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Short review

RIFM fragrance ingredient safety assessment, 5-Ethylidenebicyclo [2.2.1]hept-2-yl propionate, CAS Registry Number 73347-77-0

A.M. Api [a, *], D. Belsito [b], S. Bhatia [a], M. Bruze [c], P. Calow [d], M.L. Dagli [e], W. Dekant [f], A.D. Fryer [g], L. Kromidas [a], S. La Cava [a], J.F. Lalko [a], A. Lapczynski [a], D.C. Liebler [h], Y. Miyachi [i], V.T. Politano [a], G. Ritacco [a], D. Salvito [a], T.W. Schultz [j], J. Shen [a], I.G. Sipes [k], B. Wall [a], D.K. Wilcox [a]

[a] Research Institute for Fragrance Materials, Inc., 50 Tice Boulevard, Woodcliff Lake, NJ 07677, USA
[b] Member RIFM Expert Panel, Columbia University Medical Center, Department of Dermatology, 161 Fort Washington Ave., New York, NY 10032, USA
[c] Member RIFM Expert Panel, Malmo University Hospital, Department of Occupational & Environmental Dermatology, Sodra Forstadsgatan 101, Entrance 47, Malmo, SE 20502, Sweden
[d] Member RIFM Expert Panel, Humphrey School of Public Affairs, University of Minnesota, 301 19th Avenue South, Minneapolis, MN 55455, USA
[e] Member RIFM Expert Panel, University of Sao Paulo, School of Veterinary Medicine and Animal Science, Department of Pathology, Av. Prof. dr. Orlando Marques de Paiva, 87, Sao Paulo CEP 05508-900, Brazil
[f] Member RIFM Expert Panel, University of Wuerzburg, Department of Toxicology, Versbacher Str. 9, 97078 Würzburg, Germany
[g] Member RIFM Expert Panel, Oregon Health Science University, 3181 SW Sam Jackson Park Rd., Portland, OR 97239, USA
[h] Member RIFM Expert Panel, Vanderbilt University School of Medicine, Department of Biochemistry, Center in Molecular Toxicology, 638 Robinson Research Building, 2200 Pierce Avenue, Nashville, TN 37232-0146, USA
[i] Member RIFM Expert Panel, Department of Dermatology, Kyoto University Graduate School of Medicine, 54 Kawahara-cho, Shogoin, Sakyo-ku, Kyoto 606-8507, Japan
[j] Member RIFM Expert Panel, The University of Tennessee, College of Veterinary Medicine, Department of Comparative Medicine, 2407 River Dr., Knoxville, TN 37996-4500, USA
[k] Member RIFM Expert Panel, Department of Pharmacology, University of Arizona, College of Medicine, 1501 North Campbell Avenue, P.O. Box 245050, Tucson, AZ 85724-5050, USA

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* Corresponding author.
E-mail address: AApi@rifm.org (A.M. Api).

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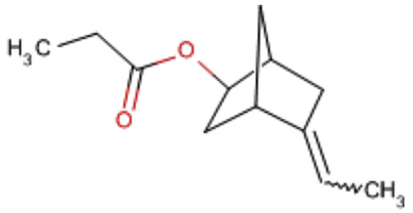


Abbreviation list:
2-Box Model – a RIFM, Inc. proprietary *in silico* tool used to calculate fragrance air exposure concentration
AF- Assessment Factor
BCF- Bioconcentration Factor
Creme RIFM model- The Creme RIFM model uses probabilistic (Monte Carlo) simulations to allow full distributions of data sets, providing a more realistic estimate of aggregate exposure to individuals across a population (Comiskey et al., 2015; Safford et al., 2015) compared to a deterministic aggregate approach.
DEREK- Derek nexus is an *in silico* tool used to identify structural alerts
DST- Dermal Sensitization Threshold
ECHA-European Chemicals Agency
EU – Europe/European Union
GLP- Good Laboratory Practice
IFRA- The International Fragrance Association
LOEL- Lowest Observable Effect Level
MOE- Margin of Exposure
MPPD - Multiple-Path Particle Dosimetry. An *in silico* model for inhaled vapors used to simulate fragrance lung deposition
NA – North America
NESIL- No Expected Sensitization Induction Level
NOAEC- No Observed Adverse Effect Concentration
NOAEL- No Observed Adverse Effect Level
NOEC- No Observed Effect Concentration
OECD- Organisation for Economic Co-operation and Development
OECD TG- Organisation for Economic Co-operation and Development Testing Guidelines
PBT- Persistent, Bioaccumulative, and Toxic
PEC/PNEC- Predicted Environmental Concentration/Predicted No Effect Concentration
QRA- quantitative risk assessment
REACH- Registration, Evaluation, Authorisation, and Restriction of Chemicals
RIFM- Research Institute for Fragrance Materials
RQ- Risk Quotient
TTC- Threshold of Toxicological Concern
UV/Vis Spectra- Ultra Violet/Visible spectra
VCF- Volatile Compounds in Food
VoU- Volume of Use
vPvB- (very) Persistent, (very) Bioaccumulative
WOE–Weight of Evidence

RIFM's Expert Panel* concludes that this material is safe under the limits described in this safety assessment.
This safety assessment is based on the RIFM Criteria Document (Api et al., 2015) which should be referred to for clarifications.
Each endpoint discussed in this safety assessment reviews the relevant data that were available at the time of writing (version number in the top box is indicative of the date of approval based on a two digit month/day/year), both in the RIFM database (consisting of publicly available and proprietary data) and through publicly available information sources (i.e., SciFinder and PubMed). Studies selected for this safety assessment were based on appropriate test criteria, such as acceptable guidelines, sample size, study duration, route of exposure, relevant animal species, most relevant testing endpoints, etc. A key study for each endpoint was selected based on the most conservative end-point value (e.g., PNEC, NOAEL, LOEL, and NESIL).
*RIFM's Expert Panel is an independent body that selects its own members and establishes its own operating procedures. The Expert Panel is comprised of internationally known scientists that provide RIFM guidance relevant to human health and environmental protection.

Summary: The use of this material under current conditions is supported by existing information.
This material was evaluated for genotoxicity, repeated dose toxicity, reproductive toxicity, local respiratory toxicity, phototoxicity/photoallergenicity, skin sensitization, as well as environmental safety. Data from the suitable read across analog isobornyl acetate (CAS # 125-12-2) show that this material is not genotoxic nor does it have skin sensitization potential. The local respiratory toxicity endpoint was completed using the TTC (Threshold of Toxicological Concern) for a Cramer Class II material (0.47 mg/day). The repeated dose, developmental and reproductive toxicity endpoints were completed using isobornyl acetate (CAS # 125-12-2) as a suitable read across analog, which provided a MOE > 100. The phototoxicity/photoallergenicity endpoint was completed based on suitable UV spectra. The environmental endpoint was completed as described in the RIFM Framework.

Human Health Safety Assessment	
Genotoxicity: Not genotoxic.	(ECHA REACH Dossier)
Repeated Dose Toxicity: NOEL = 15 mg/kg/day	(Gaunt et al., 1971)
Developmental and Reproductive Toxicity: NOAEL = 1000 and 300 mg/kg/day respectively	(ECHA REACH Dossier: exo-1,7,7-Trimethylbicyclo[2.2.1]hept-2-yl acetate; RIFM, 2011)
Skin Sensitization: Not sensitizing	(RIFM, 1968; RIFM, 1970; RIFM, 2008)
Phototoxicity/Photoallergenicity: Not phototoxic/photoallergenic	(UV Spectra, RIFM DB)
Local Respiratory Toxicity: No NOAEC available. Exposure is below the TTC.	

Environmental Safety Assessment	
Hazard Assessment:	
Persistence: Screening Level: 2.9 (Biowin 3)	(EpiSuite ver 4.1)
Bioaccumulation: Screening Level: 160 L/kg	(EpiSuite ver 4.1)
Ecotoxicity: Screening Level: Fish LC50: 6.456 mg/L	(RIFM Framework; Salvito et al., 2002)
Conclusion: Not PBT or vPvB as per IFRA Environmental Standards	

(*continued on next page*)

(*continued*)

Risk Assessment:	
Screening-Level: PEC/PNEC (North America and Europe) < 1	(RIFM Framework; Salvito et al., 2002)
Critical Ecotoxicity Endpoint: Fish LC50: 6.456 mg/L	(RIFM Framework; Salvito et al., 2002)
RIFM PNEC is: 0.006456 μg/L	
• **Revised PEC/PNECs (2011 IFRA VoU):** North America and Europe: Not Applicable; Cleared at screening level	

1. Identification

1 **Chemical Name:** 5-Ethylidenebicyclo[2.2.1]hept-2-yl propionate
2 **CAS Registry Number:** 73347-77-0
3 **Synonyms:** Bicyclo[2.2.1]heptan-2-ol, 5-ethylidene-, propanoate; 5-Ethylidenebicyclo[2.2.1]hept-2-yl propionate; Jasverate
4 **Molecular Formula:** $C_{12}H_{18}O_2$
5 **Molecular Weight:** 194.74
6 **RIFM Number:** 5984

2. Physical data

1 **Boiling Point:** 252.56 °C [EPI Suite]
2 **Flash Point:** 210.00 °F. TCC (99.10 °C)*
3 **Log K_{OW}:** 3.85 [EPI Suite]
4 **Melting Point**: 33.23 °C [EPI Suite]
5 **Water Solubility:** 24.26 mg/L [EPI Suite]
6 **Specific Gravity:** Not Available
7 **Vapor Pressure:** 0.0197 mm Hg @ 25 °C [EPI Suite], 0.0113 mmHg @ 20 °C [EPI Suite 4.0]
8 **UV Spectra:** No significant absorbance between 290 and 700 nm; molar absorption coefficient is below the benchmark (1000 L mol^{-1} cm^{-1})
9 **Appearance/Organoleptic:** Not Available
* http://www.thegoodscentscompany.com/data/rw1383791.html, retrieved 4/2/2015

3. Exposure

1 **Volume of Use (worldwide band):** < 0.1 metric tons per year (IFRA, 2011)
2 95th **Percentile Concentration in Hydroalcoholics:** 0.012% (RIFM, 2014)
3 **Inhalation Exposure*:** 0.000069 mg/kg/day or 0.0049 mg/day (RIFM, 2014)
4 **Total Systemic Exposure**:** 0.00090 mg/kg/day (RIFM, 2014)
* 95th percentile calculated exposure derived from concentration survey data in the Creme RIFM exposure model (Comiskey et al., 2015; and Safford et al., 2015).
** 95th percentile calculated exposure; assumes 100% absorption unless modified by dermal absorption data as reported in Section 4. It is derived from concentration survey data in the Creme RIFM aggregate exposure model and includes exposure via dermal, oral and inhalation routes whenever the fragrance ingredient is used in products that include these routes of exposure (Comiskey et al., 2015; and Safford et al., 2015).

4. Derivation of systemic absorption

1 **Dermal:** Assumed 100%
2 **Oral:** Assumed 100%
3 **Inhalation:** Assumed 100%

5. Computational toxicology evaluation

1 **Cramer Classification:** Class II, Intermediate (Expert Judgment)

Expert Judgment	Toxtree v 2.6	OECD QSAR Toolbox v 3.2
II[a]	III	II

[a] See Appendix below for explanation.

2 **Analogues Selected:**
a **Genotoxicity:** Isobornyl acetate (CAS # 125-12-2)
b **Repeated Dose Toxicity:** Isobornyl acetate (CAS # 125-12-2)
c **Developmental and Reproductive Toxicity:** Isobornyl acetate (CAS # 125-12-2)
d **Skin Sensitization:** Isobornyl acetate (CAS # 125-12-2)
e **Phototoxicity/Photoallergenicity:** None
f **Local Respiratory Toxicity:** None
g **Environmental Toxicity:** None
3 **Read-across Justification:** See Appendix below

6. Metabolism

Not considered for this risk assessment and therefore not reviewed except where it may pertain in specific endpoint sections as discussed below.

7. Natural occurrence (discrete chemical) or composition (NCS)

5-Ethylidenebicyclo[2.2.1]hept-2-yl propionate is not reported to occur in food by the VCF*.

*VCF Volatile Compounds in Food: database/Nijssen, L.M.; Ingen-Visscher, C.A. van; Donders, J.J.H. [eds]. – Version 15.1– Zeist (The Netherlands): TNO Triskelion, 1963–2014. A continually updated database, contains information on published volatile compounds which have been found in natural (processed) food products. Includes FEMA GRAS and EU-Flavis data.

8. IFRA standard

None.

9. REACH dossier

Pre-registered for 2013; No dossier available as of 09/13/2016.

10. Summary

1 Human Health Endpoint Summaries:

10.1. Genotoxicity

Based on the current existing data and use levels, 5-ethylidenebicyclo[2.2.1]hept-2-yl propionate does not present a concern for genetic toxicity.

10.1.1. Risk assessment

5-Ethylidenebicyclo[2.2.1]hept-2-yl propionate was found to be negative for both cytotoxicity and genotoxicity when tested in the BlueScreen assay indicating a lack for genotoxic potential (RIFM, 2013b). There are no data assessing the mutagenic activity of the target material, however read across can be made to its analog, isobornyl acetate (CAS # 125-12-2; see Section 5) which was assessed in a GLP compliant study in accordance with OECD TG 471 using the plate incorporation method. *S. typhimurium* strains TA1535, TA1537, TA1538, TA98 and TA100 were treated with isobornyl acetate in DMSO (dimethyl sulfoxide) at concentrations up to 500 μg/plate in the presence and absence of S9 mix (ECHA REACH Dossier: exo-1,7,7-trimethylbicyclo[2.2.1]hept-2-yl acetate). Under the conditions of the study, isobornyl acetate was considered not mutagenic in bacteria.

There are no studies assessing the clastogenic activity of 5-ethylidenebicyclo[2.2.1]hept-2-yl propionate. Read across material isobornyl acetate was assessed for clastogenicity in an *in vivo* mouse micronucleus assay conducted in compliance with GLP regulations and in accordance with OECD TG 474. Male and female NMRI mice were treated with isobornyl acetate dissolved in sesame oil via a single oral administration of 2000 mg/kg bodyweight. Peripheral blood was harvested 24, 48 and 72 h after administration. The number of polychromatic and normochromatic erythrocytes containing micronuclei was not increased (ECHA REACH Dossier: exo-1,7,7-trimethylbicyclo[2.2.1]hept-2-yl acetate). Under the conditions of the study, isobornyl acetate was considered negative for induction of clastogenic and aneugenic activity in mice.

Based on the available data, isobornyl acetate does not present a concern for genotoxic potential and this can be extended to 5-ethylidenebicyclo[2.2.1]hept-2-yl propionate.

Additional References: RIFM, 2013a.

Literature Search and Risk Assessment Completed on: 04/24/15.

10.2. Repeated dose toxicity

The margin of exposure for 5-ethylidenebicyclo[2.2.1]hept-2-yl propionate is adequate for the repeated dose toxicity endpoint.

10.2.1. Risk assessment

There are no repeated dose toxicity data on 5-ethylidenebicyclo [2.2.1]hept-2-yl propionate. Read across material isobornyl acetate (CAS # 125-12-2; see Section 5), has a gavage 13-week subchronic toxicity study that was conducted in rats. The NOEL was determined to be 15 mg/kg/day, based on increased urinary cell excretion (Gaunt et al., 1971). **Therefore, the MOE is equal to the isobornyl acetate NOEL in mg/kg/day divided by the total systemic exposure, 15/0.00090 or 16667.**

In addition, the total systemic exposure for 5-ethylidenebicyclo[2.2.1]hept-2-yl propionate (0.9 μg/kg/day) is below the TTC (9 μg/kg bw/day) for the repeated dose toxicity endpoint at the current level of use.

Additional References: Pinching and Doving, 1974; Schafer and Schafer, 1982

Literature Search and Risk Assessment Completed on: 04/27/15.

10.3. Developmental and reproductive toxicity

The margin of exposure for 5-ethylidenebicyclo[2.2.1]hept-2-yl propionate is adequate for the developmental and reproductive toxicity endpoint.

10.3.1. Risk assessment

There are no developmental toxicity data on 5-ethylidenebicyclo[2.2.1]hept-2-yl propionate. Read across material isobornyl acetate (CAS # 125-12-2; see Section 5) has an OECD 414 gavage limit dose study that was conducted in rats. The NOAEL was determined to be 1000 mg/kg/day, the only dosage tested (ECHA REACH Dossier: exo-1,7,7-Trimethylbicyclo[2.2.1]hept-2-yl acetate (accessed 08/12/13)). **Therefore, the MOE is equal to the isobornyl acetate NOAEL in mg/kg/day divided by the total systemic exposure, 1000/0.00090 or 1111111.**

There are no reproductive toxicity data on 5-ethylidenebicyclo [2.2.1]hept-2-yl propionate. Read across material isobornyl acetate (CAS # 125-12-2) has an enhanced OECD 415 gavage 1-generation reproductive toxicity study that was conducted in rats. The NOAEL for reproductive toxicity in the parental generation was determined to be 300 mg/kg/day, the highest dosage tested (RIFM, 2011; data also available in Politano et al., 2013). **Therefore, the MOE is equal to the isobornyl acetate NOAEL in mg/kg/day divided by the total systemic exposure, 300/0.00090 or 333333.**

In addition, the total systemic exposure to 5-ethylidenebicyclo[2.2.1]hept-2-yl propionate (0.9 μg/kg/day) is below the TTC (9 μg/kg bw/day) at the current level of use for the developmental and reproductive toxicity endpoint.

Additional References: Pinching and Doving, 1974; Schafer and Schafer, 1982.

Literature Search and Risk Assessment Completed on: 04/27/15.

10.4. Skin sensitization

Based on the existing data and the read across material isobornyl acetate (CAS # 125-12-2), 5-ethylidenebicyclo [2.2.1]hept-2-yl propionate does not present a concern for skin sensitization.

10.4.1. Risk assessment

The chemical structure of 5-ethylidenebicyclo [2.2.1] hept-2-yl propionate indicates that it would not be expected to significantly react with skin proteins (Roberts et al., 2007; Toxtree 2.6.6; OECD toolbox v3.3). In guinea pig sensitization studies and the murine local lymph node assay no reactions indicative of sensitization were observed with read across material isobornyl acetate (RIFM, 2007; Klecak, 1985, 1979). In human confirmatory studies no sensitization reactions were observed with either 5-ethylidenebicyclo [2.2.1] hept-2-yl propionate or the read across isobornyl acetate (RIFM, 1968; RIFM, 1970; RIFM, 2008).

Additional References: None.

Literature Search and Risk Assessment Completed on: 04/24/15.

10.5. Phototoxicity/photoallergenicity

Based on UV/Vis absorption spectra, 5-ethylidenebicyclo[2.2.1] hept-2-yl propionate would not be expected to present a concern for phototoxicity or photoallergenicity.

10.5.1. Risk assessment

There are no phototoxicity studies available for 5-ethylidenebicyclo[2.2.1]hept-2-yl propionate in experimental models. UV/Vis absorption spectra indicate no significant absorption between 290 and 700 nm. Corresponding molar absorption coefficient is well below the benchmark of concern for phototoxicity and photoallergenicity, 1000 L mol^{-1} cm^{-1} (Henry et al., 2009). Based on lack of absorbance, 5-ethylidenebicyclo[2.2.1]hept-2-yl propionate does not present a concern for phototoxicity or photoallergenicity.

Additional References: None.

Literature Search and Risk Assessment Completed on: 07/19/16.

10.6. Local respiratory toxicity

The margin of exposure could not be calculated due to lack of appropriate data. The material, 5-ethylidenebicyclo[2.2.1]hept-2-yl propionate, exposure level is below the Cramer Class III* TTC value for inhalation exposure local effects.

10.6.1. Risk assessment

There are no inhalation data available on 5-ethylidenebicyclo [2.2.1]hept-2-yl propionate. Based on the Creme RIFM model, the inhalation exposure is 0.0049 mg/day. This exposure is 95.9 times lower than the Cramer Class III* TTC value of 0.47 mg/day (based on human lung weight of 650 g; Carthew et al., 2009); therefore, the exposure at the current level of use is deemed safe.

*As per Carthew et al., 2009, Cramer Class II materials default to Cramer Class III.

Additional References: None.

Literature Search and Risk Assessment Completed on: 7/20/2016.

2 Environmental Endpoint Summary:

10.7. Screening-level assessment

A screening level risk assessment of 5-ethylidenebicyclo[2.2.1] hept-2-yl propionate was performed following the RIFM Environmental Framework (Salvito et al., 2002) which provides for 3 levels of screening for aquatic risk. In Tier 1, only the material's volume of use in a region, its log K_{ow} and molecular weight are needed to estimate a conservative risk quotient (RQ; Predicted Environmental Concentration/Predicted No Effect Concentration or PEC/PNEC). In Tier 1, a general QSAR for fish toxicity is used with a high uncertainty factor as discussed in Salvito et al., 2002. At Tier 2, the model ECOSAR (providing chemical class specific ecotoxicity estimates) is used and a lower uncertainty factor is applied. Finally, if needed, at Tier 3, measured biodegradation and ecotoxicity data are used to refine the RQ (again, with lower uncertainty factors applied to calculate the PNEC). Provided in the table below are the data necessary to calculate both the PEC and the PNEC determined within this Safety Assessment. For the PEC, while the actual regional tonnage is not provided, the range from the most recent IFRA Volume of Use Survey is reported. The PEC is calculated based on the actual tonnage and not the extremes noted for the range. Following the RIFM Environmental Framework, 5-ethylidenebicyclo[2.2.1]hept-2-yl propionate was identified as a fragrance material with no potential to present a possible risk to the aquatic environment (i.e., its screening level PEC/PNEC <1).

A screening-level hazard assessment using EPISUITE ver 4.1 did not identify 5-ethylidenebicyclo[2.2.1]hept-2-yl propionate as either being possibly persistent nor bioaccumulative based on its structure and physical-chemical properties. This screening level hazard assessment is a weight of evidence review of a material's physical-chemical properties, available data on environmental fate (e.g., OECD Guideline biodegradation studies or die-away studies) and fish bioaccumulation, and review of model outputs (e.g., USEPA's BIOWIN and BCFBAF found in EPISUITE ver.4.1).

10.7.1. Risk assessment

Based on current Volume of Use (2011), 5-ethylidenebicyclo [2.2.1]hept-2-yl propionate does not present a risk to the aquatic compartment in the screening level assessment.

10.8. Biodegradation

No data available.

10.9. Ecotoxicity

No data available.

10.10. Other available data

5-Ethylidenebicyclo[2.2.1]hept-2-yl propionate has been pre-registered for REACH with no additional data at this time.

10.10.1. Risk assessment refinement

Ecotoxicological data and PNEC derivation (all endpoints reported in mg/L; PNECs in μg/L).

Endpoints used to calculate PNEC are underlined.

	LC50 (Fish)	EC50 (Daphnia)	EC50 (Algae)	AF	PNEC	Chemical Class
RIFM Framework Screening Level **(Tier 1)**	6.456 mg/l			1,000,000	0.006456 μg/l	

Exposure information and PEC calculation (following RIFM Environmental Framework: Salvito et al., 2002).

Exposure	Europe (EU)	North America (NA)
Log K_{ow} used	3.85	3.85
Biodegradation Factor Used	0	0
Dilution Factor	3	3
Regional Volume of Use Tonnage Band	<1	<1
Risk Characterization: PEC/PNEC	**<1**	**<1**

Based on available data, the RQ for this material is < 1. No further assessment is necessary.

The RIFM PNEC is 0.006456 μg/L. The revised PEC/PNECs for EU and NA: Not Applicable; cleared at screening level therefore, does not present a risk to the aquatic environment at the current reported volumes of use.

Literature Search and Risk Assessment Completed on: 4/21/15.

11. Literature search*

- **RIFM database:** target, Fragrance Structure Activity Group materials, other references, JECFA, CIR, SIDS
- **ECHA:** http://echa.europa.eu/
- **NTP:** http://tools.niehs.nih.gov/ntp_tox/index.cfm
- **OECD Toolbox**
- **SciFinder:** https://scifinder.cas.org/scifinder/view/scifinder/scifinderExplore.jsf
- **PUBMED:** http://www.ncbi.nlm.nih.gov/pubmed
- **TOXNET:** http://toxnet.nlm.nih.gov/
- **IARC:** (http://monographs.iarc.fr)
- **OECD SIDS:** http://www.chem.unep.ch/irptc/sids/oecdsids/sidspub.html
- **EPA Actor:** http://actor.epa.gov/actor/faces/ACToRHome.jsp;jsessionid=0EF5C212B7906229F477472A9A4D05B7
- **US EPA HPVIS:** http://www.epa.gov/hpv/hpvis/index.html
- **US EPA Robust Summary:** http://cfpub.epa.gov/hpv-s/
- **Japanese NITE:** http://www.safe.nite.go.jp/english/db.html
- **Japan Existing Chemical Data Base:** http://dra4.nihs.go.jp/mhlw_data/jsp/SearchPageENG.jsp
- **Google:** https://www.google.com/webhp?tab=ww%26ei=KMSoUpiQK-arsQS324GwBg&ved=0CBQQ1S4

* Information sources outside of RIFM's database are noted as appropriate in the safety assessment. This is not an exhaustive list.

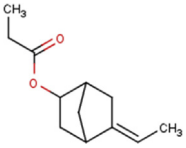
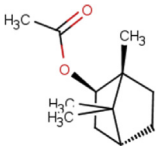
Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.fct.2016.12.009.

Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.fct.2016.12.009.

Appendix

	Target Material	Read across Material
Principal Name	5-Ethylidenebicyclo[2.2.1]hept-2-yl propionate	Isobornyl acetate
CAS No.	73347-77-0	125-12-2
Structure		
3D Structure	http://www.thegoodscentscompany.com/opl/73347-77-0.html	http://www.thegoodscentscompany.com/opl/125-12-2.html
Read-across endpoint		• Genotoxicity • Repeated Dose • Devel/Repro • Skin sensitization
Molecular Formula	C12H18O2	C12H20O2
Molecular Weight	194.28	196.29
Melting Point (°C, EPISUITE)	33.23	34.11
Boiling Point (°C, EPISUITE)	252.56	225.89
Vapor Pressure (Pa @ 25°C, EPISUITE)	2.626	14.27
Log K_{ow} (KOWWIN v1.68 in EPISUITE)	3.85	3.86
Water Solubility (mg/L, @ 25°C, WSKOW v1.42 in EPISUITE)	24.26	9.721
J_{max} (mg/cm^2/h, SAM)	83.8464125	18.65520626
Henry's Law (Pa·m^3/mol, Bond Method, EPISUITE)	45.92049	44.228362
Similarity (Tanimoto score)[a]		64%
Genotoxicity		
DNA binding (OASIS v1.1)	• No alert found	• Schiff base formers •Schiff base formers >> Direct acting Schiff base formers • Schiff base formers >> Direct acting Schiff base formers >> Specific Acetate Esters • SN1 • SN1 >> Carbenium ion formation • SN1 >> Carbenium ion formation >> Specific Acetate Esters • SN2 • SN2 >> Acylating agents • SN2 >> Acylating agents >> Specific Acetate Esters • SN2 >> SN2 at sp3-carbon atom • SN2 >> SN2 at sp3-carbon atom >> Specific Acetate Esters
DNA binding (OECD)	• No alert found	• No alert found
Carcinogenicity (genotox and non-genotox) alerts (ISS)	• No alert found	• No alert found
DNA alerts for Ames, MN, CA (OASIS v1.1)	• No alert found	• No alert found

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(*continued*)

	Target Material	Read across Material
In vitro mutagenicity (Ames test) alerts (ISS)	• No alert found	• No alert found
In vivo mutagenicity (Micronucleus) alerts (ISS)	• H-acceptor-path3-H-acceptor	• H-acceptor-path3-H-acceptor
Oncologic classification (OECD)	• Not classified	• Not classified
Repeated Dose Toxicity		
Repeated dose (HESS)	Not categorized	Not categorized
Developmental and Reproductive Toxicity		
ER binding (OECD)	Non binder, without OH or NH2 group	Non binder, without OH or NH2 group
Developmental toxicity model (CAESAR v2.1.6)	Toxicant (good reliability)	NON-Toxicant (low reliability)
Skin Sensitization		
Protein binding (OASIS v1.1)	• No alert found	• No alert found
Protein binding (OECD)	• Acylation • Acylation >> Direct Acylation Involving a Leaving group • Acylation >> Direct Acylation Involving a Leaving group >> Acetates	• Acylation • Acylation >> Direct Acylation Involving a Leaving group • Acylation >> Direct Acylation Involving a Leaving group >> Acetates
Protein binding potency (OECD)	• Not possible to classify according to these rules (GSH)	• Not possible to classify according to these rules (GSH)
Protein binding alerts for skin sensitization (OASIS v1.1)	• No alert found	• No alert found
Skin sensitization model (CAESAR v2.1.6)	Sensitizer (good reliability)	Sensitizer (good reliability)
Metabolism		
Rat liver S9 metabolism simulator (OECD)	See Supplemental Data 1	See Supplemental Data 2

[a] Values calculated using Babel with FP2 fingerprint (O'Boyle et al., 2010).

Summary

There are insufficient toxicity data on 5-ethylidenebicyclo[2.2.1]hept-2-yl propionate (RIFM# 5984, CAS# 73347-77-0). Hence, *in silico* evaluation was conducted to determine suitable read-across material. Based on structural similarity, reactivity, metabolism data, physicochemical properties and expert judgment, the above shown read-across materials were identified as proper read across for their respective toxicity endpoints.

Methods

- The identified read-across analogs were confirmed by using expert judgment
- The physicochemical properties of target and analogs were calculated using EPI Suite™ v4.11 developed by US EPA (USEPA, 2012)
- The J_{max} were calculated using RIFM skin absorption model (SAM), the parameters were calculated using consensus model (Shen et al., 2014)
- DNA binding, mutagenicity, genotoxicity alerts and oncologic classification were estimated using OECD QSAR Toolbox (v3.1) (OECD, 2012)
- ER binding and repeat dose categorization were estimated using OECD QSAR Toolbox (v3.1) (OECD, 2012)
- Developmental toxicity and skin sensitization were estimated using CAESAR (v.2.1.6) (Cassano et al., 2010)
- Protein binding were estimated using OECD QSAR Toolbox (v3.1) (OECD, 2012)
- The major metabolites for the target and read-across analogs were determined and evaluated using OECD QSAR Toolbox (v3.1) (OECD, 2012)

Conclusion/Rationale

- Isobornyl acetate (analog) was used as a read-across analog for 5-ethylidenebicyclo[2.2.1]hept-2-yl propionate (target) based on:
  - The target and analogs belong to the generic class of aliphatic esters, specifically, esters/cyclic alcohol simple acid esters/bicyclic/secondary alcohols.
  - The target and analog have similar carboxylic acid part and similar alcohol part.
  - The key difference is that the target is an acetate while the analog is a propionate. Besides, the substitutes in the alcohol part are also different. The differences between structures do not essentially change the physicochemical properties nor raise any additional structural alerts and therefore, the toxicity profiles are expected to be similar.
  - The target and analog show similar alerts for DNA binding, mutagenicity, genotoxicity and oncologic classification. An exception was noted for DNA binding alerts generated by Oasis v1.1 for isobornyl acetate.
  - The target and analog show similar alerts for Repeated Dose (HESS) Categorization and ER binding. ER binding is molecular initiating event. ER binding is not necessarily predictive of endocrine disruption given the complex pre- and post-receptor events that determine activity.
  - The target and analog show similar alerts for protein binding.
  - The target and analog are expected to be metabolized similarly. As per the OECD Toolbox, they are predicted to have similar metabolites.

Explanation of Cramer Class

Due to potential discrepancies with the current *in silico* tools (Bhatia et al., 2015), the Cramer class of the target material was determined using expert judgment based on the Cramer decision tree (Cramer et al., 1976).

Q1 Normal constituent of the body? No
Q2 Contains functional groups associated with enhanced toxicity? No
Q3 Contains elements other than C,H,O,N, divalent S? No
Q5 Simply branched aliphatic hydrocarbon or a common carbohydrate? No

Q6 Benzene derivative with certain substituents? No
Q7 Heterocyclic? No
Q16 Common terpene? No
Q17 Readily hydrolysed to a common terpene? No
Q19 Open chain? No
Q23 Aromatic? No
Q24 Monocarbocyclic with simple substituents? No
Q25 Cyclopropane, cyclobutane with certain substituents or a mono- or bicyclic sulphide or mercaptan? No
Q26 Monocycloalkanone or a bicyclocompound? Yes Class Intermediate (Class II)

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