10:	Exposure Frequency and Duration	High	All Outcomes: The exposure frequency and duration of exposure were clearly reported, and appropriate for this study type.
	Metric 11:	Number of Exposure Groups and Dose/Concentration Spacing	N/A	All Outcomes: Only one concentration of the test substance was administered. The dose was selected based on a prior study by the study authors that demonstrated androgenic activity at the selected dose. The purpose of the study was not to identify a dose-response.
	Metric 12:	Exposure Route and Method	High	All Outcomes: Test animals were administered the test substance by intraperitoneal injection daily for 30 consecutive days.
Domain 4: Test Animals				
	Metric 13:	Test Animal Characteristics	High	All Outcomes: The study used male Sprague-Dawley rats. Source, age, and initial body weights were all reported.

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Study Citation:	Li, M., Wang, P. (2023). Adverse effect of environmental androgenic compounds Galaxolide and Irgacure 369 on the male reproductive system. Reproductive Toxicology 122:108477.
Health Outcome(s):	Hepatic/Liver; Renal/Kidney;
Reported Health Effect(s):	Hepatic/Liver: Liver weight; Renal/Kidney: Kidney weight;
Duration:	Short-term (>1-30 days) Short-term ip injection
Chemical:	HHCB- Parent compound
HERO ID:	12338848

Domain	Metric		Rating	Comments
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Medium	All Outcomes: Most husbandry conditions (temperature, humidity, light-dark cycle, diet, water availability) were reported. The number of animals per cage was not reported. Animals were kept under specific pathogen free (SPF) conditions.
	Metric 15:	Number of Animals per Group	Medium	All Outcomes: The number of animals per group was adequate. The study used 10 animals per group, which is appropriate for a rodent short-term study.
Domain 5: Outcome Assessment				
	Metric 16:	Outcome Assessment Methodology	Medium	All Outcomes: The outcome assessment methodology partially addressed the intended outcomes(s) of interest. Only organ weights were assessed.
	Metric 17:	Consistency of Outcome Assessment	High	All Outcomes: Organ weights were examined consistently across groups.
	Metric 18:	Sampling Adequacy	High	All Outcomes: Organ weights were reported for all test animals.
	Metric 19:	Blinding of Assessors	N/A	All Outcomes: Blinding is not required for this endpoint.
	Metric 20:	Negative Control Response	High	All Outcomes: The biological response of the negative control group was adequate.
Domain 6: Confounding / Variable Control				
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	All Outcomes: There was no information either to support or dismiss the suggestion that confounding bias may exist among study groups that could influence the outcome assessment.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	All Outcomes: There was no information either to support or dismiss the suggestion that there were differences among groups in animal attrition or health outcomes unrelated to exposure (e.g., infection) that could influence the outcome assessment.
	Metric 23:	Data Presentation and Analysis	High	All Outcomes: Statistical analysis was performed and appropriate.
	Metric 24:	Reporting of Data	Medium	All Outcomes: Relative organ weights were reported. Absolute organ weights were not reported.

Additional Comments: None

Overall Quality Determination High

Study Citation:	Api, A.M., Ford, R.A. (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(1-2):143-149.
Health Outcome(s):	Thyroid; Cardiovascular; Renal/Kidney; Reproductive/Developmental; Hepatic/Liver; Gastrointestinal; Neurological/Behavioral; Lung/Respiratory; Mortality; Adrenal Gland, Lachrymal Glands; Nutritional/Metabolic; Immune/Hematological; Ocular/Sensory; Skin/Connective Tissue;
Reported Health Effect(s):	Thyroid: There were no treatment-related effects on thyroid weight. However individual data were not provided to allow for independent review. Histopathological analyses were not conducted on the thyroid gland.; Cardiovascular: No effects were reported on heart weights. There were no histopathological changes in the aorta.; Renal/Kidney: No effects were reported on kidney weights. No effects were reported to observed in the histopathological analyses of the kidney or urinary bladder.; Reproductive/Developmental: No adverse effects were reported in histopathological analysis of the prostate, seminal vesicles, epididymides, and testes of males and ovaries, mammary gland, uterus and vagina of females. No effects were reported on testes or ovary weights.; Hepatic/Liver: No adverse effects were reported in the histopathological analysis of the liver.; Gastrointestinal: No adverse effects were reported in the histopathological analysis of the GI tract, pancreas, or salivary glands (submaxillary and sublingual).; Neurological/Behavioral: No adverse effects were reported in the histopathological analysis of the brain. No effects were reported on pituitary gland or brain weights. Tehre were no histopathological changes reported in the brain, spinal cord, or sciatic nerve.; Lung/Respiratory: No adverse effects were reported in the histopathological analysis of the mainstem bronchi or trachea. No effects were reported on lung weights.; Mortality: There were no treatment-related deaths.; Adrenal Gland, Lachrymal Glands: No adverse effects on adrenal gland weight. No histopathological findings were reported din the lachrymal glands (exorbital).; Nutritional/Metabolic: Bodyweight gain and food consumption were increased in males, but not females, at all doses. However, there were no treatment-related effects on absolute bodyweight. Treatment-related decreases in glucose in males and females and triglycerides in males.; Immune/Hematological: No changes in hematology were observed.No treatment-related changes were reported for thymus or spleen weight. There were no histopathological changes in the mesenteric lymph node, submandibular lymph node, or femur and sternum (including marrow).; Ocular/Sensory: No changes in ophthalmologic evaluation were observed. There were no histopathological changes in the eyes (including optic nerve).; Skin/Connective Tissue: There were no histopathological changes in the skin.;
Duration:	Subchronic (>30-91 days) 90 Days
Chemical:	HHCB- Parent compound
HERO ID:	5427830

Domain		Metric	Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Thyroid: ; Cardiovascular: ; Renal/Kidney: ; Reproductive/Developmental: ; Hepatic/Liver: ; Gastrointestinal: ; Neurological/Behavioral: ; Lung/Respiratory: ; Mortality: ; Adrenal Gland, Lachrymal Glands: ; Nutritional/Metabolic: ; Immune/Hematological: ; Ocular/Sensory: ; Skin/Connective Tissue:
	Metric 2:	Test Substance Source	Low	All Outcomes: A source was not identified.
	Metric 3:	Test Substance Purity	Low	All Outcomes: Purity of test substance was not mentioned.
Domain 2: Test Design				
	Metric 4:	Negative and Vehicle Controls	High	Thyroid: ; Cardiovascular: ; Renal/Kidney: ; Reproductive/Developmental: ; Hepatic/Liver: ; Gastrointestinal: ; Neurological/Behavioral: ; Lung/Respiratory: ; Mortality: ; Adrenal Gland, Lachrymal Glands: ; Nutritional/Metabolic: ; Immune/Hematological: ; Ocular/Sensory: ; Skin/Connective Tissue:
	Metric 5:	Positive Controls	N/A	Thyroid: ; Cardiovascular: ; Renal/Kidney: ; Reproductive/Developmental: ; Hepatic/Liver: ; Gastrointestinal: ; Neurological/Behavioral: ; Lung/Respiratory: ; Mortality: ; Adrenal Gland, Lachrymal Glands: ; Nutritional/Metabolic: ; Immune/Hematological: ; Ocular/Sensory: ; Skin/Connective Tissue:
	Metric 6:	Randomized Allocation of Animals	Low	All Outcomes: Randomization not reported.

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Study Citation:	Api, A.M., Ford, R.A. (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(1-2):143-149.
Health Outcome(s):	Thyroid; Cardiovascular; Renal/Kidney; Reproductive/Developmental; Hepatic/Liver; Gastrointestinal; Neurological/Behavioral; Lung/Respiratory; Mortality; Adrenal Gland, Lachrymal Glands; Nutritional/Metabolic; Immune/Hematological; Ocular/Sensory; Skin/Connective Tissue;
Reported Health Effect(s):	Thyroid: There were no treatment-related effects on thyroid weight. However individual data were not provided to allow for independent review. Histopathological analyses were not conducted on the thyroid gland.; Cardiovascular: No effects were reported on heart weights. There were no histopathological changes in the aorta.; Renal/Kidney: No effects were reported on kidney weights. No effects were reported to observed in the histopathological analyses of the kidney or urinary bladder.; Reproductive/Developmental: No adverse effects were reported in histopathological analysis of the prostate, seminal vesicles, epididymides, and testes of males and ovaries, mammary gland, uterus and vagina of females. No effects were reported on testes or ovary weights.; Hepatic/Liver: No adverse effects were reported in the histopathological analysis of the liver.; Gastrointestinal: No adverse effects were reported in the histopathological analysis of the GI tract, pancreas, or salivary glands (submaxillary and sublingual).; Neurological/Behavioral: No adverse effects were reported in the histopathological analysis of the brain. No effects were reported on pituitary gland or brain weights. Tehre were no histopathological changes reported in the brain, spinal cord, or sciatic nerve.; Lung/Respiratory: No adverse effects were reported in the histopathological analysis of the mainstem bronchi or trachea. No effects were reported on lung weights.; Mortality: There were no treatment-related deaths.; Adrenal Gland, Lachrymal Glands: No adverse effects on adrenal gland weight. No histopathological findings were reported din the lachrymal glands (exorbital).; Nutritional/Metabolic: Bodyweight gain and food consumption were increased in males, but not females, at all doses. However, there were no treatment-related effects on absolute bodyweight. Treatment-related decreases in glucose in males and females and triglycerides in males.; Immune/Hematological: No changes in hematology were observed.No treatment-related changes were reported for thymus or spleen weight. There were no histopathological changes in the mesenteric lymph node, submandibular lymph node, or femur and sternum (including marrow).; Ocular/Sensory: No changes in ophthalmologic evaluation were observed. There were no histopathological changes in the eyes (including optic nerve).; Skin/Connective Tissue: There were no histopathological changes in the skin.;
Duration:	Subchronic (>30-91 days) 90 Days
Chemical:	HHCB- Parent compound
HERO ID:	5427830

Domain	Metric		Rating	Comments
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and Storage of Test Substance	Low	All Outcomes: No mention of test preparation or storage.
	Metric 8:	Consistency of Exposure Administration	High	Thyroid: ; Cardiovascular: ; Renal/Kidney: ; Reproductive/Developmental: ; Hepatic/Liver: ; Gastrointestinal: ; Neurological/Behavioral: ; Lung/Respiratory: ; Mortality: ; Adrenal Gland, Lachrymal Glands: ; Nutritional/Metabolic: ; Immune/Hematological: ; Ocular/Sensory: ; Skin/Connective Tissue:
	Metric 9:	Reporting of Doses/Concentrations	High	Thyroid: ; Cardiovascular: ; Renal/Kidney: ; Reproductive/Developmental: ; Hepatic/Liver: ; Gastrointestinal: ; Neurological/Behavioral: ; Lung/Respiratory: ; Mortality: ; Adrenal Gland, Lachrymal Glands: ; Nutritional/Metabolic: ; Immune/Hematological: ; Ocular/Sensory: ; Skin/Connective Tissue:
	Metric 10:	Exposure Frequency and Duration	High	Thyroid: ; Cardiovascular: ; Renal/Kidney: ; Reproductive/Developmental: ; Hepatic/Liver: ; Gastrointestinal: ; Neurological/Behavioral: ; Lung/Respiratory: ; Mortality: ; Adrenal Gland, Lachrymal Glands: ; Nutritional/Metabolic: ; Immune/Hematological: ; Ocular/Sensory: ; Skin/Connective Tissue:
	Metric 11:	Number of Exposure Groups and Dose/Concentration Spacing	High	Thyroid: ; Cardiovascular: ; Renal/Kidney: ; Reproductive/Developmental: ; Hepatic/Liver: ; Gastrointestinal: ; Neurological/Behavioral: ; Lung/Respiratory: ; Mortality: ; Adrenal Gland, Lachrymal Glands: ; Nutritional/Metabolic: ; Immune/Hematological: ; Ocular/Sensory: ; Skin/Connective Tissue:

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Study Citation:	Api, A.M., Ford, R.A. (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(1-2):143-149.
Health Outcome(s):	Thyroid; Cardiovascular; Renal/Kidney; Reproductive/Developmental; Hepatic/Liver; Gastrointestinal; Neurological/Behavioral; Lung/Respiratory; Mortality; Adrenal Gland, Lachrymal Glands; Nutritional/Metabolic; Immune/Hematological; Ocular/Sensory; Skin/Connective Tissue;
Reported Health Effect(s):	Thyroid: There were no treatment-related effects on thyroid weight. However individual data were not provided to allow for independent review. Histopathological analyses were not conducted on the thyroid gland.; Cardiovascular: No effects were reported on heart weights. There were no histopathological changes in the aorta.; Renal/Kidney: No effects were reported on kidney weights. No effects were reported to observed in the histopathological analyses of the kidney or urinary bladder.; Reproductive/Developmental: No adverse effects were reported in histopathological analysis of the prostate, seminal vesicles, epididymides, and testes of males and ovaries, mammary gland, uterus and vagina of females. No effects were reported on testes or ovary weights.; Hepatic/Liver: No adverse effects were reported in the histopathological analysis of the liver.; Gastrointestinal: No adverse effects were reported in the histopathological analysis of the GI tract, pancreas, or salivary glands (submaxillary and sublingual).; Neurological/Behavioral: No adverse effects were reported in the histopathological analysis of the brain. No effects were reported on pituitary gland or brain weights. Tehre were no histopathological changes reported in the brain, spinal cord, or sciatic nerve.; Lung/Respiratory: No adverse effects were reported in the histopathological analysis of the mainstem bronchi or trachea. No effects were reported on lung weights.; Mortality: There were no treatment-related deaths.; Adrenal Gland, Lachrymal Glands: No adverse effects on adrenal gland weight. No histopathological findings were reported din the lachrymal glands (exorbital).; Nutritional/Metabolic: Bodyweight gain and food consumption were increased in males, but not females, at all doses. However, there were no treatment-related effects on absolute bodyweight. Treatment-related decreases in glucose in males and females and triglycerides in males.; Immune/Hematological: No changes in hematology were observed.No treatment-related changes were reported for thymus or spleen weight. There were no histopathological changes in the mesenteric lymph node, submandibular lymph node, or femur and sternum (including marrow).; Ocular/Sensory: No changes in ophthalmologic evaluation were observed. There were no histopathological changes in the eyes (including optic nerve).; Skin/Connective Tissue: There were no histopathological changes in the skin.;
Duration:	Subchronic (>30-91 days) 90 Days
Chemical:	HHCB- Parent compound
HERO ID:	5427830

Domain		Metric	Rating	Comments
	Metric 12:	Exposure Route and Method	High	Thyroid: ; Cardiovascular: ; Renal/Kidney: ; Reproductive/Developmental: ; Hepatic/Liver: ; Gastrointestinal: ; Neurological/Behavioral: ; Lung/Respiratory: ; Mortality: ; Adrenal Gland, Lachrymal Glands: ; Nutritional/Metabolic: ; Immune/Hematological: ; Ocular/Sensory: ; Skin/Connective Tissue:
Domain 4: Test Animals				
	Metric 13:	Test Animal Characteristics	Medium	All Outcomes: Starting age of rats was not reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	All Outcomes: No mention of husbandry at all.
	Metric 15:	Number of Animals per Group	Medium	All Outcomes: 15 animals per group for 13 week study.
Domain 5: Outcome Assessment				
	Metric 16:	Outcome Assessment Methodology	High	Thyroid: ; Cardiovascular: ; Renal/Kidney: ; Reproductive/Developmental: ; Hepatic/Liver: ; Gastrointestinal: ; Neurological/Behavioral: ; Lung/Respiratory: ; Mortality: ; Adrenal Gland, Lachrymal Glands: ; Nutritional/Metabolic: ; Immune/Hematological: ; Ocular/Sensory: ; Skin/Connective Tissue:
	Metric 17:	Consistency of Outcome Assessment	High	Thyroid: ; Cardiovascular: ; Renal/Kidney: ; Reproductive/Developmental: ; Hepatic/Liver: ; Gastrointestinal: ; Neurological/Behavioral: ; Lung/Respiratory: ; Mortality: ; Adrenal Gland, Lachrymal Glands: ; Nutritional/Metabolic: ; Immune/Hematological: ; Ocular/Sensory: ; Skin/Connective Tissue:

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Study Citation:	Api, A.M., Ford, R.A. (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(1-2):143-149.
Health Outcome(s):	Thyroid; Cardiovascular; Renal/Kidney; Reproductive/Developmental; Hepatic/Liver; Gastrointestinal; Neurological/Behavioral; Lung/Respiratory; Mortality; Adrenal Gland, Lachrymal Glands; Nutritional/Metabolic; Immune/Hematological; Ocular/Sensory; Skin/Connective Tissue;
Reported Health Effect(s):	Thyroid: There were no treatment-related effects on thyroid weight. However individual data were not provided to allow for independent review. Histopathological analyses were not conducted on the thyroid gland.; Cardiovascular: No effects were reported on heart weights. There were no histopathological changes in the aorta.; Renal/Kidney: No effects were reported on kidney weights. No effects were reported to observed in the histopathological analyses of the kidney or urinary bladder.; Reproductive/Developmental: No adverse effects were reported in histopathological analysis of the prostate, seminal vesicles, epididymides, and testes of males and ovaries, mammary gland, uterus and vagina of females. No effects were reported on testes or ovary weights.; Hepatic/Liver: No adverse effects were reported in the histopathological analysis of the liver.; Gastrointestinal: No adverse effects were reported in the histopathological analysis of the GI tract, pancreas, or salivary glands (submaxillary and sublingual).; Neurological/Behavioral: No adverse effects were reported in the histopathological analysis of the brain. No effects were reported on pituitary gland or brain weights. Tehre were no histopathological changes reported in the brain, spinal cord, or sciatic nerve.; Lung/Respiratory: No adverse effects were reported in the histopathological analysis of the mainstem bronchi or trachea. No effects were reported on lung weights.; Mortality: There were no treatment-related deaths.; Adrenal Gland, Lachrymal Glands: No adverse effects on adrenal gland weight. No histopathological findings were reported din the lachrymal glands (exorbital).; Nutritional/Metabolic: Bodyweight gain and food consumption were increased in males, but not females, at all doses. However, there were no treatment-related effects on absolute bodyweight. Treatment-related decreases in glucose in males and females and triglycerides in males.; Immune/Hematological: No changes in hematology were observed.No treatment-related changes were reported for thymus or spleen weight. There were no histopathological changes in the mesenteric lymph node, submandibular lymph node, or femur and sternum (including marrow).; Ocular/Sensory: No changes in ophthalmologic evaluation were observed. There were no histopathological changes in the eyes (including optic nerve).; Skin/Connective Tissue: There were no histopathological changes in the skin.;
Duration:	Subchronic (>30-91 days) 90 Days
Chemical:	HHCB- Parent compound
HERO ID:	5427830

Domain		Metric	Rating	Comments
	Metric 18:	Sampling Adequacy	High	Thyroid: ; Cardiovascular: ; Renal/Kidney: ; Reproductive/Developmental: ; Hepatic/Liver: ; Gastrointestinal: ; Neurological/Behavioral: ; Lung/Respiratory: ; Mortality: ; Adrenal Gland, Lachrymal Glands: ; Nutritional/Metabolic: ; Immune/Hematological: ; Ocular/Sensory: ; Skin/Connective Tissue:
	Metric 19:	Blinding of Assessors	N/A	Thyroid: ; Cardiovascular: ; Renal/Kidney: ; Reproductive/Developmental: ; Hepatic/Liver: ; Gastrointestinal: ; Neurological/Behavioral: ; Lung/Respiratory: ; Mortality: ; Adrenal Gland, Lachrymal Glands: ; Nutritional/Metabolic: ; Immune/Hematological: ; Ocular/Sensory: ; Skin/Connective Tissue:
	Metric 20:	Negative Control Response	High	Thyroid: ; Cardiovascular: ; Renal/Kidney: ; Reproductive/Developmental: ; Hepatic/Liver: ; Gastrointestinal: ; Neurological/Behavioral: ; Lung/Respiratory: ; Mortality: ; Adrenal Gland, Lachrymal Glands: ; Nutritional/Metabolic: ; Immune/Hematological: ; Ocular/Sensory: ; Skin/Connective Tissue:
Domain 6: Confounding / Variable Control				
	Metric 21:	Confounding Variables in Test Design and Procedures	High	Thyroid: ; Cardiovascular: ; Renal/Kidney: ; Reproductive/Developmental: ; Hepatic/Liver: ; Gastrointestinal: ; Neurological/Behavioral: ; Lung/Respiratory: ; Mortality: ; Adrenal Gland, Lachrymal Glands: ; Nutritional/Metabolic: ; Immune/Hematological: ; Ocular/Sensory: ; Skin/Connective Tissue:
	Metric 22:	Health Outcomes Unrelated to Exposure	High	Thyroid: ; Cardiovascular: ; Renal/Kidney: ; Reproductive/Developmental: ; Hepatic/Liver: ; Gastrointestinal: ; Neurological/Behavioral: ; Lung/Respiratory: ; Mortality: ; Adrenal Gland, Lachrymal Glands: ; Nutritional/Metabolic: ; Immune/Hematological: ; Ocular/Sensory: ; Skin/Connective Tissue:

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Study Citation:	Api, A.M., Ford, R.A. (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(1-2):143-149.
Health Outcome(s):	Thyroid; Cardiovascular; Renal/Kidney; Reproductive/Developmental; Hepatic/Liver; Gastrointestinal; Neurological/Behavioral; Lung/Respiratory; Mortality; Adrenal Gland, Lachrymal Glands; Nutritional/Metabolic; Immune/Hematological; Ocular/Sensory; Skin/Connective Tissue;
Reported Health Effect(s):	Thyroid: There were no treatment-related effects on thyroid weight. However individual data were not provided to allow for independent review. Histopathological analyses were not conducted on the thyroid gland.; Cardiovascular: No effects were reported on heart weights. There were no histopathological changes in the aorta.; Renal/Kidney: No effects were reported on kidney weights. No effects were reported to observed in the histopathological analyses of the kidney or urinary bladder.; Reproductive/Developmental: No adverse effects were reported in histopathological analysis of the prostate, seminal vesicles, epididymides, and testes of males and ovaries, mammary gland, uterus and vagina of females. No effects were reported on testes or ovary weights.; Hepatic/Liver: No adverse effects were reported in the histopathological analysis of the liver.; Gastrointestinal: No adverse effects were reported in the histopathological analysis of the GI tract, pancreas, or salivary glands (submaxillary and sublingual).; Neurological/Behavioral: No adverse effects were reported in the histopathological analysis of the brain. No effects were reported on pituitary gland or brain weights. Tehre were no histopathological changes reported in the brain, spinal cord, or sciatic nerve.; Lung/Respiratory: No adverse effects were reported in the histopathological analysis of the mainstem bronchi or trachea. No effects were reported on lung weights.; Mortality: There were no treatment-related deaths.; Adrenal Gland, Lachrymal Glands: No adverse effects on adrenal gland weight. No histopathological findings were reported din the lachrymal glands (exorbital).; Nutritional/Metabolic: Bodyweight gain and food consumption were increased in males, but not females, at all doses. However, there were no treatment-related effects on absolute bodyweight. Treatment-related decreases in glucose in males and females and triglycerides in males.; Immune/Hematological: No changes in hematology were observed.No treatment-related changes were reported for thymus or spleen weight. There were no histopathological changes in the mesenteric lymph node, submandibular lymph node, or femur and sternum (including marrow).; Ocular/Sensory: No changes in ophthalmologic evaluation were observed. There were no histopathological changes in the eyes (including optic nerve).; Skin/Connective Tissue: There were no histopathological changes in the skin.;
Duration:	Subchronic (>30-91 days) 90 Days
Chemical:	HHCB- Parent compound
HERO ID:	5427830

Domain	Metric		Rating	Comments
	Metric 23:	Data Presentation and Analysis	High	Thyroid: ; Cardiovascular: ; Renal/Kidney: ; Reproductive/Developmental: ; Hepatic/Liver: ; Gastrointestinal: ; Neurological/Behavioral: ; Lung/Respiratory: ; Mortality: ; Adrenal Gland, Lachrymal Glands: ; Nutritional/Metabolic: ; Immune/Hematological: ; Ocular/Sensory: ; Skin/Connective Tissue:
	Metric 24:	Reporting of Data	Low	All Outcomes: Insufficient data reporting across many outcomes.

Additional Comments: None

Overall Quality Determination Medium

Study Citation:	IFF, (n.d.). Twenty-six week subacute dermal toxicity study in rats. Book 1 of 4. Galaxolide 50. (sanitized).
Health Outcome(s):	Immune/Hematological; Cardiovascular; Reproductive/Developmental; Hepatic/Liver; Nutritional/Metabolic; Clinical signs; Mortality;
Reported Health Effect(s):	Immune/Hematological: Gross necropsy; Histology on mesenteric lymph nodes and spleen; Hematology (RBC, WBC, differential WBC, hematocrit and hemoglobin); Cardiovascular: Gross necropsy; Heart weight and histology; Reproductive/Developmental: Weight, gross necropsy, and histology of ovaries and uterus, or testes.; Hepatic/Liver: Liver weight, gross necropsy, and histology; serum SGPT, SGPT and SAP levels; Nutritional/Metabolic: Body weight and food intake; Clinical signs: Clinical signs of toxicity; Mortality: Mortality;
Duration:	Subchronic (>30-91 days) Up to 13 weeks (interim sacrifices)
Chemical:	HHCB- Parent compound
HERO ID:	8785658

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	All Outcomes: The test substance was identified as Galaxolide 50. This is a common tradename for the chemical 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8,-hexamethylcyclopenta[g]benzopyran or HHCB.
	Metric 2:	Test Substance Source	High	All Outcomes: The source of the test substance was International Flavours and Fragrances, Inc., New York, NY. The Batch was not reported.
	Metric 3:	Test Substance Purity	Low	All Outcomes: The purity of the test substance was not reported.
Domain 2: Test Design				
	Metric 4:	Negative and Vehicle Controls	High	All Outcomes: Negative control groups were appropriate. The study included an untreated control group and a vehicle control group.
	Metric 5:	Positive Controls	N/A	All Outcomes: A positive control group is not required for this study type.
	Metric 6:	Randomized Allocation of Animals	Medium	All Outcomes: Animals were randomly allocated into study groups using a computer-generated randomization procedure. The study text also noted that animals were randomly selected for the interim sacrifices and for assignment to the recovery group.
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and Storage of Test Substance	Medium	All Outcomes: Storage information of the test substance was not reported, but the test substance was prepared fresh weekly by dissolving appropriate amount of test material in 95% ethanol. The ethanol was warmed in a water bath to facilitate dissolving.
	Metric 8:	Consistency of Exposure Administration	Low	All Outcomes: The test substance concentration in ethanol (10% w/v) was consistent across groups; however, the volume applied to the skin varied to achieve the desired dose. For example, the 200 mg/kg/day group received 2.0 ml/kg/day of test substance solution, whereas the 50 mg/kg/day group received 0.5 ml/kg/day. The vehicle control group received a volume equal to the maximum volume administered (2 ml/kg/day).
	Metric 9:	Reporting of Doses/Concentrations	Medium	All Outcomes: Nominal doses were reported. Concentrations of the test substance were not analytically verified, but this is unlikely to have a substantial impact on the study results.
	Metric 10:	Exposure Frequency and Duration	High	All Outcomes: The exposure frequency and duration were appropriate for the study's aim.
	Metric 11:	Number of Exposure Groups and Dose/Concentration Spacing	Medium	All Outcomes: The doses and dose-spacing were sufficient for identification of a study-wide NOAEL and LOAEL following 26 weeks of exposure. The dose selections were not justified by the study authors.

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Study Citation:	IFF, (n.d.). Twenty-six week subacute dermal toxicity study in rats. Book 1 of 4. Galaxolide 50. (sanitized).
Health Outcome(s):	Immune/Hematological; Cardiovascular; Reproductive/Developmental; Hepatic/Liver; Nutritional/Metabolic; Clinical signs; Mortality;
Reported Health Effect(s):	Immune/Hematological: Gross necropsy; Histology on mesenteric lymph nodes and spleen; Hematology (RBC, WBC, differential WBC, hematocrit and hemoglobin); Cardiovascular: Gross necropsy; Heart weight and histology; Reproductive/Developmental: Weight, gross necropsy, and histology of ovaries and uterus, or testes.; Hepatic/Liver: Liver weight, gross necropsy, and histology; serum SGPT, SGPT and SAP levels; Nutritional/Metabolic: Body weight and food intake; Clinical signs: Clinical signs of toxicity; Mortality: Mortality;
Duration:	Subchronic (>30-91 days) Up to 13 weeks (interim sacrifices)
Chemical:	HHCB- Parent compound
HERO ID:	8785658

Domain		Metric	Rating	Comments
	Metric 12:	Exposure Route and Method	Low	All Outcomes: One part of the study text indicated that the test substance was applied to clipped skin via syringe. Later, it was reported that the test substance was applied to clipped backs of animals by gentle inunction. The test substance was not covered; therefore test substance may have rubbed off, or possibly been licked by animals. The test substance was dissolved in ethanol; ethanol would have likely evaporated from the skin.
Domain 4: Test Animals				
	Metric 13:	Test Animal Characteristics	High	All Outcomes: The sex, age, starting body weight, strain, species and source were reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Medium	All Outcomes: Light/dark cycle, number of animals/cage and diet/water availability were reported. Animals were placed in temperature- and humidity-controlled room (details not reported).
	Metric 15:	Number of Animals per Group	Low	All Outcomes: The initial number of animals/group included 15 males and 35 females/group. For a chronic duration study, OECD guidelines recommend 20/sex/group. According to OECD guidelines, if interim sacrifices are planned, the number of animals should be increased by the number to be sacrificed before study completion. This study included two interim sacrifices (at weeks 6 and 13) without increasing the number of animals. Additionally, a subset of animals was assigned to a recovery group and stopped treatment at week 13. Therefore, the number of animals left at study termination was significantly lower than recommended for a chronic duration study.
Domain 5: Outcome Assessment				
	Metric 16:	Outcome Assessment Methodology	High	All Outcomes: The outcome methodology was sensitive and appropriate for the outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	High	All Outcomes: Outcome assessment was consistent across all animals.
	Metric 18:	Sampling Adequacy	High	All Outcomes: Sampling was adequate in that no mortality was observed and all animals designated for evaluation at each specific timepoint were used. In some cases, the numbers were low (e.g., as few as 5 animals). This was sufficient to conduct statistical analysis, but, for some endpoints like histopathology, lacked statistical power.
	Metric 19:	Blinding of Assessors	N/A	All Outcomes: Blinding was not reported; however, the endpoints were simple measures, not subjective, or consisted of clinical signs, gross observations or initial histopathology, and blinding is not required.
	Metric 20:	Negative Control Response	High	All Outcomes: Negative control responses were appropriate for the outcomes of interest.
Domain 6: Confounding / Variable Control				

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Study Citation:	IFF, (n.d.). Twenty-six week subacute dermal toxicity study in rats. Book 1 of 4. Galaxolide 50. (sanitized).
Health Outcome(s):	Immune/Hematological; Cardiovascular; Reproductive/Developmental; Hepatic/Liver; Nutritional/Metabolic; Clinical signs; Mortality;
Reported Health Effect(s):	Immune/Hematological: Gross necropsy; Histology on mesenteric lymph nodes and spleen; Hematology (RBC, WBC, differential WBC, hematocrit and hemoglobin); Cardiovascular: Gross necropsy; Heart weight and histology; Reproductive/Developmental: Weight, gross necropsy, and histology of ovaries and uterus, or testes.; Hepatic/Liver: Liver weight, gross necropsy, and histology; serum SGPT, SGPT and SAP levels; Nutritional/Metabolic: Body weight and food intake; Clinical signs: Clinical signs of toxicity; Mortality: Mortality;
Duration:	Subchronic (>30-91 days) Up to 13 weeks (interim sacrifices)
Chemical:	HHCB- Parent compound
HERO ID:	8785658

Domain	Metric		Rating	Comments
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	All Outcomes: Most information to assess potential confounding was provided. The study did not report whether measures were taken to prevent the possibility of oral exposures, which could impact the study results.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	All Outcomes: There was no information either to support or dismiss the suggestion that there were differences among groups in animal attrition or health outcomes unrelated to exposure (e.g., infection) that could influence the outcome assessment.
	Metric 23:	Data Presentation and Analysis	High	All Outcomes: Statistical analysis was appropriate, or data were provided to conduct independent statistical analysis.
	Metric 24:	Reporting of Data	High	All Outcomes: Data are fully reported.

Additional Comments: None

Overall Quality Determination High

Study Citation:	IFF, (n.d.). Twenty-six week subacute dermal toxicity study in rats. Book 1 of 4. Galaxolide 50. (sanitized).
Health Outcome(s):	Renal/Kidney
Reported Health Effect(s):	Renal/Kidney: Kidney weight, gross necropsy, histology on kidney and urinary bladder; serum glucose and BUN; urinalysis (color, pH, specific gravity, glucose, ketones and blood)
Duration:	Subchronic (>30-91 days) Up to 13 weeks (interim sacrifices)
Chemical:	HHCB- Parent compound
HERO ID:	8785658

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The test substance was identified as Galaxolide 50. This is a common tradename for the chemical 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8,-hexamethyl-cyclopenta[g]benzopyran or HHCB.
	Metric 2:	Test Substance Source	High	The source of the test substance was International Flavours and Fragrances, Inc., New York, NY. The Batch was not reported.
	Metric 3:	Test Substance Purity	Low	The purity of the test substance was not reported.
Domain 2: Test Design				
	Metric 4:	Negative and Vehicle Controls	High	Negative control groups were appropriate. The study included an untreated control group and a vehicle control group.
	Metric 5:	Positive Controls	N/A	A positive control group is not required for this study type.
	Metric 6:	Randomized Allocation of Animals	Medium	Animals were randomly allocated into study groups using a computer-generated randomization procedure. The study text also noted that animals were randomly selected for the interim sacrifices and for assignment to the recovery group.
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and Storage of Test Substance	Medium	Storage information of the test substance was not reported, but the test substance was prepared fresh weekly by dissolving appropriate amount of test material in 95% ethanol. The ethanol was warmed in a water bath to facilitate dissolving.
	Metric 8:	Consistency of Exposure Administration	Low	The test substance concentration in ethanol (10% w/v) was consistent across groups; however, the volume applied to the skin varied to achieve the desired dose. For example, the 200 mg/kg/day group received 2.0 ml/kg/day of test substance solution, whereas the 50 mg/kg/day group received 0.5 ml/kg/day. The vehicle control group received a volume equal to the maximum volume administered (2 ml/kg/day).
	Metric 9:	Reporting of Doses/Concentrations	Medium	Nominal doses were reported. Concentrations of the test substance were not analytically verified, but this is unlikely to have a substantial impact on the study results.
	Metric 10:	Exposure Frequency and Duration	High	The exposure frequency and duration were appropriate for the study's aim.
	Metric 11:	Number of Exposure Groups and Dose/Concentration Spacing	Medium	The doses and dose-spacing were sufficient for identification of a study-wide NOAEL and LOAEL following 26 weeks of exposure. The dose selections were not justified by the study authors.
	Metric 12:	Exposure Route and Method	Low	One part of the study text indicated that the test substance was applied to clipped skin via syringe. Later, it was reported that the test substance was applied to clipped backs of animals by gentle inunction. The test substance was not covered; therefore test substance may have rubbed off, or possibly been licked by animals. The test substance was dissolved in ethanol; ethanol would have likely evaporated from the skin.
Domain 4: Test Animals				

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Study Citation:	IFF, (n.d.). Twenty-six week subacute dermal toxicity study in rats. Book 1 of 4. Galaxolide 50. (sanitized).
Health Outcome(s):	Renal/Kidney
Reported Health Effect(s):	Renal/Kidney: Kidney weight, gross necropsy, histology on kidney and urinary bladder; serum glucose and BUN; urinalysis (color, pH, specific gravity, glucose, ketones and blood)
Duration:	Subchronic (>30-91 days) Up to 13 weeks (interim sacrifices)
Chemical:	HHCB- Parent compound
HERO ID:	8785658

Domain	Metric		Rating	Comments
	Metric 13:	Test Animal Characteristics	High	The sex, age, starting body weight, strain, species and source were reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Medium	Light/dark cycle, number of animals/cage and diet/water availability were reported. Animals were placed in temperature- and humidity-controlled room (details not reported).
	Metric 15:	Number of Animals per Group	Low	The initial number of animals/group included 15 males and 35 females/group. For a chronic duration study, OECD guidelines recommend 20/sex/group. According to OECD guidelines, if interim sacrifices are planned, the number of animals should be increased by the number to be sacrificed before study completion. This study included two interim sacrifices (at weeks 6 and 13) without increasing the number of animals. Additionally, a subset of animals was assigned to a recovery group and stopped treatment at week 13. Therefore, the number of animals left at study termination was significantly lower than recommended for a chronic duration study.
Domain 5: Outcome Assessment				
	Metric 16:	Outcome Assessment Methodology	High	The outcome methodology was sensitive and appropriate for the outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	High	Outcome assessment was consistent across all animals.
	Metric 18:	Sampling Adequacy	High	Sampling was adequate in that no mortality was observed and all animals designated for evaluation at each specific timepoint were used. In some cases, the numbers were low (e.g., as few as 5 animals). This was sufficient to conduct statistical analysis, but, for some endpoints like histopathology, lacked statistical power.
	Metric 19:	Blinding of Assessors	N/A	Blinding was not reported; however, the endpoints were simple measures, not subjective, or consisted of clinical signs, gross observations or initial histopathology, and blinding is not required.
	Metric 20:	Negative Control Response	Medium	Significant differences were observed between untreated and vehicle controls for female urine volume and specific gravity (at week 12)
Domain 6: Confounding / Variable Control				
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	Most information to assess potential confounding was provided. The study did not report whether measures were taken to prevent the possibility of oral exposures, which could impact the study results.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	There was no information either to support or dismiss the suggestion that there were differences among groups in animal attrition or health outcomes unrelated to exposure (e.g., infection) that could influence the outcome assessment.
	Metric 23:	Data Presentation and Analysis	High	Statistical analysis was appropriate, or data were provided to conduct independent statistical analysis.
	Metric 24:	Reporting of Data	High	Data are fully reported.

Additional Comments: None

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Study Citation:	IFF, (n.d.). Twenty-six week subacute dermal toxicity study in rats. Book 1 of 4. Galaxolide 50. (sanitized).
Health Outcome(s):	Renal/Kidney
Reported Health Effect(s):	Renal/Kidney: Kidney weight, gross necropsy, histology on kidney and urinary bladder; serum glucose and BUN; urinalysis (color, pH, specific gravity, glucose, ketones and blood)
Duration:	Subchronic (>30-91 days) Up to 13 weeks (interim sacrifices)
Chemical:	HHCB- Parent compound
HERO ID:	8785658

Domain	Metric	Rating	Comments
Overall Quality Determination		**High**	

Study Citation:	IFF, (n.d.). Twenty-six week subacute dermal toxicity study in rats. Book 1 of 4. Galaxolide 50. (sanitized).
Health Outcome(s):	Neurological/Behavioral
Reported Health Effect(s):	Neurological/Behavioral: Brain weight and histology, gross necropsy,Assessment of startle response, limb rotation, righting reflex, locomotor activity, tremors, alley progression, ataxia, and grip strength
Duration:	Subchronic (>30-91 days) Up to 13 weeks (interim sacrifices)
Chemical:	HHCB- Parent compound
HERO ID:	8785658

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The test substance was identified as Galaxolide 50. This is a common tradename for the chemical 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8,-hexamethyl-cyclopenta[g]benzopyran or HHCB.
	Metric 2:	Test Substance Source	High	The source of the test substance was International Flavours and Fragrances, Inc., New York, NY. The Batch was not reported.
	Metric 3:	Test Substance Purity	Low	The purity of the test substance was not reported.
Domain 2: Test Design				
	Metric 4:	Negative and Vehicle Controls	High	Negative control groups were appropriate. The study included an untreated control group and a vehicle control group.
	Metric 5:	Positive Controls	N/A	A positive control group is not required for this study type.
	Metric 6:	Randomized Allocation of Animals	Medium	Animals were randomly allocated into study groups using a computer-generated randomization procedure. The study text also noted that animals were randomly selected for the interim sacrifices and for assignment to the recovery group.
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and Storage of Test Substance	Medium	Storage information of the test substance was not reported, but the test substance was prepared fresh weekly by dissolving appropriate amount of test material in 95% ethanol. The ethanol was warmed in a water bath to facilitate dissolving.
	Metric 8:	Consistency of Exposure Administration	Low	The test substance concentration in ethanol (10% w/v) was consistent across groups; however, the volume applied to the skin varied to achieve the desired dose. For example, the 200 mg/kg/day group received 2.0 ml/kg/day of test substance solution, whereas the 50 mg/kg/day group received 0.5 ml/kg/day. The vehicle control group received a volume equal to the maximum volume administered (2 ml/kg/day).
	Metric 9:	Reporting of Doses/Concentrations	Medium	Nominal doses were reported. Concentrations of the test substance were not analytically verified, but this is unlikely to have a substantial impact on the study results.
	Metric 10:	Exposure Frequency and Duration	High	The exposure frequency and duration were appropriate for the study's aim.
	Metric 11:	Number of Exposure Groups and Dose/Concentration Spacing	Medium	The doses and dose-spacing were sufficient for identification of a study-wide NOAEL and LOAEL following 26 weeks of exposure. The dose selections were not justified by the study authors.
	Metric 12:	Exposure Route and Method	Low	One part of the study text indicated that the test substance was applied to clipped skin via syringe. Later, it was reported that the test substance was applied to clipped backs of animals by gentle inunction. The test substance was not covered; therefore test substance may have rubbed off, or possibly been licked by animals. The test substance was dissolved in ethanol; ethanol would have likely evaporated from the skin.
Domain 4: Test Animals				

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Study Citation:	IFF, (n.d.). Twenty-six week subacute dermal toxicity study in rats. Book 1 of 4. Galaxolide 50. (sanitized).
Health Outcome(s):	Neurological/Behavioral
Reported Health Effect(s):	Neurological/Behavioral: Brain weight and histology, gross necropsy,Assessment of startle response, limb rotation, righting reflex, locomotor activity, tremors, alley progression, ataxia, and grip strength
Duration:	Subchronic (>30-91 days) Up to 13 weeks (interim sacrifices)
Chemical:	HHCB- Parent compound
HERO ID:	8785658

Domain	Metric		Rating	Comments
	Metric 13:	Test Animal Characteristics	High	The sex, age, starting body weight, strain, species and source were reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Medium	Light/dark cycle, number of animals/cage and diet/water availability were reported. Animals were placed in temperature- and humidity-controlled room (details not reported).
	Metric 15:	Number of Animals per Group	Low	The initial number of animals/group included 15 males and 35 females/group. For a chronic duration study, OECD guidelines recommend 20/sex/group. According to OECD guidelines, if interim sacrifices are planned, the number of animals should be increased by the number to be sacrificed before study completion. This study included two interim sacrifices (at weeks 6 and 13) without increasing the number of animals. Additionally, a subset of animals was assigned to a recovery group and stopped treatment at week 13. Therefore, the number of animals left at study termination was significantly lower than recommended for a chronic duration study.
Domain 5: Outcome Assessment				
	Metric 16:	Outcome Assessment Methodology	High	The outcome methodology was sensitive and appropriate for the outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	High	Outcome assessment was consistent across all animals.
	Metric 18:	Sampling Adequacy	High	Sampling was adequate in that no mortality was observed and all animals designated for evaluation at each specific timepoint were used. In some cases, the numbers were low (e.g., as few as 5 animals). This was sufficient to conduct statistical analysis, but, for some endpoints like histopathology, lacked statistical power.
	Metric 19:	Blinding of Assessors	High	Neurobehavioral assessments were conducted blinded. Blinding was not reported for brain weight or initial histology.
	Metric 20:	Negative Control Response	High	Negative control responses were appropriate for the outcomes of interest.
Domain 6: Confounding / Variable Control				
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	Most information to assess potential confounding was provided. The study did not report whether measures were taken to prevent the possibility of oral exposures, which could impact the study results.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	There was no information either to support or dismiss the suggestion that there were differences among groups in animal attrition or health outcomes unrelated to exposure (e.g., infection) that could influence the outcome assessment.
	Metric 23:	Data Presentation and Analysis	High	Statistical analysis was appropriate, or data were provided to conduct independent statistical analysis.
	Metric 24:	Reporting of Data	High	Data are fully reported.

Additional Comments: None

Overall Quality Determination High

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Study Citation:	IFF, (n.d.). Twenty-six week subacute dermal toxicity study in rats. Book 1 of 4. Galaxolide 50. (sanitized).
Health Outcome(s):	Neurological/Behavioral
Reported Health Effect(s):	Neurological/Behavioral: Brain weight and histology, gross necropsy,Assessment of startle response, limb rotation, righting reflex, locomotor activity, tremors, alley progression, ataxia, and grip strength
Duration:	Subchronic (>30-91 days) Up to 13 weeks (interim sacrifices)
Chemical:	HHCB- Parent compound
HERO ID:	8785658

Domain	Metric	Rating	Comments

Study Citation:	IFF, (n.d.). Twenty-six week subacute dermal toxicity study in rats. Book 1 of 4. Galaxolide 50. (sanitized).
Health Outcome(s):	Thyroid; Lung/Respiratory; Ocular/Sensory; Skin/Connective Tissue; Gastrointestinal; Endocrine (Endocrine);
Reported Health Effect(s):	Thyroid: Thyroid histology; Lung/Respiratory: Gross necropsy and Histology on lungs and trachea; Ocular/Sensory: Gross necropsy and Histology of the eye; Skin/Connective Tissue: Gross necropsy and Histology of the skin; Gastrointestinal: Histology on large intestine, salivary gland, small intestine, stomach, esophagus; Endocrine (Endocrine): Histology on adrenals, pancreas, and pituitary;
Duration:	Subchronic (>30-91 days) Up to 13 weeks (interim sacrifices)
Chemical:	HHCB- Parent compound
HERO ID:	8785658

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	All Outcomes: The test substance was identified as Galaxolide 50. This is a common tradename for the chemical 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8,-hexamethyl-cyclopenta[g]benzopyran or HHCB.
	Metric 2:	Test Substance Source	High	All Outcomes: The source of the test substance was International Flavours and Fragrances, Inc., New York, NY. The Batch was not reported.
	Metric 3:	Test Substance Purity	Low	All Outcomes: The purity of the test substance was not reported.
Domain 2: Test Design				
	Metric 4:	Negative and Vehicle Controls	High	All Outcomes: Negative control groups were appropriate. The study included an untreated control group and a vehicle control group.
	Metric 5:	Positive Controls	N/A	All Outcomes: A positive control group is not required for this study type.
	Metric 6:	Randomized Allocation of Animals	Medium	All Outcomes: Animals were randomly allocated into study groups using a computer-generated randomization procedure. The study text also noted that animals were randomly selected for the interim sacrifices and for assignment to the recovery group.
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and Storage of Test Substance	Medium	All Outcomes: Storage information of the test substance was not reported, but the test substance was prepared fresh weekly by dissolving appropriate amount of test material in 95% ethanol. The ethanol was warmed in a water bath to facilitate dissolving.
	Metric 8:	Consistency of Exposure Administration	Low	All Outcomes: The test substance concentration in ethanol (10% w/v) was consistent across groups; however, the volume applied to the skin varied to achieve the desired dose. For example, the 200 mg/kg/day group received 2.0 ml/kg/day of test substance solution, whereas the 50 mg/kg/day group received 0.5 ml/kg/day. The vehicle control group received a volume equal to the maximum volume administered (2 ml/kg/day).
	Metric 9:	Reporting of Doses/Concentrations	Medium	All Outcomes: Nominal doses were reported. Concentrations of the test substance were not analytically verified, but this is unlikely to have a substantial impact on the study results.
	Metric 10:	Exposure Frequency and Duration	High	All Outcomes: The exposure frequency and duration were appropriate for the study's aim.
	Metric 11:	Number of Exposure Groups and Dose/Concentration Spacing	Medium	All Outcomes: The doses and dose-spacing were sufficient for identification of a study-wide NOAEL and LOAEL following 26 weeks of exposure. The dose selections were not justified by the study authors.

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Study Citation:	IFF, (n.d.). Twenty-six week subacute dermal toxicity study in rats. Book 1 of 4. Galaxolide 50. (sanitized).
Health Outcome(s):	Thyroid; Lung/Respiratory; Ocular/Sensory; Skin/Connective Tissue; Gastrointestinal; Endocrine (Endocrine);
Reported Health Effect(s):	Thyroid: Thyroid histology; Lung/Respiratory: Gross necropsy and Histology on lungs and trachea; Ocular/Sensory: Gross necropsy and Histology of the eye; Skin/Connective Tissue: Gross necropsy and Histology of the skin; Gastrointestinal: Histology on large intestine, salivary gland, small intestine, stomach, esophagus; Endocrine (Endocrine): Histology on adrenals, pancreas, and pituitary;
Duration:	Subchronic (>30-91 days) Up to 13 weeks (interim sacrifices)
Chemical:	HHCB- Parent compound
HERO ID:	8785658

Domain	Metric		Rating	Comments
	Metric 12:	Exposure Route and Method	Low	All Outcomes: One part of the study text indicated that the test substance was applied to clipped skin via syringe. Later, it was reported that the test substance was applied to clipped backs of animals by gentle inunction. The test substance was not covered; therefore test substance may have rubbed off, or possibly been licked by animals. The test substance was dissolved in ethanol; ethanol would have likely evaporated from the skin.
Domain 4: Test Animals				
	Metric 13:	Test Animal Characteristics	High	All Outcomes: The sex, age, starting body weight, strain, species and source were reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Medium	All Outcomes: Light/dark cycle, number of animals/cage and diet/water availability were reported. Animals were placed in temperature- and humidity-controlled room (details not reported).
	Metric 15:	Number of Animals per Group	Low	All Outcomes: The initial number of animals/group included 15 males and 35 females/group. For a chronic duration study, OECD guidelines recommend 20/sex/group. According to OECD guidelines, if interim sacrifices are planned, the number of animals should be increased by the number to be sacrificed before study completion. This study included two interim sacrifices (at weeks 6 and 13) without increasing the number of animals. Additionally, a subset of animals was assigned to a recovery group and stopped treatment at week 13. Therefore, the number of animals left at study termination was significantly lower than recommended for a chronic duration study.
Domain 5: Outcome Assessment				
	Metric 16:	Outcome Assessment Methodology	High	All Outcomes: The outcome methodology was sensitive and appropriate for the outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	High	All Outcomes: Outcome assessment was consistent across all animals.
	Metric 18:	Sampling Adequacy	Medium	All Outcomes: Sampling was adequate in that no mortality was observed and all animals designated for evaluation at each specific timepoint were used. In some cases, the numbers were low (e.g., as few as 5 animals). This was sufficient to conduct statistical analysis, but, for some endpoints like histopathology, lacked statistical power.
	Metric 19:	Blinding of Assessors	N/A	All Outcomes: Blinding was not reported; however, the endpoints were simple measures, not subjective, or consisted of clinical signs, gross observations or initial histopathology, and blinding is not required.
	Metric 20:	Negative Control Response	High	All Outcomes: Negative control responses were appropriate for the outcomes of interest.
Domain 6: Confounding / Variable Control				

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Study Citation:	IFF, (n.d.). Twenty-six week subacute dermal toxicity study in rats. Book 1 of 4. Galaxolide 50. (sanitized).
Health Outcome(s):	Thyroid; Lung/Respiratory; Ocular/Sensory; Skin/Connective Tissue; Gastrointestinal; Endocrine (Endocrine);
Reported Health Effect(s):	Thyroid: Thyroid histology; Lung/Respiratory: Gross necropsy and Histology on lungs and trachea; Ocular/Sensory: Gross necropsy and Histology of the eye; Skin/Connective Tissue: Gross necropsy and Histology of the skin; Gastrointestinal: Histology on large intestine, salivary gland, small intestine, stomach, esophagus; Endocrine (Endocrine): Histology on adrenals, pancreas, and pituitary;
Duration:	Subchronic (>30-91 days) Up to 13 weeks (interim sacrifices)
Chemical:	HHCB- Parent compound
HERO ID:	8785658

Domain	Metric		Rating	Comments
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	All Outcomes: Most information to assess potential confounding was provided. The study did not report whether measures were taken to prevent the possibility of oral exposures, which could impact the study results.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	All Outcomes: There was no information either to support or dismiss the suggestion that there were differences among groups in animal attrition or health outcomes unrelated to exposure (e.g., infection) that could influence the outcome assessment.
	Metric 23:	Data Presentation and Analysis	High	All Outcomes: Statistical analysis was appropriate, or data were provided to conduct independent statistical analysis.
	Metric 24:	Reporting of Data	High	All Outcomes: Data are fully reported.

Additional Comments: None

Overall Quality Determination High

Study Citation:	IFF, (n.d.). Twenty-six week subacute dermal toxicity study in rats. Book 1 of 4. Galaxolide 50. (sanitized).
Health Outcome(s):	Mortality; Clinical signs (Clinical signs); Nutritional/Metabolic; Renal/Kidney; Hepatic/Liver; Reproductive/Developmental; Cardiovascular; Immune/Hematological;
Reported Health Effect(s):	Mortality: Mortality; Clinical signs (Clinical signs): Clinical signs of toxicity; Nutritional/Metabolic: Body weight and food intake; Renal/Kidney: Kidney weight, gross necropsy, histology on kidney and urinary bladder; serum glucose and BUN; urinalysis (color, pH, specific gravity, glucose, ketones and blood); Hepatic/Liver: Liver weight, gross necropsy, and histology; serum SGPT, SGPT and SAP levels; Reproductive/Developmental: Weight, gross necropsy, and histology of ovaries and uterus, or testes.; Cardiovascular: Gross necropsy; Heart weight and histology; Immune/Hematological: Gross necropsy; Histology on mesenteric lymph nodes and spleen; Hematology (RBC, WBC, differential WBC, hematocrit and hemoglobin);
Duration:	Chronic (>91 days) 26-weeks
Chemical:	HHCB- Parent compound
HERO ID:	8785658

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	All Outcomes: The test substance was identified as Galaxolide 50. This is a common tradename for the chemical 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8,-hexamethylcyclopenta[g]benzopyran or HHCB.
	Metric 2:	Test Substance Source	High	All Outcomes: The source of the test substance was International Flavours and Fragrances, Inc., New York, NY. The Batch was not reported.
	Metric 3:	Test Substance Purity	Low	All Outcomes: The purity of the test substance was not reported.
Domain 2: Test Design				
	Metric 4:	Negative and Vehicle Controls	High	All Outcomes: Negative control groups were appropriate. The study included an untreated control group and a vehicle control group.
	Metric 5:	Positive Controls	N/A	All Outcomes: A positive control group is not required for this study type.
	Metric 6:	Randomized Allocation of Animals	Medium	All Outcomes: Animals were randomly allocated into study groups using a computer-generated randomization procedure. The study text also noted that animals were randomly selected for the interim sacrifices and for assignment to the recovery group.
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and Storage of Test Substance	Medium	All Outcomes: Storage information of the test substance was not reported, but the test substance was prepared fresh weekly by dissolving appropriate amount of test material in 95% ethanol. The ethanol was warmed in a water bath to facilitate dissolving.
	Metric 8:	Consistency of Exposure Administration	Low	All Outcomes: The test substance concentration in ethanol (10% w/v) was consistent across groups; however, the volume applied to the skin varied to achieve the desired dose. For example, the 200 mg/kg/day group received 2.0 ml/kg/day of test substance solution, whereas the 50 mg/kg/day group received 0.5 ml/kg/day. The vehicle control group received a volume equal to the maximum volume administered (2 ml/kg/day).
	Metric 9:	Reporting of Doses/Concentrations	Medium	All Outcomes: Nominal doses were reported. Concentrations of the test substance were not analytically verified, but this is unlikely to have a substantial impact on the study results.
	Metric 10:	Exposure Frequency and Duration	High	All Outcomes: The exposure frequency and duration were appropriate for the study's aim.
	Metric 11:	Number of Exposure Groups and Dose/Concentration Spacing	Medium	All Outcomes: The doses and dose-spacing were sufficient for identification of a study-wide NOAEL and LOAEL following 26 weeks of exposure. The dose selections were not justified by the study authors.

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Study Citation:	IFF, (n.d.). Twenty-six week subacute dermal toxicity study in rats. Book 1 of 4. Galaxolide 50. (sanitized).
Health Outcome(s):	Mortality; Clinical signs (Clinical signs); Nutritional/Metabolic; Renal/Kidney; Hepatic/Liver; Reproductive/Developmental; Cardiovascular; Immune/Hematological;
Reported Health Effect(s):	Mortality: Mortality; Clinical signs (Clinical signs): Clinical signs of toxicity; Nutritional/Metabolic: Body weight and food intake; Renal/Kidney: Kidney weight, gross necropsy, histology on kidney and urinary bladder; serum glucose and BUN; urinalysis (color, pH, specific gravity, glucose, ketones and blood); Hepatic/Liver: Liver weight, gross necropsy, and histology; serum SGPT, SGPT and SAP levels; Reproductive/Developmental: Weight, gross necropsy, and histology of ovaries and uterus, or testes.; Cardiovascular: Gross necropsy; Heart weight and histology; Immune/Hematological: Gross necropsy; Histology on mesenteric lymph nodes and spleen; Hematology (RBC, WBC, differential WBC, hematocrit and hemoglobin);
Duration:	Chronic (>91 days) 26-weeks
Chemical:	HHCB- Parent compound
HERO ID:	8785658

Domain		Metric	Rating	Comments
	Metric 12:	Exposure Route and Method	Low	All Outcomes: One part of the study text indicated that the test substance was applied to clipped skin via syringe. Later, it was reported that the test substance was applied to clipped backs of animals by gentle inunction. The test substance was not covered; therefore test substance may have rubbed off, or possibly been licked by animals. The test substance was dissolved in ethanol; ethanol would have likely evaporated from the skin.
Domain 4: Test Animals				
	Metric 13:	Test Animal Characteristics	High	All Outcomes: The sex, age, starting body weight, strain, species and source were reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Medium	All Outcomes: Light/dark cycle, number of animals/cage and diet/water availability were reported. Animals were placed in temperature- and humidity-controlled room (details not reported).
	Metric 15:	Number of Animals per Group	Low	All Outcomes: The initial number of animals/group included 15 males and 35 females/group. For a chronic duration study, OECD guidelines recommend 20/sex/group. According to OECD guidelines, if interim sacrifices are planned, the number of animals should be increased by the number to be sacrificed before study completion. This study included two interim sacrifices (at weeks 6 and 13) without increasing the number of animals. Additionally, a subset of animals was assigned to a recovery group and stopped treatment at week 13. Therefore, the number of animals left at study termination was significantly lower than recommended for a chronic duration study.
Domain 5: Outcome Assessment				
	Metric 16:	Outcome Assessment Methodology	High	All Outcomes: The outcome methodology was sensitive and appropriate for the outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	High	All Outcomes: Outcome assessment was consistent across all animals.
	Metric 18:	Sampling Adequacy	High	All Outcomes: Sampling was adequate in that no mortality was observed and all animals designated for evaluation at each specific timepoint were used. In some cases, the numbers were low (e.g., as few as 5 animals). This was sufficient to conduct statistical analysis, but, for some endpoints like histopathology, lacked statistical power.
	Metric 19:	Blinding of Assessors	N/A	All Outcomes: Blinding was not reported; however, the endpoints were simple measures, not subjective, or consisted of clinical signs, gross observations or initial histopathology, and blinding is not required.
	Metric 20:	Negative Control Response	High	All Outcomes: Negative control responses were appropriate for the outcomes of interest.

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Study Citation:	IFF, (n.d.). Twenty-six week subacute dermal toxicity study in rats. Book 1 of 4. Galaxolide 50. (sanitized).
Health Outcome(s):	Mortality; Clinical signs (Clinical signs); Nutritional/Metabolic; Renal/Kidney; Hepatic/Liver; Reproductive/Developmental; Cardiovascular; Immune/Hematological;
Reported Health Effect(s):	Mortality: Mortality; Clinical signs (Clinical signs): Clinical signs of toxicity; Nutritional/Metabolic: Body weight and food intake; Renal/Kidney: Kidney weight, gross necropsy, histology on kidney and urinary bladder; serum glucose and BUN; urinalysis (color, pH, specific gravity, glucose, ketones and blood); Hepatic/Liver: Liver weight, gross necropsy, and histology; serum SGPT, SGPT and SAP levels; Reproductive/Developmental: Weight, gross necropsy, and histology of ovaries and uterus, or testes.; Cardiovascular: Gross necropsy; Heart weight and histology; Immune/Hematological: Gross necropsy; Histology on mesenteric lymph nodes and spleen; Hematology (RBC, WBC, differential WBC, hematocrit and hemoglobin);
Duration:	Chronic (>91 days) 26-weeks
Chemical:	HHCB- Parent compound
HERO ID:	8785658

Domain	Metric	Rating	Comments
Domain 6: Confounding / Variable Control			
	Metric 21: Confounding Variables in Test Design and Procedures	Medium	All Outcomes: Most information to assess potential confounding was provided. The study did not report whether measures were taken to prevent the possibility of oral exposures, which could impact the study results.
	Metric 22: Health Outcomes Unrelated to Exposure	Medium	All Outcomes: There was no information either to support or dismiss the suggestion that there were differences among groups in animal attrition or health outcomes unrelated to exposure (e.g., infection) that could influence the outcome assessment.
	Metric 23: Data Presentation and Analysis	High	All Outcomes: Statistical analysis was appropriate, or data were provided to conduct independent statistical analysis.
	Metric 24: Reporting of Data	High	All Outcomes: Data are fully reported.

Additional Comments: None

Overall Quality Determination High

Study Citation:	IFF, (n.d.). Twenty-six week subacute dermal toxicity study in rats. Book 1 of 4. Galaxolide 50. (sanitized).
Health Outcome(s):	Neurological/Behavioral
Reported Health Effect(s):	Neurological/Behavioral: Brain weight and histology, gross necropsy,Assessment of startle response, limb rotation, righting reflex, locomotor activity, tremors, alley progression, ataxia, and grip strength
Duration:	Chronic (>91 days) 26-weeks
Chemical:	HHCB- Parent compound
HERO ID:	8785658

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The test substance was identified as Galaxolide 50. This is a common tradename for the chemical 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8,-hexamethyl-cyclopenta[g]benzopyran or HHCB.
	Metric 2:	Test Substance Source	High	The source of the test substance was International Flavours and Fragrances, Inc., New York, NY. The Batch was not reported.
	Metric 3:	Test Substance Purity	Low	The purity of the test substance was not reported.
Domain 2: Test Design				
	Metric 4:	Negative and Vehicle Controls	High	Negative control groups were appropriate. The study included an untreated control group and a vehicle control group.
	Metric 5:	Positive Controls	N/A	A positive control group is not required for this study type.
	Metric 6:	Randomized Allocation of Animals	Medium	Animals were randomly allocated into study groups using a computer-generated randomization procedure. The study text also noted that animals were randomly selected for the interim sacrifices and for assignment to the recovery group.
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and Storage of Test Substance	Medium	Storage information of the test substance was not reported, but the test substance was prepared fresh weekly by dissolving appropriate amount of test material in 95% ethanol. The ethanol was warmed in a water bath to facilitate dissolving.
	Metric 8:	Consistency of Exposure Administration	Low	The test substance concentration in ethanol (10% w/v) was consistent across groups; however, the volume applied to the skin varied to achieve the desired dose. For example, the 200 mg/kg/day group received 2.0 ml/kg/day of test substance solution, whereas the 50 mg/kg/day group received 0.5 ml/kg/day. The vehicle control group received a volume equal to the maximum volume administered (2 ml/kg/day).
	Metric 9:	Reporting of Doses/Concentrations	Medium	Nominal doses were reported. Concentrations of the test substance were not analytically verified, but this is unlikely to have a substantial impact on the study results.
	Metric 10:	Exposure Frequency and Duration	High	The exposure frequency and duration were appropriate for the study's aim.
	Metric 11:	Number of Exposure Groups and Dose/Concentration Spacing	Medium	The doses and dose-spacing were sufficient for identification of a study-wide NOAEL and LOAEL following 26 weeks of exposure. The dose selections were not justified by the study authors.
	Metric 12:	Exposure Route and Method	Low	One part of the study text indicated that the test substance was applied to clipped skin via syringe. Later, it was reported that the test substance was applied to clipped backs of animals by gentle inunction. The test substance was not covered; therefore test substance may have rubbed off, or possibly been licked by animals. The test substance was dissolved in ethanol; ethanol would have likely evaporated from the skin.
Domain 4: Test Animals				

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Study Citation:	IFF, (n.d.). Twenty-six week subacute dermal toxicity study in rats. Book 1 of 4. Galaxolide 50. (sanitized).
Health Outcome(s):	Neurological/Behavioral
Reported Health Effect(s):	Neurological/Behavioral: Brain weight and histology, gross necropsy,Assessment of startle response, limb rotation, righting reflex, locomotor activity, tremors, alley progression, ataxia, and grip strength
Duration:	Chronic (>91 days) 26-weeks
Chemical:	HHCB- Parent compound
HERO ID:	8785658

Domain	Metric		Rating	Comments
	Metric 13:	Test Animal Characteristics	High	The sex, age, starting body weight, strain, species and source were reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Medium	Light/dark cycle, number of animals/cage and diet/water availability were reported. Animals were placed in temperature- and humidity-controlled room (details not reported).
	Metric 15:	Number of Animals per Group	Low	The initial number of animals/group included 15 males and 35 females/group. For a chronic duration study, OECD guidelines recommend 20/sex/group. According to OECD guidelines, if interim sacrifices are planned, the number of animals should be increased by the number to be sacrificed before study completion. This study included two interim sacrifices (at weeks 6 and 13) without increasing the number of animals. Additionally, a subset of animals was assigned to a recovery group and stopped treatment at week 13. Therefore, the number of animals left at study termination was significantly lower than recommended for a chronic duration study.
Domain 5: Outcome Assessment				
	Metric 16:	Outcome Assessment Methodology	High	The outcome methodology was sensitive and appropriate for the outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	High	Outcome assessment was consistent across all animals.
	Metric 18:	Sampling Adequacy	High	Sampling was adequate in that no mortality was observed and all animals designated for evaluation at each specific timepoint were used. In some cases, the numbers were low (e.g., as few as 5 animals). This was sufficient to conduct statistical analysis, but, for some endpoints like histopathology, lacked statistical power.
	Metric 19:	Blinding of Assessors	High	Neurobehavioral assessments were conducted blinded. Blinding was not reported for brain weight or initial histology.
	Metric 20:	Negative Control Response	High	Negative control responses were appropriate for the outcomes of interest.
Domain 6: Confounding / Variable Control				
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	Most information to assess potential confounding was provided. The study did not report whether measures were taken to prevent the possibility of oral exposures, which could impact the study results.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	There was no information either to support or dismiss the suggestion that there were differences among groups in animal attrition or health outcomes unrelated to exposure (e.g., infection) that could influence the outcome assessment.
	Metric 23:	Data Presentation and Analysis	High	Statistical analysis was appropriate, or data were provided to conduct independent statistical analysis.
	Metric 24:	Reporting of Data	High	Data are fully reported.

Additional Comments: None

Overall Quality Determination High

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Study Citation:	IFF, (n.d.). Twenty-six week subacute dermal toxicity study in rats. Book 1 of 4. Galaxolide 50. (sanitized).
Health Outcome(s):	Neurological/Behavioral
Reported Health Effect(s):	Neurological/Behavioral: Brain weight and histology, gross necropsy,Assessment of startle response, limb rotation, righting reflex, locomotor activity, tremors, alley progression, ataxia, and grip strength
Duration:	Chronic (>91 days) 26-weeks
Chemical:	HHCB- Parent compound
HERO ID:	8785658

Domain	Metric	Rating	Comments

Study Citation:	IFF, (n.d.). Twenty-six week subacute dermal toxicity study in rats. Book 1 of 4. Galaxolide 50. (sanitized).
Health Outcome(s):	Endocrine (Endocrine); Gastrointestinal; Skin/Connective Tissue; Ocular/Sensory; Lung/Respiratory; Thyroid (Endocrine);
Reported Health Effect(s):	Endocrine (Endocrine): Histology on adrenals, pancreas, and pituitary; Gastrointestinal: Histology on large intestine, salivary gland, small intestine, stomach, esophagus; Skin/Connective Tissue: Gross necropsy and Histology of the skin; Ocular/Sensory: Gross necropsy and Histology of the eye; Lung/Respiratory: Gross necropsy and Histology on lungs and trachea; Thyroid (Endocrine): Thyroid histology;
Duration:	Chronic (>91 days) 26-weeks
Chemical:	HHCB- Parent compound
HERO ID:	8785658

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	All Outcomes: The test substance was identified as Galaxolide 50. This is a common tradename for the chemical 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8,-hexamethyl-cyclopenta[g]benzopyran or HHCB.
	Metric 2:	Test Substance Source	High	All Outcomes: The source of the test substance was International Flavours and Fragrances, Inc., New York, NY. The Batch was not reported.
	Metric 3:	Test Substance Purity	Low	All Outcomes: The purity of the test substance was not reported.
Domain 2: Test Design				
	Metric 4:	Negative and Vehicle Controls	High	All Outcomes: Negative control groups were appropriate. The study included an untreated control group and a vehicle control group.
	Metric 5:	Positive Controls	N/A	All Outcomes: A positive control group is not required for this study type.
	Metric 6:	Randomized Allocation of Animals	Medium	All Outcomes: Animals were randomly allocated into study groups using a computer-generated randomization procedure. The study text also noted that animals were randomly selected for the interim sacrifices and for assignment to the recovery group.
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and Storage of Test Substance	Medium	All Outcomes: Storage information of the test substance was not reported, but the test substance was prepared fresh weekly by dissolving appropriate amount of test material in 95% ethanol. The ethanol was warmed in a water bath to facilitate dissolving.
	Metric 8:	Consistency of Exposure Administration	Low	All Outcomes: The test substance concentration in ethanol (10% w/v) was consistent across groups; however, the volume applied to the skin varied to achieve the desired dose. For example, the 200 mg/kg/day group received 2.0 ml/kg/day of test substance solution, whereas the 50 mg/kg/day group received 0.5 ml/kg/day. The vehicle control group received a volume equal to the maximum volume administered (2 ml/kg/day).
	Metric 9:	Reporting of Doses/Concentrations	Medium	All Outcomes: Nominal doses were reported. Concentrations of the test substance were not analytically verified, but this is unlikely to have a substantial impact on the study results.
	Metric 10:	Exposure Frequency and Duration	High	All Outcomes: The exposure frequency and duration were appropriate for the study's aim.
	Metric 11:	Number of Exposure Groups and Dose/Concentration Spacing	Medium	All Outcomes: The doses and dose-spacing were sufficient for identification of a study-wide NOAEL and LOAEL following 26 weeks of exposure. The dose selections were not justified by the study authors.

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Study Citation:	IFF, (n.d.). Twenty-six week subacute dermal toxicity study in rats. Book 1 of 4. Galaxolide 50. (sanitized).
Health Outcome(s):	Endocrine (Endocrine); Gastrointestinal; Skin/Connective Tissue; Ocular/Sensory; Lung/Respiratory; Thyroid (Endocrine);
Reported Health Effect(s):	Endocrine (Endocrine): Histology on adrenals, pancreas, and pituitary; Gastrointestinal: Histology on large intestine, salivary gland, small intestine, stomach, esophagus; Skin/Connective Tissue: Gross necropsy and Histology of the skin; Ocular/Sensory: Gross necropsy and Histology of the eye; Lung/Respiratory: Gross necropsy and Histology on lungs and trachea; Thyroid (Endocrine): Thyroid histology;
Duration:	Chronic (>91 days) 26-weeks
Chemical:	HHCB- Parent compound
HERO ID:	8785658

Domain	Metric		Rating	Comments
	Metric 12:	Exposure Route and Method	Low	All Outcomes: One part of the study text indicated that the test substance was applied to clipped skin via syringe. Later, it was reported that the test substance was applied to clipped backs of animals by gentle inunction. The test substance was not covered; therefore test substance may have rubbed off, or possibly been licked by animals. The test substance was dissolved in ethanol; ethanol would have likely evaporated from the skin.
Domain 4: Test Animals				
	Metric 13:	Test Animal Characteristics	High	All Outcomes: The sex, age, starting body weight, strain, species and source were reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Medium	All Outcomes: Light/dark cycle, number of animals/cage and diet/water availability were reported. Animals were placed in temperature- and humidity-controlled room (details not reported).
	Metric 15:	Number of Animals per Group	Low	All Outcomes: The initial number of animals/group included 15 males and 35 females/group. For a chronic duration study, OECD guidelines recommend 20/sex/group. According to OECD guidelines, if interim sacrifices are planned, the number of animals should be increased by the number to be sacrificed before study completion. This study included two interim sacrifices (at weeks 6 and 13) without increasing the number of animals. Additionally, a subset of animals was assigned to a recovery group and stopped treatment at week 13. Therefore, the number of animals left at study termination was significantly lower than recommended for a chronic duration study.
Domain 5: Outcome Assessment				
	Metric 16:	Outcome Assessment Methodology	High	All Outcomes: The outcome methodology was sensitive and appropriate for the outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	High	All Outcomes: Outcome assessment was consistent across all animals.
	Metric 18:	Sampling Adequacy	Medium	All Outcomes: Sampling was adequate in that no mortality was observed and all animals designated for evaluation at each specific timepoint were used. In some cases, the numbers were low (e.g., as few as 5 animals). This was sufficient to conduct statistical analysis, but, for some endpoints like histopathology, lacked statistical power.
	Metric 19:	Blinding of Assessors	N/A	All Outcomes: Blinding was not reported; however, the endpoints were simple measures, not subjective, or consisted of clinical signs, gross observations or initial histopathology, and blinding is not required.
	Metric 20:	Negative Control Response	High	All Outcomes: Negative control responses were appropriate for the outcomes of interest.
Domain 6: Confounding / Variable Control				

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Study Citation:	IFF, (n.d.). Twenty-six week subacute dermal toxicity study in rats. Book 1 of 4. Galaxolide 50. (sanitized).
Health Outcome(s):	Endocrine (Endocrine); Gastrointestinal; Skin/Connective Tissue; Ocular/Sensory; Lung/Respiratory; Thyroid (Endocrine);
Reported Health Effect(s):	Endocrine (Endocrine): Histology on adrenals, pancreas, and pituitary; Gastrointestinal: Histology on large intestine, salivary gland, small intestine, stomach, esophagus; Skin/Connective Tissue: Gross necropsy and Histology of the skin; Ocular/Sensory: Gross necropsy and Histology of the eye; Lung/Respiratory: Gross necropsy and Histology on lungs and trachea; Thyroid (Endocrine): Thyroid histology;
Duration:	Chronic (>91 days) 26-weeks
Chemical:	HHCB- Parent compound
HERO ID:	8785658

Domain	Metric		Rating	Comments
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	All Outcomes: Most information to assess potential confounding was provided. The study did not report whether measures were taken to prevent the possibility of oral exposures, which could impact the study results.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	All Outcomes: There was no information either to support or dismiss the suggestion that there were differences among groups in animal attrition or health outcomes unrelated to exposure (e.g., infection) that could influence the outcome assessment.
	Metric 23:	Data Presentation and Analysis	High	All Outcomes: Statistical analysis was appropriate, or data were provided to conduct independent statistical analysis.
	Metric 24:	Reporting of Data	High	All Outcomes: Data are fully reported.

Additional Comments: None

Overall Quality Determination High

Study Citation:	Argus Research Laboratories, (1997). Initial submission: audited draft report, oral (gavage) developmental toxicity study of (HHCB) in rats with attachments and cover letter dated 5/22/1997.
Health Outcome(s):	Reproductive/Developmental; Mortality; Neurological/Behavioral; Nutritional/Metabolic;
Reported Health Effect(s):	Reproductive/Developmental: No treatment-related abortions or premature deliveries. There were 25, 24, 24 and 21 pregnant dams Caesarean-sectioned on GD 20 in the O (Vehicle), 50, 150 and 500 mg/kg/day dosage groups, respectively.The 500 mg/kg/day dosage group had significantly reduced (p~0.01) fetal body weights, as compared with the control group values. No other Caesarea.n-sectioning and litter parameters were affected by dosages of the test article as high as 500 mg/kg/day.Malformations (incidences of axial skeleton (vertebral/rib) malformations) occurred in one, zero, two and six (ps0.01) fetuses from one, zero, two and five (p~0.01) litters in the four respective dosage groups. The finding was considered to be adverse at 500 mg/kg/day.; Mortality: No treatment-related maternal deaths were reported.; Neurological/Behavioral: The 500 mg/kg/day dosage group had four to nine rats with excess salivation, urine-stained abdominal fur, red or brown substance on the forepaws and alopecia.; Nutritional/Metabolic: Maternal body weight gains were reduced and significantly reduced in the 150 and 500 mg/kg/day dosage groups, respectively. Significant reductions in maternal body weight gains were generally evident in the 500 mg/kg/day dosage group on GDs B through 20.Feed consumption was also reduced for the 150 and 500 mg/kg/day animals.;
Duration:	Reproductive/Developmental 13 days
Chemical:	HHCB- Parent compound
HERO ID:	5431330

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Reproductive/Developmental: ; Mortality: ; Neurological/Behavioral: ; Nutritional/Metabolic:
	Metric 2:	Test Substance Source	Low	All Outcomes: Source not specified.
	Metric 3:	Test Substance Purity	Low	All Outcomes: Purity not specified.
Domain 2: Test Design				
	Metric 4:	Negative and Vehicle Controls	Low	All Outcomes: Individual data and/or summary data were not provided. Only information provided was study author conclusions. Reviewer cannot verify results.
	Metric 5:	Positive Controls	N/A	Reproductive/Developmental: ; Mortality: ; Neurological/Behavioral: ; Nutritional/Metabolic:
	Metric 6:	Randomized Allocation of Animals	Low	All Outcomes: Allocation method not specified.
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and Storage of Test Substance	Low	All Outcomes: Not specified.
	Metric 8:	Consistency of Exposure Administration	High	Reproductive/Developmental: ; Mortality: ; Neurological/Behavioral: ; Nutritional/Metabolic:
	Metric 9:	Reporting of Doses/Concentrations	Medium	All Outcomes: Nominal doses.
	Metric 10:	Exposure Frequency and Duration	High	Reproductive/Developmental: ; Mortality: ; Neurological/Behavioral: ; Nutritional/Metabolic:
	Metric 11:	Number of Exposure Groups and Dose/Concentration Spacing	High	Reproductive/Developmental: ; Mortality: ; Neurological/Behavioral: ; Nutritional/Metabolic:
	Metric 12:	Exposure Route and Method	High	Reproductive/Developmental: ; Mortality: ; Neurological/Behavioral: ; Nutritional/Metabolic:
Domain 4: Test Animals				

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Study Citation:	Argus Research Laboratories, (1997). Initial submission: audited draft report, oral (gavage) developmental toxicity study of (HHCB) in rats with attachments and cover letter dated 5/22/1997.
Health Outcome(s):	Reproductive/Developmental; Mortality; Neurological/Behavioral; Nutritional/Metabolic;
Reported Health Effect(s):	Reproductive/Developmental: No treatment-related abortions or premature deliveries. There were 25, 24, 24 and 21 pregnant dams Caesarean-sectioned on GD 20 in the O (Vehicle), 50, 150 and 500 mg/kg/day dosage groups, respectively.The 500 mg/kg/day dosage group had significantly reduced (p~0.01) fetal body weights, as compared with the control group values. No other Caesarea.n-sectioning and litter parameters were affected by dosages of the test article as high as 500 mg/kg/day.Malformations (incidences of axial skeleton (vertebral/rib) malformations) occurred in one, zero, two and six (ps0.01) fetuses from one, zero, two and five (p~0.01) litters in the four respective dosage groups. The finding was considered to be adverse at 500 mg/kg/day.; Mortality: No treatment-related maternal deaths were reported.; Neurological/Behavioral: The 500 mg/kg/day dosage group had four to nine rats with excess salivation, urine-stained abdominal fur, red or brown substance on the forepaws and alopecia.; Nutritional/Metabolic: Maternal body weight gains were reduced and significantly reduced in the 150 and 500 mg/kg/day dosage groups, respectively. Significant reductions in maternal body weights were generally evident in the 500 mg/kg/day dosage group on GDs B through 20.Feed consumption was also reduced for the 150 and 500 mg/kg/day animals.;
Duration:	Reproductive/Developmental 13 days
Chemical:	HHCB- Parent compound
HERO ID:	5431330

Domain	Metric		Rating	Comments
	Metric 13:	Test Animal Characteristics	Low	All Outcomes: Test animal characteristics not provided.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	All Outcomes: Not specified.
	Metric 15:	Number of Animals per Group		All Outcomes: 25 animals per group.
Domain 5: Outcome Assessment				
	Metric 16:	Outcome Assessment Methodology	High	Reproductive/Developmental: ; Mortality: ; Neurological/Behavioral: ; Nutritional/Metabolic:
	Metric 17:	Consistency of Outcome Assessment	High	Reproductive/Developmental: ; Mortality: ; Neurological/Behavioral: ; Nutritional/Metabolic:
	Metric 18:	Sampling Adequacy	High	Reproductive/Developmental: ; Mortality: ; Neurological/Behavioral: ; Nutritional/Metabolic:
	Metric 19:	Blinding of Assessors	Medium	All Outcomes: Not likely to impact results.
	Metric 20:	Negative Control Response	Low	All Outcomes: Individual data and/or summary data were not provided. Only information provided was study author conclusions. Reviewer cannot verify results.
Domain 6: Confounding / Variable Control				
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	All Outcomes: Individual data and/or summary data were not provided. Only information provided was study author conclusions. Reviewer cannot verify results.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	All Outcomes: Individual data and/or summary data were not provided. Only information provided was study author conclusions. Reviewer cannot verify results.
	Metric 23:	Data Presentation and Analysis	High	Reproductive/Developmental: ; Mortality: ; Neurological/Behavioral: ; Nutritional/Metabolic:
	Metric 24:	Reporting of Data	Low	All Outcomes: Individual data and/or summary data were not provided. Only information provided was study author conclusions. Reviewer cannot verify results.

Additional Comments: Individual data and/or summary data were not provided. Only information provided was study author conclusions. Reviewer cannot verify results.

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Study Citation:	Argus Research Laboratories, (1997). Initial submission: audited draft report, oral (gavage) developmental toxicity study of (HHCB) in rats with attachments and cover letter dated 5/22/1997.
Health Outcome(s):	Reproductive/Developmental; Mortality; Neurological/Behavioral; Nutritional/Metabolic;
Reported Health Effect(s):	Reproductive/Developmental: No treatment-related abortions or premature deliveries. There were 25, 24, 24 and 21 pregnant dams Caesarean-sectioned on GD 20 in the O (Vehicle), 50, 150 and 500 mg/kg/day dosage groups, respectively.The 500 mg/kg/day dosage group had significantly reduced (p~0.01) fetal body weights, as compared with the control group values. No other Caesarea.n-sectioning and litter parameters were affected by dosages of the test article as high as 500 mg/kg/day.Malformations (incidences of axial skeleton (vertebral/rib) malformations) occurred in one, zero, two and six (ps0.01) fetuses from one, zero, two and five (p~0.01) litters in the four respective dosage groups. The finding was considered to be adverse at 500 mg/kg/day.; Mortality: No treatment-related maternal deaths were reported.; Neurological/Behavioral: The 500 mg/kg/day dosage group had four to nine rats with excess salivation, urine-stained abdominal fur, red or brown substance on the forepaws and alopecia.; Nutritional/Metabolic: Maternal body weight gains were reduced and significantly reduced in the 150 and 500 mg/kg/day dosage groups, respectively. Significant reductions in maternal body weights were generally evident in the 500 mg/kg/day dosage group on GDs B through 20.Feed consumption was also reduced for the 150 and 500 mg/kg/day animals.;
Duration:	Reproductive/Developmental 13 days
Chemical:	HHCB- Parent compound
HERO ID:	5431330

Domain	Metric	Rating	Comments
Overall Quality Determination		**Low**	

Study Citation:	Christian, M.S., Parker, R.M., Hoberman, A.M., Diener, R.M., Api, A.M. (1999). Developmental toxicity studies of four fragrances in rats. Toxicology Letters 111(1-2):169-174.
Health Outcome(s):	Reproductive/Developmental
Reported Health Effect(s):	Reproductive/Developmental: maternal toxicity effects (clinical signs, reduced weight gain and feed consumption) and developmental toxicity effects (axial skeletal malformations)
Duration:	Reproductive/Developmental Prenatal
Chemical:	HHCB- Parent compound
HERO ID:	4955361

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Test substance was identified by nomenclature and CASRN
	Metric 2:	Test Substance Source	Low	Source and purity were not reported, but a purity > 95% is reported for this study in the 2014 OPPT risk evaluation and study authors report that chemical analysis verified concentration and stability of the prepared solutions.
	Metric 3:	Test Substance Purity	Low	No mention of purity of test substance.
Domain 2: Test Design				
	Metric 4:	Negative and Vehicle Controls	High	Control 1 (vehicle only) was concurrent with HHCB
	Metric 5:	Positive Controls	N/A	Positive controls are not needed for this study.
	Metric 6:	Randomized Allocation of Animals	Low	The study did not report how animals were allocated to study groups.
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and Storage of Test Substance	High	Solutions were prepared twice during the treatment period. Chemical analysis verified concentration and stability of the prepared solutions.
	Metric 8:	Consistency of Exposure Administration	Medium	Control and test articles were administered once daily by gavage at a dosage volume of 5 ml/kg, adjusted daily according to individualbody weights recorded immediately before intubation. Because there is not info on body weights, it could not be confirmed that the dosage volume was not excessive.
	Metric 9:	Reporting of Doses/Concentrations	High	Study reported doses/concentrations of test substance used in study.
	Metric 10:	Exposure Frequency and Duration	High	Exposure frequency and duration were explained in the study.
	Metric 11:	Number of Exposure Groups and Dose/Concentration Spacing	High	Dosage-range finding studies in rats were conducted at the following dosage levels for HHCB: 1000, 500, 250 100 mg/kg per day
	Metric 12:	Exposure Route and Method	High	The route and method of exposure were reported and were suited to the test substance.
Domain 4: Test Animals				
	Metric 13:	Test Animal Characteristics	Medium	Starting body weights were not reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	Animal husbandry was reported in the study in detail.
	Metric 15:	Number of Animals per Group	Medium	The number of animals per study group was reported, appropriate for the study type and outcome analysis, and consistent with similar studies.
Domain 5: Outcome Assessment				

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Study Citation:	Christian, M.S., Parker, R.M., Hoberman, A.M., Diener, R.M., Api, A.M. (1999). Developmental toxicity studies of four fragrances in rats. Toxicology Letters 111(1-2):169-174.
Health Outcome(s):	Reproductive/Developmental
Reported Health Effect(s):	Reproductive/Developmental: maternal toxicity effects (clinical signs, reduced weight gain and feed consumption) and developmental toxicity effects (axial skeletal malformations)
Duration:	Reproductive/Developmental Prenatal
Chemical:	HHCB- Parent compound
HERO ID:	4955361

Domain	Metric		Rating	Comments
	Metric 16:	Outcome Assessment Methodology	High	The methodology of the study was appropriate for the endpoints measured.
	Metric 17:	Consistency of Outcome Assessment	High	Protocols were consistent among each study group.
	Metric 18:	Sampling Adequacy	High	Study used adequate sampling for purpose of study.
	Metric 19:	Blinding of Assessors	N/A	There was no need to blind assessors in this study.
	Metric 20:	Negative Control Response	High	Responses from negative control were adequate.
Domain 6: Confounding / Variable Control				
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	Paper identified minor differences between groups.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	There was no information either to support or dismiss the suggestion that there were differences among groups in animal attrition or health outcomes unrelated to exposure that could influence the assessment.
	Metric 23:	Data Presentation and Analysis	High	Statistical methods were explained and sufficient for study.
	Metric 24:	Reporting of Data	Low	Data for exposure-related findings were not shown for each study group, but results were described in the text.

Additional Comments: None

Overall Quality Determination High

Study Citation:	IFF, (2021). Extended one generation reproductive toxicity study (including cohorts 1 and F2 - generation of HHCB by the oral route (dietary admixture) in the rat (OECD 443) (sanitized).
Health Outcome(s):	Reproductive/Developmental; Renal/Kidney; Hepatic/Liver; Endocrine (Endocrine); Neurological/Behavioral; Cardiovascular; Musculoskeletal; Gastrointestinal; Mortality; Nutritional/Metabolic; Nutritional/Metabolic; Immune/Hematological; Thyroid;
Reported Health Effect(s):	Reproductive/Developmental: F0 and F1 (cohort 1a, 1b, and surplus): Organ weights: cervix, epidiymides, ovaries, oviducts (F0 only), prostate, seminal vesicles with coagulating gland, testes and uterus; F0 and F1 (cohort 1a and unscheduled deaths) Histopathology: cervix, ovaries, oviducts, epididymides, mammary gland, prostate, seminal vesicles with coagulating gland, testes, uterus, vagina and vas deferens;F0: estrous cycles, mating performance and fertility, delivery and litter data, sperm qualityF1: mortality, clinical signs, body weights, food consumption, clinical pathology, thyroid hormone levels (PND 4 and 21), anogenital distance, pup number of aerola/nipples, estrous cycles, sexual maturation, sperm quality, macroscopic observations, organ weights and histopathology, splenic lymphocyte subpopulationF1: mating performance and fertility, delivery and litter dataF2 offspring: postnatal development, mortality, clinical signs, body weights, sex, anogenital distance, number of aerola/nipples, organ weights and macroscopy. F2 offspring sacrificed on PND21-23, organ weights (brain, liver, thyroid glands, spleen, thymus); Renal/Kidney: F0 and F1 (cohort 1a): Serum urea, creatinine, albumin, globulin, glucose, and total protein; urinalysis;F0 and F1 (cohort 1a, 1b, and surplus): kidney weight;F0 and F1 (cohort 1a and unscheduled deaths): histopathology on kidney and urinary bladder.; Hepatic/Liver: F0 and F1 (cohort 1a): Serum ALT, AST, ALP, total cholesterol, triglycerides; F0 and F1 (cohort 1a, 1b, and surplus): liver weight F0 and F1 (cohort 1a and unscheduled deaths): histopathology on liver; Endocrine (Endocrine): F0 and F1 (cohort 1a, 1b, and surplus): adrenal gland and pituitary weightF0: pancreas weightF0 and F1 (cohort 1a and unscheduled deaths): histopathology on adrenal and pituitary gland; Neurological/Behavioral: Clinical signs (piloerection); F0 and F1 (cohort 1a, 1b, and surplus): Brain weight; F0 and F1 (cohort 1a and unscheduled death): histopathology on brain, peripheral nerve, and spinal cord; Cardiovascular: F0 and F1 (cohort 1a, 1b, and surplus): Heart weight;F0 and F1 (cohort 1a and unscheduled deaths): histopathology on heart; Musculoskeletal: Clinical signs (malocclusion); F0 and F1 (cohort 1a and unscheduled deaths): Histopathology on bone (sternum) with bone marrow; Gastrointestinal: Clinical signs (hypersalivation, soft or no feces); F0 and F1 (cohort 1a and unscheduled deaths): Histopathology on caecum, colon, duodenum, esophagus, ileum, jejunum, rectum, and stomach; Mortality: F0 and all F1 (adult treated) cohorts: Mortality; Nutritional/Metabolic: F0 and all F1 adult treated cohorts: Body weight and food consumption; Nutritional/Metabolic: F0 and all F1 adult treated cohorts: Body weight and food consumption; Immune/Hematological: F0 and F1 (cohort 1a) Hematology. Fo and F1 (cohort 1a, 1b, and surplus): Spleen and thymus weight F1 (cohort 1a): lymph node weight (mandibular and mesenteric)F0 and F1 (cohort 1a and unscheduled deaths): histopathology on spleen, thymus, and lymph nodes (F1 only)F1 (cohort 1a): splenic lymphocyte population analysis; Thyroid: F0, F1 (cohort 1a, surplus PND4 pups): serum thyroid hormone levels (T4, TSH);F0, F1 (cohort 1a, 1b, and surplus): Thyroid and parathyroid weights; F0 and F1 (cohort 1a and unscheduled deaths, surplus PND4 pups): histopathology on thyroid and parathyroid;;
Duration:	Reproductive/Developmental Extended one generation Repro/Dev study
Chemical:	HHCB- Parent compound
HERO ID:	8785683

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	All Outcomes: The test substance was identified as Galaxolide, undiluted. This is a common tradename for the chemical 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8,-hexamethyl-cyclopenta[g]benzopyran or HHCB. The test substance consisted of 252933-49-6 and 252933-48-5.
	Metric 2:	Test Substance Source	High	All Outcomes: The test substance source (International Flavors & Fragrances) and batch/lot number were provided. The chemical identity was analytically verified, and this information was reported in Appendix A.
	Metric 3:	Test Substance Purity	Low	All Outcomes: The purity of the test substance was not reported.
Domain 2: Test Design				

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Domain		Metric	Rating	Comments
	Metric 4:	Negative and Vehicle Controls	High	All Outcomes: A concurrent negative control group was included and appropriate (basal diet).
	Metric 5:	Positive Controls	N/A	All Outcomes: A positive control is not required for this study type.
	Metric 6:	Randomized Allocation of Animals	Medium	All Outcomes: F0 animals were randomly allocated into study groups. The study authors do not report if F1 animals were randomly assigned to cohorts, but do state that selective elimination of pups based on body weight or AGD was not done.
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and Storage of Test Substance	Low	All Outcomes: Storage of the test substance and preparation of diet were fully reported. The diet was formulated weekly. The concentration and homogeneity (low and high dose only) of the diet mixture was analyzed using UPLC. The stability of the test substance in the diet (low and high-dose groups) was assessed. Stability testing showed a loss of test item up to 25.1% in formulations stored at RT for 8 days, and a loss of up to 18.6% when stored for 4 days. This suggests that the frequency of preparation (weekly) may not be appropriate.

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Domain	Metric		Rating	Comments
	Metric 8:	Consistency of Exposure Administration	Medium	All Outcomes: All animals were provided diets ad libitum; however, issues with the stability of the test substance in the diet suggest a lack of consistency over time in the dietary doses received. There could also be within-group or across-group variability due to differences in the rate of degradation.

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Domain	Metric	Rating	Comments
	Metric 9: Reporting of Doses/Concentrations	Medium	All Outcomes: Nominal concentrations in diet were reported. The diets were made up fresh weekly. Analytical analysis of diets was done on day 1, week 11, 16, 22, and 33. There are weeks when analytical concentrations deviated >20% from nominal. The study authors calculated doses in mg/kg/day for F0 and F1 generations. Concentrations during lactation were adapted based on historical control food consumption and body weight data for females rather than body weight and food consumption data from animals used in the study. It does not appear that in-use animal data at other times (e.g., premating) were used for any dose calculations, and it is unclear where the body weight and food consumption values they provided came from (e.g., default or historical?). This could have a significant impact on dosing. The study reported significant reductions in food consumption and in animal body weights for some groups, and these changes are presumably not reflected in the doses reported. It was noted that the reported doses in mg/kg-day accounted for a loss of approximately 25% in the diet mix. Overall, there is some uncertainty about the reported doses; however, raw data (food intake and body weights) were provided in most cases, allowing for independent calculations to be performed.

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Domain	Metric		Rating	Comments
	Metric 10:	Exposure Frequency and Duration	High	All Outcomes: The exposure frequency and duration were appropriate for the study's aim (10 weeks pre-mating, during mating, gestation and lactation for F0 and F1 breeding experiments; and 13 additional weeks after weaning for some F1 cohorts).
	Metric 11:	Number of Exposure Groups and Dose/Concentration Spacing	Medium	All Outcomes: The number of exposure groups and spacing were appropriate. Doses were chosen based on a 90-day repeated dose study, an OECD TG 414 study, and a DRF for this EOGRT study. A NOAEL was not obtained.
	Metric 12:	Exposure Route and Method	Medium	All Outcomes: There were minor limitations regarding the route and method of exposure, but the researchers took appropriate steps to mitigate the problem. According to the study authors findings on stability, approximately 25% of the test compound was lost after 4 days. The author therefore deliberately set dose levels +25% in order to compensate rather than increasing the frequency of preparation.
Domain 4: Test Animals				
	Metric 13:	Test Animal Characteristics	Medium	All Outcomes: The sex, age, starting body weight, strain, and species were reported. The source of the animals was redacted in the copy available for review.

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Chemical: HHCB- Parent compound

HERO ID: 8785683

Domain		Metric	Rating	Comments
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	All Outcomes: All husbandry conditions were reported (temperature, humidity, light-dark cycle, diet, water availability, number of animals per cage) and appropriate.
	Metric 15:	Number of Animals per Group	Medium	All Outcomes: The number of animals/group was appropriate (F0 generation 25/sex/group; F1 generation 20/sex/group).
Domain 5: Outcome Assessment				
	Metric 16:	Outcome Assessment Methodology	High	All Outcomes: The outcome methodology was sensitive and appropriate for the outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	High	All Outcomes: Outcome assessment was consistent across all animals.
	Metric 18:	Sampling Adequacy	High	All Outcomes: The sampling was sufficient. All animals are accounted for in the results.
	Metric 19:	Blinding of Assessors	N/A	All Outcomes: Blinding was not reported; however, endpoints were evaluated quantitatively, clinical signs, or histopathology.
	Metric 20:	Negative Control Response	High	All Outcomes: The negative control response was appropriate. Study authors also included historic control data.

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Study Citation:	IFF, (2021). Extended one generation reproductive toxicity study (including cohorts 1 and F2 - generation of HHCB by the oral route (dietary admixture) in the rat (OECD 443) (sanitized).
Health Outcome(s):	Reproductive/Developmental; Renal/Kidney; Hepatic/Liver; Endocrine (Endocrine); Neurological/Behavioral; Cardiovascular; Musculoskeletal; Gastrointestinal; Mortality; Nutritional/Metabolic; Nutritional/Metabolic; Immune/Hematological; Thyroid;
Reported Health Effect(s):	Reproductive/Developmental: F0 and F1 (cohort 1a, 1b, and surplus): Organ weights: cervix, epidiymides, ovaries, oviducts (F0 only), prostate, seminal vesicles with coagulating gland, testes and uterus; F0 and F1 (cohort 1a and unscheduled deaths) Histopathology: cervix, ovaries, oviducts, epididymides, mammary gland, prostate, seminal vesicles with coagulating gland, testes, uterus, vagina and vas deferens;F0: estrous cycles, mating performance and fertility, delivery and litter data, sperm qualityF1: mortality, clinical signs, body weights, food consumption, clinical pathology, thyroid hormone levels (PND 4 and 21), anogenital distance, pup number of aerola/nipples, estrous cycles, sexual maturation, sperm quality, macroscopic observations, organ weights and histopathology, splenic lymphocyte subpopulationF1: mating performance and fertility, delivery and litter dataF2 offspring: postnatal development, mortality, clinical signs, body weights, sex, anogenital distance, number of aerola/nipples, organ weights and macroscopy. F2 offspring sacrificed on PND21-23, organ weights (brain, liver, thyroid glands, spleen, thymus); Renal/Kidney: F0 and F1 (cohort 1a): Serum urea, creatinine, albumin, globulin, glucose, and total protein; urinalysis;F0 and F1 (cohort 1a, 1b, and surplus): kidney weight;F0 and F1 (cohort 1a and unscheduled deaths): histopathology on kidney and urinary bladder.; Hepatic/Liver: F0 and F1 (cohort 1a): Serum ALT, AST, ALP, total cholesterol, triglycerides; F0 and F1 (cohort 1a, 1b, and surplus): liver weight F0 and F1 (cohort 1a and unscheduled deaths): histopathology on liver; Endocrine (Endocrine): F0 and F1 (cohort 1a, 1b, and surplus): adrenal gland and pituitary weightF0: pancreas weightF0 and F1 (cohort 1a and unscheduled deaths): histopathology on adrenal and pituitary gland; Neurological/Behavioral: Clinical signs (piloerection); F0 and F1 (cohort 1a, 1b, and surplus): Brain weight; F0 and F1 (cohort 1a and unscheduled death): histopathology on brain, peripheral nerve, and spinal cord; Cardiovascular: F0 and F1 (cohort 1a, 1b, and surplus): Heart weight;F0 and F1 (cohort 1a and unscheduled deaths): histopathology on heart; Musculoskeletal: Clinical signs (malocclusion); F0 and F1 (cohort 1a and unscheduled deaths): Histopathology on bone (sternum) with bone marrow; Gastrointestinal: Clinical signs (hypersalivation, soft or no feces); F0 and F1 (cohort 1a and unscheduled deaths): Histopathology on caecum, colon, duodenum, esophagus, ileum, jejunum, rectum, and stomach; Mortality: F0 and all F1 (adult treated) cohorts: Mortality; Nutritional/Metabolic: F0 and all F1 adult treated cohorts: Body weight and food consumption; Nutritional/Metabolic: F0 and all F1 adult treated cohorts: Body weight and food consumption; Immune/Hematological: F0 and F1 (cohort 1a) Hematology. Fo and F1 (cohort 1a, 1b, and surplus): Spleen and thymus weight F1 (cohort 1a): lymph node weight (mandibular and mesenteric)F0 and F1 (cohort 1a and unscheduled deaths): histopathology on spleen, thymus, and lymph nodes (F1 only)F1 (cohort 1a): splenic lymphocyte population analysis; Thyroid: F0, F1 (cohort 1a, surplus PND4 pups): serum thyroid hormone levels (T4, TSH);F0, F1 (cohort 1a, 1b, and surplus): Thyroid and parathyroid weights; F0 and F1 (cohort 1a and unscheduled deaths, surplus PND4 pups): histopathology on thyroid and parathyroid;;
Duration:	Reproductive/Developmental Extended one generation Repro/Dev study
Chemical:	HHCB- Parent compound
HERO ID:	8785683

Domain	Metric		Rating	Comments
Domain 6: Confounding / Variable Control				
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	All Outcomes: Most information to determine confounding was reported (body weights and food intake). Water intake was not reported. The text indicates significant issues with food spillage during the study, resulting in animals going a full day without food and erroneous reporting of food intake. In several cases, exclusions had to be made in calculating food intake due to this issue. However, overall, it is unclear whether this had any major impact on the study results.
	Metric 22:	Health Outcomes Unrelated to Exposure	High	All Outcomes: The study reported most information that could identify attrition or other health outcomes unrelated to exposure (e.g., haematological analysis and histopathology, as well as mortality, and clinical signs). Based on the data provided, there were no differences across groups suggestive of attrition.
	Metric 23:	Data Presentation and Analysis	High	All Outcomes: Statistical methods were described and appropriate. The litter was used as the basic sample unit.

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Study Citation:	IFF, (2021). Extended one generation reproductive toxicity study (including cohorts 1 and F2 - generation of HHCB by the oral route (dietary admixture) in the rat (OECD 443) (sanitized).
Health Outcome(s):	Reproductive/Developmental; Renal/Kidney; Hepatic/Liver; Endocrine (Endocrine); Neurological/Behavioral; Cardiovascular; Musculoskeletal; Gastrointestinal; Mortality; Nutritional/Metabolic; Nutritional/Metabolic; Immune/Hematological; Thyroid;
Reported Health Effect(s):	Reproductive/Developmental: F0 and F1 (cohort 1a, 1b, and surplus): Organ weights: cervix, epidiymides, ovaries, oviducts (F0 only), prostate, seminal vesicles with coagulating gland, testes and uterus; F0 and F1 (cohort 1a and unscheduled deaths) Histopathology: cervix, ovaries, oviducts, epididymides, mammary gland, prostate, seminal vesicles with coagulating gland, testes, uterus, vagina and vas deferens;F0: estrous cycles, mating performance and fertility, delivery and litter data, sperm qualityF1: mortality, clinical signs, body weights, food consumption, clinical pathology, thyroid hormone levels (PND 4 and 21), anogenital distance, pup number of aerola/nipples, estrous cycles, sexual maturation, sperm quality, macroscopic observations, organ weights and histopathology, splenic lymphocyte subpopulationF1: mating performance and fertility, delivery and litter dataF2 offspring: postnatal development, mortality, clinical signs, body weights, sex, anogenital distance, number of aerola/nipples, organ weights and macroscopy. F2 offspring sacrificed on PND21-23, organ weights (brain, liver, thyroid glands, spleen, thymus); Renal/Kidney: F0 and F1 (cohort 1a): Serum urea, creatinine, albumin, globulin, glucose, and total protein; urinalysis;F0 and F1 (cohort 1a, 1b, and surplus): kidney weight;F0 and F1 (cohort 1a and unscheduled deaths): histopathology on kidney and urinary bladder.; Hepatic/Liver: F0 and F1 (cohort 1a): Serum ALT, AST, ALP, total cholesterol, triglycerides; F0 and F1 (cohort 1a, 1b, and surplus): liver weight F0 and F1 (cohort 1a and unscheduled deaths): histopathology on liver; Endocrine (Endocrine): F0 and F1 (cohort 1a, 1b, and surplus): adrenal gland and pituitary weightF0: pancreas weightF0 and F1 (cohort 1a and unscheduled deaths): histopathology on adrenal and pituitary gland; Neurological/Behavioral: Clinical signs (piloerection); F0 and F1 (cohort 1a, 1b, and surplus): Brain weight; F0 and F1 (cohort 1a and unscheduled death): histopathology on brain, peripheral nerve, and spinal cord; Cardiovascular: F0 and F1 (cohort 1a, 1b, and surplus): Heart weight;F0 and F1 (cohort 1a and unscheduled deaths): histopathology on heart; Musculoskeletal: Clinical signs (malocclusion); F0 and F1 (cohort 1a and unscheduled deaths): Histopathology on bone (sternum) with bone marrow; Gastrointestinal: Clinical signs (hypersalivation, soft or no feces); F0 and F1 (cohort 1a and unscheduled deaths): Histopathology on caecum, colon, duodenum, esophagus, ileum, jejunum, rectum, and stomach; Mortality: F0 and all F1 (adult treated) cohorts: Mortality; Nutritional/Metabolic: F0 and all F1 adult treated cohorts: Body weight and food consumption; Nutritional/Metabolic: F0 and all F1 adult treated cohorts: Body weight and food consumption; Immune/Hematological: F0 and F1 (cohort 1a) Hematology. Fo and F1 (cohort 1a, 1b, and surplus): Spleen and thymus weight F1 (cohort 1a): lymph node weight (mandibular and mesenteric)F0 and F1 (cohort 1a and unscheduled deaths): histopathology on spleen, thymus, and lymph nodes (F1 only)F1 (cohort 1a): splenic lymphocyte population analysis; Thyroid: F0, F1 (cohort 1a, surplus PND4 pups): serum thyroid hormone levels (T4, TSH);F0, F1 (cohort 1a, 1b, and surplus): Thyroid and parathyroid weights; F0 and F1 (cohort 1a and unscheduled deaths, surplus PND4 pups): histopathology on thyroid and parathyroid;;
Duration:	Reproductive/Developmental Extended one generation Repro/Dev study
Chemical:	HHCB- Parent compound
HERO ID:	8785683

Domain	Metric	Rating	Comments
	Metric 24: Reporting of Data	High	All Outcomes: Data were fully reported and individual animal data for all endpoints were provided in the study appendices.

Additional Comments: None

Overall Quality Determination High

Study Citation:	IFF, (1975). Galaxolide 50: Instilled into rabbit's eyes. HH06_HHCB (sanitized).
Health Outcome(s):	Irritation (Eye damage)
Reported Health Effect(s):	Irritation (Eye damage): No ocular irritation was reported.
Duration:	Short-term (>1-30 days) 4 Days
Chemical:	HHCB- Mixture: HHCB and DEP
HERO ID:	8785656

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	Medium	The mixture ratio was written at top of manuscript. No other in-depth characterization of test substance.
	Metric 2:	Test Substance Source	Low	I did not see any information on the manufacturer or analytical verification.
	Metric 3:	Test Substance Purity	Low	Purity was not reported.
Domain 2: Test Design				
	Metric 4:	Negative and Vehicle Controls	High	Tested in one eye and the other was used as negative control
	Metric 5:	Positive Controls	N/A	
	Metric 6:	Randomized Allocation of Animals	Low	No mention of randomization of animals.
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and Storage of Test Substance	Medium	The ratio of the test substance mixture was reported but no other information was stated.
	Metric 8:	Consistency of Exposure Administration	High	
	Metric 9:	Reporting of Doses/Concentrations	Uninformative	Doses/concentrations were not reported. No way to determine how much HHCB was actually given.
	Metric 10:	Exposure Frequency and Duration	High	
	Metric 11:	Number of Exposure Groups and Dose/Concentration Spacing	Low	No changes in dosage between the 3 rabbits.
	Metric 12:	Exposure Route and Method	High	
Domain 4: Test Animals				
	Metric 13:	Test Animal Characteristics	Low	No distinctive information on animlas used.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	
	Metric 15:	Number of Animals per Group	Medium	
Domain 5: Outcome Assessment				
	Metric 16:	Outcome Assessment Methodology	High	
	Metric 17:	Consistency of Outcome Assessment	High	
	Metric 18:	Sampling Adequacy	High	
	Metric 19:	Blinding of Assessors	N/A	

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Health Outcome(s):	Irritation (Eye damage)
Reported Health Effect(s):	Irritation (Eye damage): No ocular irritation was reported.
Duration:	Short-term (>1-30 days) 4 Days
Chemical:	HHCB- Mixture: HHCB and DEP
HERO ID:	8785656

Domain	Metric		Rating	Comments
	Metric 20:	Negative Control Response	High	
Domain 6: Confounding / Variable Control				
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	
	Metric 22:	Health Outcomes Unrelated to Exposure	High	
	Metric 23:	Data Presentation and Analysis	N/A	No statistical analysis needed, as there was no outcome from the study. HHDB is not an eye irritant
	Metric 24:	Reporting of Data	Medium	

Additional Comments: None

Overall Quality Determination Medium

Study Citation:	IFF, (2020). A GLP simplified reproduction/developmental toxicity screening test of HHCB by the oral route (dietary admixture) in the rat (OECD 421) (sanitized).
Health Outcome(s):	Reproductive/Developmental (Systemic effects)
Reported Health Effect(s):	Reproductive/Developmental (Systemic effects): Developmental toxicity
Duration:	Subchronic (>30-91 days) 29 days for Males and 71 for females
Chemical:	HHCB- Mixture: Galaxolide racemic mixture Cas 1222-05-5
HERO ID:	8785662

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	
	Metric 2:	Test Substance Source	High	
	Metric 3:	Test Substance Purity	High	Racemic mixture was tested, both components were identified by CAS#s.
Domain 2: Test Design				
	Metric 4:	Negative and Vehicle Controls	Uninformative	This was a dose finding study for EOGRT, historical control data would be sufficient, my preference would be N/A
	Metric 5:	Positive Controls	N/A	Guidelines do not explicitly call for a positive control
	Metric 6:	Randomized Allocation of Animals	Medium	
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and Storage of Test Substance	High	
	Metric 8:	Consistency of Exposure Administration	High	Dose levels were set to 25% to account for loss of test item of 25% in feed
	Metric 9:	Reporting of Doses/Concentrations	High	
	Metric 10:	Exposure Frequency and Duration	High	
	Metric 11:	Number of Exposure Groups and Dose/Concentration Spacing		This was a dose determining study for EOGRT
	Metric 12:	Exposure Route and Method	High	
Domain 4: Test Animals				
	Metric 13:	Test Animal Characteristics	High	
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	
	Metric 15:	Number of Animals per Group	Medium	
Domain 5: Outcome Assessment				
	Metric 16:	Outcome Assessment Methodology	High	
	Metric 17:	Consistency of Outcome Assessment	Medium	Female dosing is a range low dose of (305-690 ppm) and high dose of (1090-2480ppm)
	Metric 18:	Sampling Adequacy	High	
	Metric 19:	Blinding of Assessors	N/A	

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Study Citation:	IFF, (2020). A GLP simplified reproduction/developmental toxicity screening test of HHCB by the oral route (dietary admixture) in the rat (OECD 421) (sanitized).
Health Outcome(s):	Reproductive/Developmental (Systemic effects)
Reported Health Effect(s):	Reproductive/Developmental (Systemic effects): Developmental toxicity
Duration:	Subchronic (>30-91 days) 29 days for Males and 71 for females
Chemical:	HHCB- Mixture: Galaxolide racemic mixture Cas 1222-05-5
HERO ID:	8785662

Domain	Metric		Rating	Comments
	Metric 20:	Negative Control Response	N/A	Negative controls were historical test data
Domain 6: Confounding / Variable Control				
	Metric 21:	Confounding Variables in Test Design and Procedures	High	
	Metric 22:	Health Outcomes Unrelated to Exposure	High	
	Metric 23:	Data Presentation and Analysis	High	
	Metric 24:	Reporting of Data	High	

Additional Comments: None

Overall Quality Determination High

Study Citation:	IFF, (n.d.). A prenatal developmental toxicity study of HHCB by the oral (gavage) route in the rabbit. (sanitized).
Health Outcome(s):	Nutritional/Metabolic; Reproductive/Developmental;
Reported Health Effect(s):	Nutritional/Metabolic: Maternal body weight gain and food consumption; Reproductive/Developmental: Outcomes evaluated include number of implantation sites, live litter size fetal body weights, sex ratio, and external, visceral and skeletal malformations;
Duration:	Reproductive/Developmental Developmental study GD 6-28
Chemical:	HHCB- Mixture: HHCB
HERO ID:	8785663

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	All Outcomes: The test substance is identified as HHCB (multiconstituent); the cas number, structures of constituents, and molecular formula are also provided.
	Metric 2:	Test Substance Source	High	All Outcomes: The source was identified as the study sponsor. The batch/lot number is provided and a product certificate of analysis is provided in the appendix.
	Metric 3:	Test Substance Purity	N/A	All Outcomes: authors report purity as not applicable because HHCB is a mixture
Domain 2: Test Design				
	Metric 4:	Negative and Vehicle Controls	Low	All Outcomes: A concurrent vehicle control group was included, but not concurrent untreated controls were included. Comparisons with historical controls indicate potential vehicle effects and there is no concurrent untreated control group to use as a reference.
	Metric 5:	Positive Controls	N/A	Nutritional/Metabolic: ; Reproductive/Developmental:
	Metric 6:	Randomized Allocation of Animals	Medium	All Outcomes: The authors report animals were randomly assigned to treatment groups using random allocation
Domain 3: Exposure Characterization				
	Metric 7:	Preparation and Storage of Test Substance	High	All Outcomes: The authors describe preparation and storage. Samples were prepared in vehicle in test aliquots to be dispensed on each dosing occasion. Samples were stored 60-70 degrees and mixed well before use
	Metric 8:	Consistency of Exposure Administration	High	Nutritional/Metabolic: ; Reproductive/Developmental:
	Metric 9:	Reporting of Doses/Concentrations	High	All Outcomes: Gavage doses were confirmed analytically and results are presented in Appendix 3; deviations from the nominal concentrations ranged from -2.3 to 2.1%.
	Metric 10:	Exposure Frequency and Duration	High	All Outcomes: Exposure frequency and duration were consistent with OECD 414
	Metric 11:	Number of Exposure Groups and Dose/Concentration Spacing	High	All Outcomes: The number of exposure groups and doses are consistent with OECD TG 414 and were selected based on previous dose range finding studies. The volume of corn oil vehicle used (0.8 ml/kg bw) is also based on a previous range finding study reporting that 100 mg/kg/day in 0.8 ml/kg/day was associated with "tolerable effects on mean food consumption and body weight gain". This is consistent with OECD TG 414 which states "when corn oil is used, volume should not exceed 0.4ml/100g body weight."
	Metric 12:	Exposure Route and Method	High	All Outcomes: Exposure route and method are appropriate and consistent with methods in OECD TG 414
Domain 4: Test Animals				
	Metric 13:	Test Animal Characteristics	Low	All Outcomes: The source of the test animals was redacted. Sex, age and range of starting weights of animals were reported.

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Health Outcome(s):	Nutritional/Metabolic; Reproductive/Developmental;
Reported Health Effect(s):	Nutritional/Metabolic: Maternal body weight gain and food consumption; Reproductive/Developmental: Outcomes evaluated include number of implantation sites, live litter size fetal body weights, sex ratio, and external, visceral and skeletal malformations;
Duration:	Reproductive/Developmental Developmental study GD 6-28
Chemical:	HHCB- Mixture: HHCB
HERO ID:	8785663

Domain	Metric		Rating	Comments
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	All Outcomes: Temperature, humidity, light/dark, ventilation were reported and appropriate. Authors also report providing items for psychological/environmental enrichment
	Metric 15:	Number of Animals per Group	Medium	All Outcomes: The number of animals per group (22) is appropriate and consistent with OECD TG 414
Domain 5: Outcome Assessment				
	Metric 16:	Outcome Assessment Methodology	High	All Outcomes: Assessment methodology is appropriate and generally consistent w/ OECD 414
	Metric 17:	Consistency of Outcome Assessment	High	All Outcomes: Details of outcome assessment were reported and appear consistent with OECD TG 414
	Metric 18:	Sampling Adequacy	Low	All Outcomes: For some dose group and endpoints, the number of animals available for evaluation was not sufficient. For the 30 mg/kg/day dose group, the number of animals available for evaluation for most endpoints was 15. The OECD TG 414 states that ”groups with fewer than 16 animals with implantation sites may be inappropriate”
	Metric 19:	Blinding of Assessors	N/A	Nutritional/Metabolic: ; Reproductive/Developmental:
	Metric 20:	Negative Control Response	Uninformative	All Outcomes: Authors report reduced maternal food consumption and body weight gain and reduced fetal body weights in vehicle controls relative to historical controls. The authors attribute these effects to vehicle-related toxicity. For example, maternal body weight gain in vehicle controls is 51% less than the mean historical control values (see p.28), maternal food consumption in vehicle controls is 28% less than mean historical controls (p. 29) and fetal body weight in test groups ranges 33.1-37.3 while the historical control range is 38.4-43.2 (p. 30).
Domain 6: Confounding / Variable Control				
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	All Outcomes: The authors do not report any differences among treatment groups that would suggest potential for confounding. However, authors report reduced maternal food consumption, reduced maternal body weight gain and reduced fetal body weights in vehicle controls relative to historical controls, suggesting potential for confounding by an unknown factor. The authors attribute these effects to vehicle-related toxicity, but do not provide information about other potential sources of confounding.
	Metric 22:	Health Outcomes Unrelated to Exposure	Low	All Outcomes: The authors do not report differences in study groups that are unrelated to exposure or serious attrition. However, authors report reduced maternal food consumption, reduced maternal body weight gain and reduced fetal body weights in vehicle controls relative to historical controls, suggesting potential for confounding by an unknown factor. The authors attribute these effects to vehicle-related toxicity, but do not provide information about other potential sources of confounding.
	Metric 23:	Data Presentation and Analysis	High	Nutritional/Metabolic: ; Reproductive/Developmental:
	Metric 24:	Reporting of Data	High	Nutritional/Metabolic: ; Reproductive/Developmental:

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Reported Health Effect(s):	Nutritional/Metabolic: Maternal body weight gain and food consumption; Reproductive/Developmental: Outcomes evaluated include number of implantation sites, live litter size fetal body weights, sex ratio, and external, visceral and skeletal malformations;
Duration:	Reproductive/Developmental Developmental study GD 6-28
Chemical:	HHCB- Mixture: HHCB
HERO ID:	8785663

Domain	Metric	Rating	Comments

Additional Comments: None

Overall Quality Determination Uninformative