SILIFOOD application

User Manual

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1. Introduction

1.1. Objective of the tool

The SILIFOOD application is a stand-alone, publicly accessible software to collect regulatory and hazard data of **single organic (non-)intentionally added substances** present in Food Contact Materials (FCM).

The application combines existing toxicological data with (quantitative) structure-activity relationship ((Q)SAR) predictions, developed to ensure a fast risk assessment of non-evaluated FCM substances.

1.2. General information

Regulation (EU) No 1935/2004 requires that all materials and articles intended to come into contact directly or indirectly with food (FCM) must be safe for human health (Regulation (EC) No 1935/2004). At present, the safety assessment of non-evaluated food contact material (FCM) substances is hampered by the lack of a legislative framework and clear guidance. In 2019, EFSA proposed a methodology[1] for the fast evaluation of food contaminants, but its application to non-evaluated FCM substances is challenging, especially when only limited toxicological data are available. Furthermore, the potential of *in silico* models to fill gaps in toxicological knowledge is not exploited in the EFSA methodology.

In order to support the safety assessment of non-evaluated FCM, an automated workflow for data collection was developed integrating also *in silico* models. In line with the EFSA methodology, information sources for chemicals related to genotoxicity, carcinogenicity, health-based guidance values (HBGV) or reference points were identified. Additionally, other data sources providing information on the evaluation status and migration limits for FCM substances were selected. In order to further support the risk assessment of non-evaluated FCM substances, (Q)SAR models from the VEGA Hub (https://www.vegahub.eu/) predicting several relevant toxicological endpoints were also included in the workflow.

The selected toxicological information sources and (Q)SAR models were integrated into an automated workflow consisting of the following steps:

Step 1. Molecule input / Identification of the substance

Step 2. Data collection: Toxicological data in the context of FCM use

Step 3. Data collection: Toxicological data in a non-FCM context

Step 4. Presence on lists of substances with toxicological concern

Step 5. Bioavailability/bioaccumulation potential

Step 6. Hazard identification by (Q)SAR predictions

[1] EFSA (European Food Safety Authority), 2019. Scientific technical assistance to RASFF on chemical contaminants: Risk evaluation of chemical contaminants in food in the context of RASFF notifications. EFSA supporting publication 2019:EN-1625. 108 pp. doi:10.2903/sp.efsa.2019.EN-1625

- Genotoxicity
- Carcinogenicity
- Reproductive and developmental toxicity
- NOAEL (subchronic oral toxicity data: 90-day study)
- Endocrine activity

Step 7. Information to support the Threshold of Toxicological Concern (TTC) approach

2. Scope of the tool

This version of the SILIFOOD application has been developed specifically to support the assessment of non-evaluated FCM substances and focuses on single organic substances.

The following substances are outside the scope of the tool:

- Inorganic chemicals
- Substances containing "unusual elements" different from C, O, N, S, P, Cl, Br, F
- Stereoisomers since SMILES do not take into account the 3D structure
- Mixtures that can contain several compounds (isomers or not) as the SMILES will be referring only to one constituent of the mixture.
- Salts

Athough the SILIFOOD application may create a report for these groups of substances, the report may be incomplete or contain incorrect *in silico* predictions. The user must therefore be careful and ensure that the substance of interest does not belong to one of these categories.

3. How to install the SILIFOOD application

The SILIFOOD application is a Java stand-alone application that does not connect to any remote server or share information with a remote machine. After its installation, the application can be used without internet connection.

To install the application, the users must download the file.zip on their computer. Then, the zipped file has to be unpacked by selecting the file and clicking on 'Extract all'. The application is started after a double click on the file 'starter' in the **unpacked** application folder.

Remark: The application does not require the installation of Java for its use on Windows (due to the inclusion of OpenJdk 11 distribution). However, its use on other operating systems (e.g. Linux or Mac OS X) requires the installation of the appropriate JAVA version.

4. How to run the SILIFOOD application

The application can be run in just three steps (Figure 1):

1. **Insert the 'Input compound' (chemical identifier) and select the 'Input type'**
   The input type can be the CAS number, the SMILES (in case of salt, the original SMILES, i.e. the non-neutralized form, has to be inserted) or the FCM number (i.e. the number associated to the FCM compound present on the Annex I of the Regulation No 10/2011) of the target compound.
2. **Select the directory for the output on your computer by clicking on 'Select directory'**
   The pdf report generated by the tool will appear in the selected folder.
3. Click on **'Start calculation'** to run the workflow, and the report will be generated.

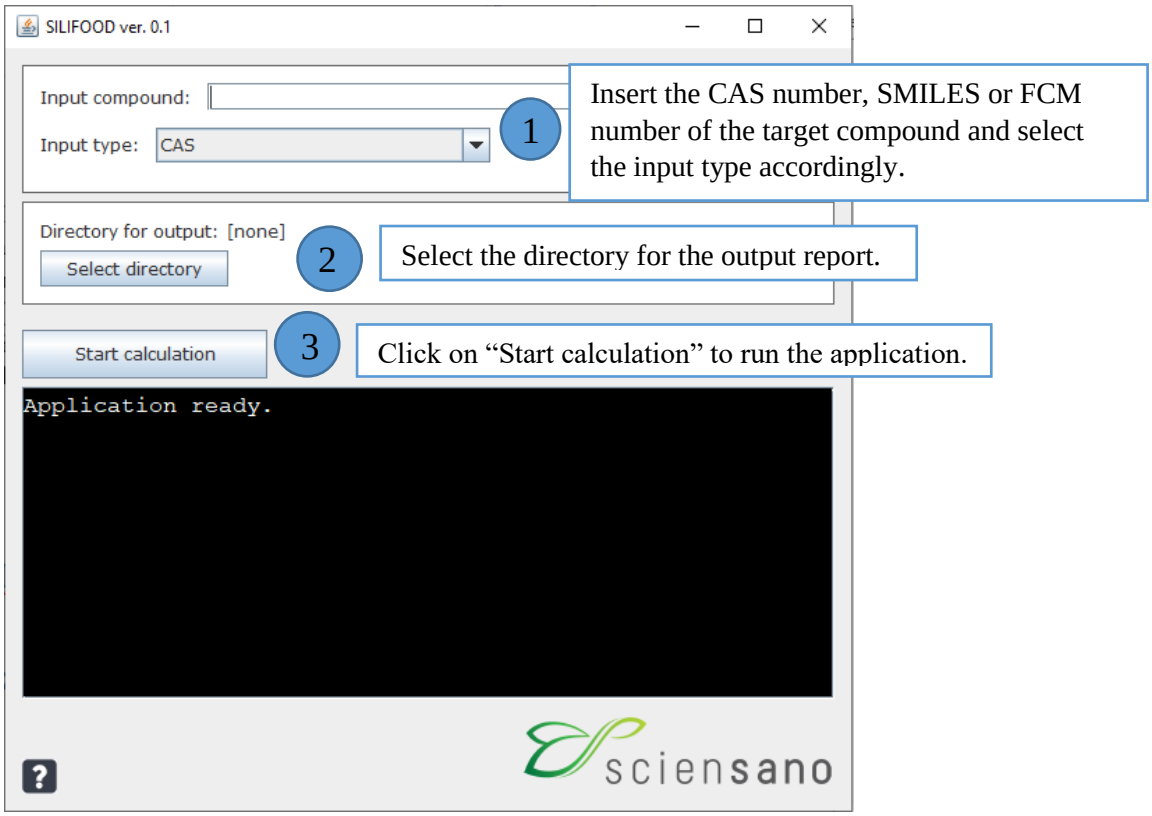


Figure 1: Interface of the SILIFOOD application

While the application is running, the following messages appear successively: "Starting calculation… Creating pdf report…done!" (Figure 2). Once the last message is displayed, the application is ready to be run with a new chemical identifier. Thus, the application can be used continuously by repeating the process from step 1. A pdf report will be generated for each "calculation". The generated pdf reports can be found in the selected folder.

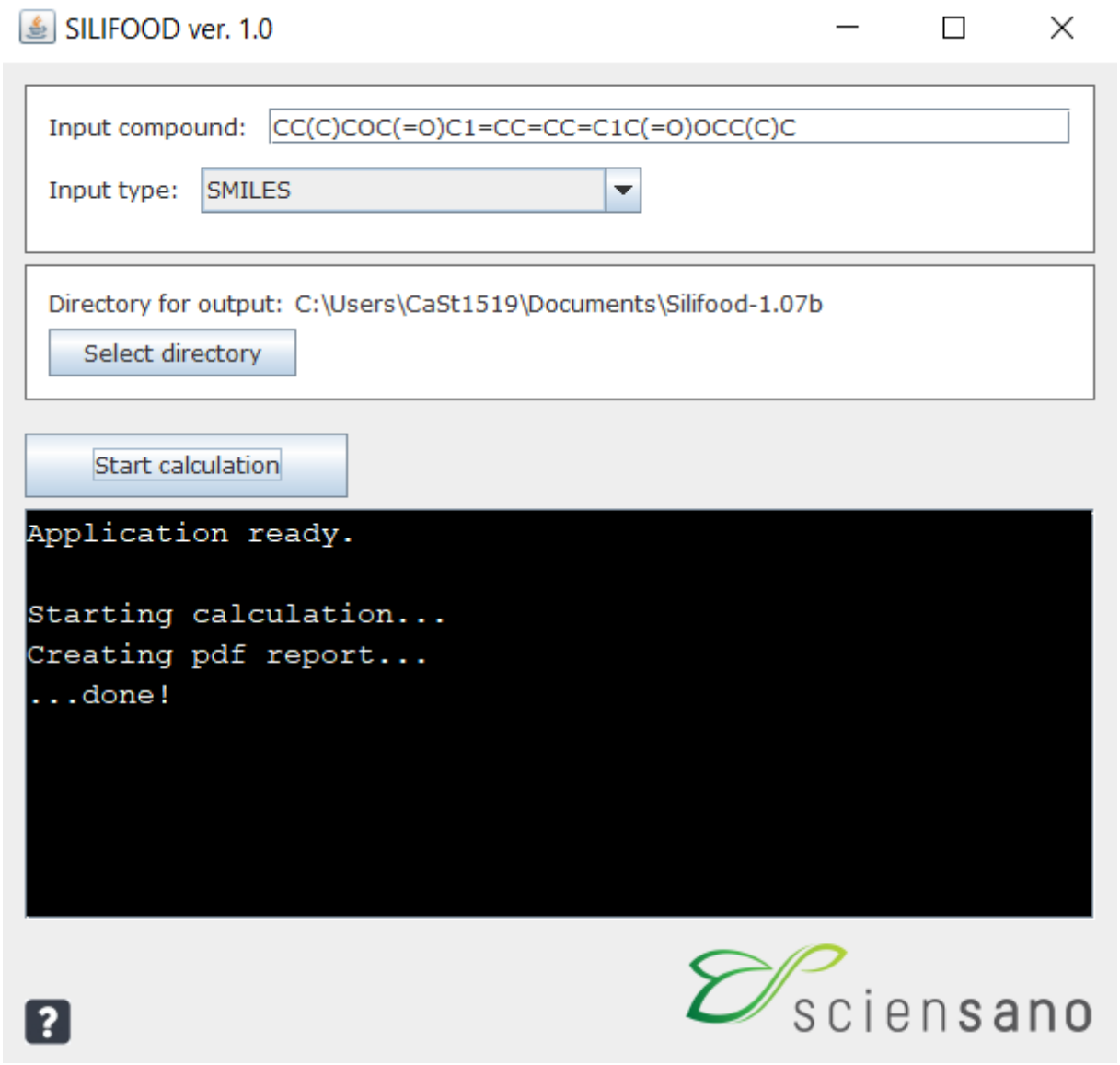


Figure 2: SILIFOOD application interface after successive runs

5. How to collect toxicological data from the SILIFOOD report

5.1. Substance identification

The first part of the report generated by the workflow (Annex 1) is the 'Identification of the substance'. In this first part, an overview is provided of all the information sources containing data on the substance of interest together with the substance name(s) used in the respective source(s). Other information on the substance listed in this table includes the molecular structure, synonyms if available, CAS number(s), EC number(s), molecular formula and (Original and VEGA) SMILES, allowing the user to check the identity of the compound. Original SMILES refer to any non-curated SMILES corresponding to the substance while the VEGA SMILES correspond to neutralized curated SMILES.

5.2. Data collection

The second step of the report summarizes the information collected from the different inventories included in the in-house FCM database of Sciensano. Parts three and four summarize the information collected from evaluations done in a non-FCM context or from lists with substances of concern, respectively.

5.2.1. Information from the in-house Food Contact Material database

This in-house database developed by Sciensano lists the compounds that can be used in different types of FCM and their associated information on restrictions of use, legislation, and evaluation status. The database includes information from the following inventories:

- The Annex I of authorized substances for plastic FCM;
- The Synoptic Document (SANCO D3/AS D(2005)) constituting an inventory of monomers and additives used in plastics or coating;
- The Annex 10 of Swiss Ordinance on FCM including lists of permitted substances for the manufacture of packaging inks;
- The resolutions of the Council of Europe about silicones, paper and board, coatings and cork;
- The EFSA ESCO reports which are collection of substances used in non-plastic FCM and present in positives lists of European countries. The types of FCM considered by the EFSA ESCO reports are paper and board, coatings, printing inks, colorants, rubber, silicones, cork and wood .

Consultation of the FCM database allows to check if an evaluation for use in a specific FCM has been done for the substance of interest, if a legal limit or reference value exists such as a specific migration limit (SML) together with specification(s) and/or restriction(s) as well as a health based guidance value (HBGV). In some cases, information about the carcinogenicity, mutagenicity or reproductive toxicity (CMR) of a substance can also be found in this database.

5.2.2. Evaluation in a non-FCM context

In this third section, information on the substance of interest, if available, from four data sources is provided i.e. the EFSA OpenFoodTox database, the Annex VI of the CLP Regulation (EC) No 1272/2008, the CoRAP list and the biocidal active substances list.

EFSA OpenFoodTox database

The European Food Safety Authority (EFSA) regularly provides risk assessment reports and/or opinions on different substances that can contaminate food or feed. More than 4,950 substances have been evaluated in over 2,000 scientific opinions, statements and/or conclusions. Since 2021, data for each substance have been collected and structured in the EFSA's chemical hazards database, namely the OpenFoodTox database. The OpenFoodTox database provides open-source data for the substance

characterisation, the links to EFSA's related output, background European legislation, and a summary of the critical toxicological endpoints and reference values (Chemical hazards | EFSA).

The SILIFOOD application checks if the substance of interest is present in the OpenFoodTox database and provides the reference of the related EFSA output(s). The output(s) can then be manually consulted through the link provided by the SILIFOOD application, or the user can consult the freely available OpenFoodTox database to collect the genotoxicity study results, the critical toxicological endpoints and reference values available (https://www.efsa.europa.eu/en/data-report/chemical-hazards-database-openfoodtox)

Annex VI of the CLP Regulation (EC) No 1272/2008

The harmonized CLP classifications for CMR (carcinogenic, mutagenic and reproductive toxic) compounds is provided. If available, other CLP classifications for these CMR compounds are provided as well. The different (sub)categories of CMR according to the CLP Regulation (EC) No 1272/2008 are described below:

- ♣ Cat 1A: Substances known to have CMR potential for humans, mainly based on human evidence;
- ♣ Cat 1B: Substances presumed to have CMR potential for humans, mainly based on experimental animal data;
- ♣ Cat 2: Substances suspected to have CMR potential for humans based on the evidence obtained from human and/or animal studies but which is not sufficiently convincing to place the substance in Category 1A or 1B.

CoRAP list and biocidal active substances list

The CoRAP (Community Rolling Action Plan) list and the biocidal active substances list provide information on the evaluation status of substances according to REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals) Regulation (EC) No 1907/2006 and the Biocidal Product Regulation (EU) No 528/2012, respectively. If the substance is present on the list, the link(s) to the assessment document(s) are provided. In the CoRAP list, the initial ground of concern is also indicated, including (suspected) CMR or ED properties.

5.2.3. Inclusion in lists of substances of concern

Section 4 of the report generated by the SILIFOOD application includes information found in three lists (or groups of lists) containing substances of concern i.e. the Candidate list of ECHA, the SIN list and the Endocrine Disruptor Lists.

Candidate list (ECHA)

The Candidate list of ECHA gives an overview of the Substances of Very High Concern (SVHC) produced or imported in Europe. The substances identified as SVHC are substances meeting one of the following criteria: (i) the substance is classified as CMR category 1A or 1B according to the CLP Regulation, (ii) the substance is persistent, bioaccumulative and toxic (PBT) or very persistent and very bioaccumulative (vPvB) or (iii) substance that causes an equivalent level of concern, e.g. substance with endocrine disrupting properties. The process of inclusion of substances on this list starts when a Member State or the ECHA, at the request of the Commission, proposes a substance to be identified as SVHC. The proposal is subject to a consultation for 45 days before being voted by the Member State Committee (MSC). If the MSC reaches a unanimous agreement, the substance is added to the Candidate List (Substances of Very High Concern - ECHA).

SIN List

The SIN (Substitute It Now!)-list 2.1 contains substances and substance groups that were identified by the International Chemical Secretariat (ChemSec, Gothenburg, Sweden) to be of 'very high concern' based on the criteria established in article 57 of Regulation (EC) No 1907/2006 (Regulation (EC) No 1907/2006). In addition to chemicals characterized as CMR, persistent, bioaccumulative and toxic (PBT), very persistent and very bioaccumulative (vPvB), the list contains a category of chemicals posing an 'equivalent environmental or health threat'. The latter includes, besides chemicals that are less toxic but highly bioaccumulative and/or persistent, also chemicals with ED properties (article 57(f), Regulation (EC) No. 1907/2006). Identification of chemicals as EDs by ChemSec was based on the criteria specified by the Danish Centre on Endocrine Disrupters (2012) (Geueke, Wagner, and Muncke 2014; SIN List).

Endocrine Disruptor Lists

The Endocrine Disruptor lists (https://edlists.org/) are an initiative from different national authorities, including Belgium, Denmark, France, The Netherlands, Sweden and Spain, consisting of 3 lists of substances identified or suspected to be EDs by European or National authorities. Substances on these lists have been evaluated (list I) or are under evaluation (list II) for (suspected) ED properties in the frame of an EU Regulation (such as REACH Regulation No 1907/2006, the Plant Protection Products Regulation (PPPR) (EC) No 1107/2009 and (EU) No 2018/605, the biocidal products regulation (EU) No 528/2012 and (EU) No 2017/2100 or the Cosmetic Regulation (EC) No 1223/2009) or following an evaluation by the National Authority of a EU member states supporting the initiative (List III).

5.3. *In silico* methods and (Q)SAR predictions

In the last three sections of the report, the results of the *in silico* predictions are provided. Section 5 of the report summarizes (predicted/calculated) parameters related to the bioavailability (and bioaccumulation) potential of the compounds of interest, whereas sections 6 includes the predictions for

the toxicological endpoints of interest. In section 7, infomation to support the application of the Threshold of Toxicological Concern (TTC) approach is included.

Bioavailability/bioaccumulation potential

In this section, the molecular properties (molecular weight, number of hydrogen bond donor and acceptor, polar surface area and number of rotating bond) automatically calculated by descriptors are combined with the Log P value (predicted by the Meylan/Kowwin VEGA model) to evaluate whether the criteria for bioavailability (Van Bossuyt et al., 2016) are fulfilled.

A compound is likely to be bioavailable if :

- The Lipinski rules (Lipinski et al. 1997)is respected i.e. there is no more than one violation of the following criteria (Schilter et al., 2013): (i) a molecular weight ≤ 500 g/mol, (ii) a logP ≤ 5, (iii) maximum 5 hydrogen bond donors and (iv) maximum 10 hydrogen bond acceptors.
- The polar surface area of the target compound is below or equal to 140 Angström (Ghose et al., 1999; Veber et al., 2002)
- The number of rotating bonds of the target compound is below or equal to 10 (Veber et al., 2002).

In case all these criteria are met, 'Yes' will be displayed after 'Bioavailability'. If one of the criteria is not fulfilled, 'No' will be visible after 'Bioavailability' to reflect that at least one criterium compromises/disagrees with the bioavailability potential.

Hazard predictions using VEGA models

The endpoints covered by the QSAR models are genotoxicity, carcinogenicity, reproductive and developmental toxicity, subchronic oral toxicity (NOAEL calculation) and endocrine activity (Table 1).

The color of the boxes containing the model's output depends on the hazard level of concern: green for no concern, yellow for possible concern and red for concern.

Additionally, for all the VEGA models, the prediction is associated with the level of reliability (good, moderate or low reliability), except for the mutagenicity consensus model, where the reliability is reflected in the consensus score (CS). The CS takes into account the predictions of the four mutagenicity models included in the consensus model and their associated level of reliability. The CS is a value from 0 to 1 and can be interpreted as low reliability if CS ≤ 0.15, moderate reliability if CS is between 0.15 and 0.5, and good reliability if CS ≥ 0.5. The categorization into good, moderate and low reliability for the other models is based on the applicability domain index (ADI). The ADI (not indicated in the report) is a global index from 0 (worst case) to 1 (best case) calculated by a specific algorithm taking into account several parameters, also expressed numerically, such as: the degree of similarity of the 3 most similar compounds from the dataset to the assessed compound (similarity index); the ability of the model

to provide an exact prediction for these similar compounds (accuracy index); the concordance between the predictions provided for these similar compounds and the assessed compound (concordance index) and the identification of molecular fragments present in the assessed compound that would be rare or absent among the compounds of the dataset (Atom Centered Fragment index) (Benfenati E. 2016).

For all models, the message 'Experimental value' is provided when an experimental value for the substance of interest was included in the dataset used to build the model.

More information on the models can be found on the VEGA website https://www.vegahub.eu/download/vega-interpretation/. For clarity and to keep the report easy to read, the ADI and the other calculated indexes used to determine the prediction's level of reliability do not appear in the output report. In order to obtain the ADI associated with each VEGA model prediction, the VEGA application can be run separately with the VEGA SMILES provided by the workflow.

Table 1 : Overview of the VEGA models included in the SILIFOOD application

Toxicological data required	**Models selected from VEGA**
➢ Data to demonstrate absence of accumulation potential in man	• LogP model (Meylan/Kowwin)
➢ Genotoxicity	
• Gene mutations	• Mutagenicity (Ames test) CONSENSUS model
• Structural and numerical chromosome aberration	• Chromosomal aberration model (CORAL) • *In vitro* Micronucleus activity (IRFMN/Vermeer) • *In vivo* Micronucleus activity (IRFMN)
➢ Data from long term toxicity/carcinogenicity studies	• Carcinogenicity model (CAESAR) • Carcinogenicity model (ISS) • Carcinogenicity model (IRFMN/ISSCAN-CGX) • Carcinogenicity model (IRFMN/Antares) • Carcinogenicity oral classification model (IRFMN)
➢ Data on reproductive and developmental toxicity	• Developmental Toxicity model (CAESAR) • Developmental/Reproductive Toxicity library (PG)
➢ Subchronic oral toxicity data (90-day study)	• NOAEL (IRFMN/CORAL)
➢ Endocrine disruption	• Estrogen Receptor Relative binding Affinity Model (IRFMN) • Estrogen receptor-mediated effect (IRFMN/CERAPP) • Androgen receptor-mediated effect (IRFMN/COMPARA) • Thyroid receptor alpha effect (NRME) • Thyroid receptor beta effect (NRME)

Information to support the Threshold of Toxicological Concern (TTC) approach

The Threshold of Toxicological Concern (TTC) approach assigns non-evaluated substances to a class based on their chemical structure. Each class is associated with a threshold (in µg/person/day or µg/body weight/day) of exposure, below which there is expected to be no appreciable risk to human health.

Importantly, this approach can only be applied in absence of toxicological data and is not applicable to some specific groups of substances such as inorganic substances, proteins, nanomaterials, radioactive substances, organosilicon substances, metals, substances with special properties such as high potency carcinogens (i.e aflatoxin-like, azoxy- or N-nitroso substances and benzidines), steroids and substances with a potential for bioaccumulation (including substances like polyhalogenated, -dibenzodioxins, -dibenzofurans and –biphenyls) (EFSA, 2019).

If the compound of interest is not included in one of the categories mentioned above, the Cramer Class provided by the VEGA model in the SILIFOOD application can be used to derive the TTC value. The VEGA implementation of the Cramer classification model is a structure-based model using the same Cramer classification scheme (or decision tree) as implemented in the Toxtree software (Patlewicz et al. 2008). The model classifies the compound of interest based on its structure in the 3 Cramer classes related to the expected severity of the effect: Class I (lower concern), Class II (intermediate concern) and Class III (higher concern). All the values applying for food contact materials (FCM) compounds are summarized in Table 2. More information can be found in the EFSA document 'Guidance on the use of the Threshold of Toxicological Concern approach'.

Important note: Potential DNA-reactive mutagens and/or carcinogens and organophosphates and carbamates have their own TTC values, which is not attributed automatically by the VEGA model! The information provided by the SILIFOOD application should thus be carefully interpreted when used to support the application of the TTC approach.

*Table 2: Overview of the threshold values for applying the Threshold of Toxicological Concern (TTC) approach to FCM compounds; *excluding aflatoxin-like, azoxy- or N-nitroso compounds; bw : body weight.*

Classification	**TTC in µg/person per day**	**TTC in µg/kg bw per day**
Potential DNA-reactive mutagens and/or carcinogens*	0.15	0.0025
Organophosphates and carbamates	18	0.3
Cramer Class III	90	1.5
Cramer Class II	540	9
Cramer Class I	1800	30

5.4. Common abbreviations used in the reports

ADI : Applicability Domain Index

ADI : Acceptable Daily Intake

BfR : Bundesinstitut für Risikobewertung / German Federal Institute for Risk Assessment

BIBRA : British Industrial Biological Research Association (UK)

CIVO-TNO : Central Institute for Nutrition and Food Research (NL)

CLP : Classification, Labelling and Packaging

CMR : Carcinogenic, Mutagenic or toxic for Reproduction

CoE : Council of Europe

CoRAP : Community Rolling Action Plan

EC : European Commission

ECHA : European Chemicals Agency

ESCO : EFSA Scientific Cooperation

ED : Endocrine Disruptor(s)

EFSA : European Food Safety Authority

EU : European Union

FAO : Food and Agriculture Organization (UN)

FCC : Food Contact Chemical(s)

FCM : Food Contact Material(s)

FRF : Fat Reduction Factor

HRC : Huntingdon Research Centre (UK)

IARC : International Agency for Research on Cancer

IRFMN : IRCCS - Istituto di Recerche Farmacologiche Mario Negro / Mario Negri Institute for Pharmacological research

JECFA : Joint FAO/WHO Expert Committee on Food Additive (UN)

LMS : Limite de Migration Spécifique

LOAEL : Lowest Observed Adverse Effect Level

MS : Member State(s)

MSC : Member State Committee

MTDI : Maximum Tolerable Daily Intake

NIAS : Non-Intentionally Added Substances

NOAEL : No Observed Adverse Effect Level

NTP : National Toxicology Program (USA)

NS : Not specified

OECD : Organisation for Economic Cooperation and Development

PAH : Polycyclic Aromatic Hydrocarbons

PBT : Persistent, Bioaccumulative and Toxic

PMTDI : Provisional Maximum Tolerable Daily Intake

PT : Product Type

PTWI : Provisional Tolerable Weekly Intake

(Q)SAR : (Quantitative) Structure Activity Relationship

RASFF : Rapid Alert System for Food and Feed

REACH : Registration, Evaluation, Authorisation and Restriction of Chemicals

RIVM : National Institute for Public Health and Environmental Protection (NL)

SCC : Scientific Committee for Cosmetology (EEC)

SCF : Scientific Committee on Food (EEC)

SMILES : Simplified Molecular Input Line Entry System

SML(T) : Specific Migration Limit (Total)

SIN List : Substitute It Now! List

SVHC : Substances of Very High Concern

t-ADI : Temporary ADI

t-TDI : Temporary TDI

TDI : Tolerable Daily Intake

TTC : Threshold of Toxicological Concern

vPvB: very Persistent, very Bioaccumulative

WG : Working Group

WHO : World Health Organization

6. References

Annex 6 of the Ordinance of the FDHA on articles and materials of 23 November 2005 (RS 817.023.21)

Benfenati, Emilio, ed. 2016. 1425 In Silico Methods for Predicting Drug Toxicity. New York, NY: Springer. http://link.springer.com/10.1007/978-1-4939-3609-0 (August 24, 2023)

"Chemical Hazards | EFSA." https://www.efsa.europa.eu/en/microstrategy/openfoodtox (October 28, 2022).

Council of Europe Resolutions https://www.edqm.eu/en/food-contact-materials-and-articles

EC, 2005. "Synoptic Document": Provisional List of Monomers and Additives Notified to European Commission as Substances Which May Be Used in the Manufacture of Plastics or Coatings Intended to Come into Contact with Foodstuffs. Sanco D3/AS D, p. 2005.

"Endocrine Disruptor List." https://edlists.org/ (March 30, 2023).

European Commission. 2004. Regulation (EC) No 1935/2004 of the European Parliament and of the Council of 27 October 2004 on Materials and Articles Intended to Come into Contact with Food and Repealing Directives 80/590/EEC and 89/109/EEC. (OP_DATPRO) http://data.europa.eu/eli/reg/2004/1935/2021-03-27/eng (October 28, 2022).

European Commission. 2011. Commission Regulation (EU) No 10/2011 of 14 January 2011 on Plastic Materials and Articles Intended to Come into Contact with Food . *Text with EEA Relevance*. 012 OJ L (COM) http://data.europa.eu/eli/reg/2011/10/oj/eng (October 28, 2022).

European Food Safety Authority; Report of ESCO WG on non-plastic Food Contact Materials. Supporting Publications 2012:139 [63 pp.]. Available online: www.efsa.europa.eu

EFSA (European Food Safety Authority), 2019. Scientific technical assistance to RASFF on chemical contaminants: Risk evaluation of chemical contaminants in food in the context of RASFF notifications. EFSA supporting publication 2019:EN-1625. 108 pp. doi:10.2903/sp.efsa.2019.EN-1625

EFSA Scientific Committee et al. 2019. "Guidance on the Use of the Threshold of Toxicological Concern Approach in Food Safety Assessment." *EFSA Journal* 17(6). https://data.europa.eu/doi/10.2903/j.efsa.2019.5708 (October 27, 2022).

Fürst, Peter et al. 2019. "Risk Evaluation of Chemical Contaminants in Food in the Context of RASFF Notifications." *EFSA Supporting Publications* 16(5): 1625E.

Geueke B, Wagner CC, Muncke J. 2014. Food contact substances and chemicals of concern: a comparison of inventories. Food Addit Contam Part A Chem Anal Control Expo Risk Assess.31 (8):1438e1450.

Ghose, A.K., Viswanadhan, V.N., Wendoloski, J.J., 1999. A knowledge-based approach in designing combinatorial or medicinal chemistry libraries for drug discovery. 1. A qualitative and quantitative characterization of known drug databases. J. Comb. Chem. 1, 55e68.

"Information on Biocides - ECHA." https://echa.europa.eu/information-on-chemicals/biocidal-active-substances (March 30, 2023).

Lipinski, C.A., Lombardo, F., Dominy, B.W., Feeney, P.J., 1997. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv. Drug Deliv. Rev. 23, 3e25.

Patlewicz G, Jeliazkova N, Safford RJ, Worth AP, Aleksiev B. An evaluation of the implementation of the Cramer classification scheme in the Toxtree software. SAR QSAR Environ Res. 2008;19(5-6):495-524. doi: 10.1080/10629360802083871. PMID: 18853299.

"Resolutions & Policy Statements - European Directorate for the Quality of Medicines & HealthCare - EDQM." *European Directorate for the Quality of Medicines & HealthCare*. https://www.edqm.eu/en/ (March 30, 2023).

Schilter, B., Benigni, R., Boobis, A., Chiodini, A., Cockburn, A., Cronin, M.T.D., Lo Piparo, E., Modi, S., Thiel, A., Worth, A., 2013. Establishing the level of safety concern for chemicals in food without the need for toxicity testing. Reg. Tox. Pharm. 68, 275e296.

"SIN List." https://sinlist.chemsec.org/ (October 28, 2022).

"Substances of Very High Concern - ECHA." https://echa.europa.eu/substances-of-very-high-concern-identification-explained (October 27, 2022).

"Substance Evaluation - CoRAP - ECHA." https://echa.europa.eu/information-on-chemicals/evaluation/community-rolling-action-plan/corap-table (March 30, 2023).

"Substances of Very High Concern - ECHA." https://echa.europa.eu/substances-of-very-high-concern-identification-explained (October 27, 2022).

"Table of Harmonised Entries in Annex VI to CLP - ECHA." https://echa.europa.eu/information-on-chemicals/annex-vi-to-clp (March 30, 2023).

Van Bossuyt, Melissa et al. 2016. "Printed Paper and Board Food Contact Materials as a Potential Source of Food Contamination." *Regulatory Toxicology and Pharmacology* 81: 10–19.

Veber, D.F., Johnson, S.R., Cheng, H.Y., Smith, B.R., Ward, K.W., Kopple, K.D., 2002. Molecular properties that influence the oral bioavailability of drug candidates. J. Med. Chem. 45, 2615e2623.

Annex 1: Report generated by the SILIFOOD application for Diisobutylphthalate (CAS number 84-69-5)

SILIFOOD FCM REPORT

SEARCH RESULTS FOR TARGET CAS NUMBER 84-69-5

Engine version: 1.0

Created on: 17 10 2023

This application consists of an automated workflow developed to streamline and speed up the risk assessment of (non-evaluated) FCM substances. The workflow combines existing toxicological data with (quantitative) structure-activity relationship ((Q)SAR) predictions.

Contact

More detailed information can be found in the user manual. For any questions, please contact us through the following email address: Silifood@sciensano.be

Disclaimer

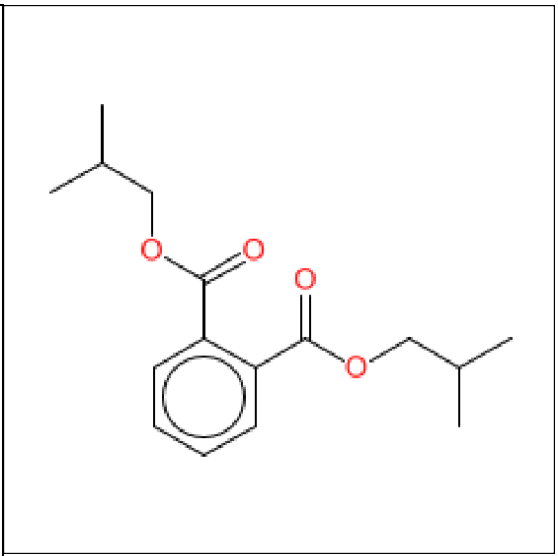
Sciensano ensures the accurate introduction of information in its in-house FCM database as well as the correct extraction of the toxicological data from the various data sources included in this automated workflow (references on the last page of this report), but cannot be held responsible for the scientific accuracy of this information. The presence of a substance in a data source is not necessarily an agreement for its use in materials and articles intended to come into contact with foodstuffs.

The chemical properties, hazard and Cramer classification provided by the automated workflow are predicted using in silico software. In case the text ‘experimental data’ is displayed for an in silico prediction, this means that the compound was included in the training set of that model. These data are not generated or checked by Sciensano.

Acknowledgement

This work was conducted with the financial support of the Federal Public Service Health, Food Chain, Safety and Environment (SILIFOOD project number RF21 6349). This application has been developed by Kode Chemoinformatics under the supervision of Sciensano and in collaboration with the Instituto di Ricerche Farmacologiche Mario Negri (IRFMN).

1. Identification of the substance

Molecular Structure	
Substance name	Phthalic acid, diisobutyl ester **(A, B)** Diisobutyl phthalate (DIBP) **(H)** diisobutyl phthalate **(E, F, G)**
Synonyms	Synonyms not found in data source(s)
CAS number	84-69-5 **(A, B, E, F, G, H)**
EC List number	201-553-2 **(A, E, F, G, H)**
Molecular formula	C16H22O4 **(A)**
Original SMILES	CC(C)COC(=O)C1=CC=CC=C1C(=O)OCC(C)C **(A, B)** CC(C)COC(=O)c1c(cccc1)C(=O)OCC(C)C CC(C)COC(=O)c1ccccc1C(=O)OCC(C)C **(H)** CC(C)COC(=O)c1ccccc1C(=O)OCC(C)C **(E, F, G)**
VEGA SMILES	O=C(OCC(C)C)c1ccccc1(C(=O)OCC(C)C) **(A, B, E, F, H)** CC(C)COC(=O)c1ccccc1C(=O)OCC(C)C **(G)**

Data Sources
A - in-house FCM Database
B - EFSA OpenFoodTox Database
C - CORAP list (ECHA database)
D - Biocidal active substances list (ECHA database)
E - Annex VI CLP Regulation

2. Information from Food Contact Material Database

(Last review: 17/10/2023)

Results for compound Phthalic acid, diisobutyl ester

- EU Regulation 10/2011 Annex I

The compound is not present in Annex I of EU Regulation 10/2011

- Synoptic Document 2005

Restrictions : -

SCF List : 8 - Substances for which no or only scanty and inadequate data were available

EFSA/SCF Opinion : Due to the lack of data according to new SCF guidelines, the substance is transferred from List 6B to List 8. In first instance more data on the migration and use are needed in order to judge if further toxicity data are also needed. Group R: 0.05 mg/kg b.w. Needed: toxicological data depending on migration level (see SCF guidelines) and, if migration exceeds 0.05 mg/kg, peroxisome proliferation studies too. http://www.efsa.eu.int/science/afc/afc_documents/469/statement01_afc_phthalates_en1. pdf

- Swiss Ordinance Annex 10 (previously Annex 6)

Evaluation : Part B - Non-evaluated substances

SML [mg/Kg] : -

Notice : -

CMR (preposition amendment) : R1B

- ESCO Reports

The compound is present in the following ESCO reports:

1) Coatings (ESCO Reports)

1 entry found

SCF List : -

MS : NL

Safety Evaluation MS : B - Substances used for the manufacture of paper and board, printing inks, coatings, rubber, colorants, wood and cork and evaluated at national level before the publication of SCF Guidelines for Food Contact Materials (1991)

Regulations/recommendations : NL X 3k, 5d, 7h(More information can be found in the EFSA external scientific report of ESCO WG on non-plastic FCM (2012))

Substance Positive List : Y

Restrictions : SML=1, together with phthalic acid, dibutyl ester

Remarks : as plasticizer

2) Paper & Board (ESCO Reports)

1 entry found

SCF List : -

MS : NL

Safety Evaluation MS : A - Substances used for the manufacture of paper and board, printing inks, coatings, rubber, colorants, wood and cork and evaluated at national level after the publication of SCF Guidelines for Food Contact Materials (1991)
Regulations/recommendations : NL II 1.2.2 o(More information can be found in the EFSA external scientific report of ESCO WG on non-plastic FCM (2012))
Substance Positive List : Y

Restrictions : SML = 1 mg/kg alone or with dibutyl phthalate. Will be revised to 0.3 mg/kg within a few years

Remarks : -

- Council of Europe (CoE) Resolutions

The compound is present in the following CoE Resolutions:

1) Coatings (CoE)

1 entry found

List/Appendix : Temporary appendix to list 1 of additives: list of additives approved by Partial Agreement member states or by FDA, applying evaluation criteria at the time of their approval

SCF List : 6B: Substances suspected to have toxic properties (other than carcinogenic). Restrictions may be indicated

Restrictions : To be fixed

ADI/TDI [mg/kg bw] : -

2) Paper & Board (CoE)

1 entry found

List/Appendix : List 2 of additives: list of additives not yet assessed

SCF List : 6B: Substances suspected to have toxic properties (other than carcinogenic). Restrictions may be indicated

Restrictions : To be fixed

ADI/TDI [mg/kg bw] : -

3. Evaluation in a non-FCM context

- EFSA OpenFoodTox database

(Last review: 25/10/2022)

If the compound is present in the OpenFoodTox database, a summary of the critical toxicological endpoints and reference values can be available here:
https://www.efsa.europa.eu/en/microstrategy/openfoodtox

Present in EFSA OpenFoodTox database	Yes
Substance name in EFSA Database	Phthalic acid, diisobutyl ester

1 entry found in EFSA OpenFoodTox database

Title	Statement of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (AFC Panel) on the re-classification of some phthalates for consistency with the new SCF guidelines for food contact materials
Publication date	09-06-2004
DOI	doi:10.2903/j.efsa.2004.1062
URL	http://dx.doi.org/10.2903/j.efsa.2004.1062

- CLP Regulation (EC) No 1272/2008

(Last review: 10/01/2023)

Present in CLP list as CMR	Yes
CLP details	Repr. 1B

- CoRAP List

(Last review: 15/12/2022)

Present in CoRAP list	No

- Biocidal Active substances list

(Last review: 10/01/2023)

Present in the biocidal active substances list	No

4. Inclusion in lists of substances of concern

- Candidate List (ECHA)

(Last review: 10/01/2023)

Present in the Candidate list (ECHA)?	Yes
Substance Name	Diisobutyl phthalate
Reason for inclusion	Toxic for reproduction (Article 57c)#Endocrine disrupting properties (Article 57(f) - human health)

- SIN List

(Last review: 19/12/2022)

Present in the SIN list?	Yes
Substance Name	diisobutyl phthalate
Reason for inclusion	Classified CMR according to Annex VI of Regulation 1272/2008

- Endocrine Disruptor Lists

(Last review: 11/10/2023)

Present in the ED lists?	Yes
Substance Name	Diisobutyl phthalate (DIBP)
Which list?	List I- Substances as endocrine disruptors at EU level
Reason for inclusion?	Health Effect

5. Bioavailability/Bioaccumulation potential

Molecular Weight [Da]	278.38
Number of hydrogen bond donor	0
Number of hydrogen bond acceptor	4
Polar Surface area [Angstroms^2]	52.6
Rotating bond	8
LogP model (Meylan/Kowwin)	4.11 (EXPERIMENTAL value)

Bioavailability: Yes

6. Hazard predictions using vega models

Genotoxicity Data

Mutagenicity - Ames test [Consensus model]	**NON-Mutagenic (Consensus score: 1)**
Chromosomal aberration [CORAL model]	Inactive (GOOD reliability)
In vitro Micronucleus activity [IRFMN/Vermeer model]	Inactive (MODERATE reliability)
In vivo Micronucleus activity [IRFMN model]	NON-genotoxic (GOOD reliability)

Carcinogenicity studies

Carcinogenicity [CAESAR model]	NON-Carcinogen (MODERATE reliability)
Carcinogenicity [ISS/Benigni-Bossa alerts model]	Carcinogen (GOOD reliability)
Carcinogenicity [ISSCAN-CGX model]	Carcinogen (MODERATE reliability)
Carcinogenicity [Antares model]	Carcinogen (MODERATE reliability)
Carcinogenicity oral Slope Factor model [IRFMN]	NON-Carcinogen (GOOD reliability)

Reproductive and developmental toxicity

Developmental Toxicity [CAESAR model]	NON-Toxicant (LOW reliability)
Developmental/Reproductive Toxicity library [P&G model]	Toxicant (GOOD reliability)

NOAEL

Subchronic oral toxicity data (90-day study) (NOAEL) [CORAL model]	38.07 mg/kg (GOOD reliability)

Endocrine activity

Estrogen Receptor Relative Binding Affinity Model [IRFMN model]	**Active (EXPERIMENTAL value)**
Estrogen receptor-mediated effect [CERAPP model]	Possible NON-active (GOOD reliability)
Androgen receptor-mediated effect [COMPARA model]	**NON-active (EXPERIMENTAL value)**
Thyroid receptor alpha effect [NRMEA model]	**Inactive (EXPERIMENTAL value)**
Thyroid receptor beta effect [NRMEA model]	**Inactive (EXPERIMENTAL value)**

7. Information to support the Threshold for Toxicological Concern (TTC) approach

The TTC approach can only be applied in some conditions, for more information, please refer to the software user

guide

Cramer Classification

Cramer class [ToxTree model]	Low (Class I)

Reference List

Source's name	References	Last update
EU Regulation 10/2011 - Annex I	European Commission. 2011. Commission Regulation (EU) No.10/2011 of 14 January 2011 on Plastic Materials and Articles Intended to Come into Contact with Food . Text with EEA Relevance. 012 OJ L (COM) http://data.europa.eu/eli/reg/2011/10/oj/eng (September 04, 2023).	17/10/2023
Synoptic Document (2005)	EC, 2005. "Synoptic Document": Provisional List of Monomers and Additives Notified to European Commission as Substances Which May Be Used in the Manufacture of Plastics or Coatings Intended to Come into Contact with Foodstuffs. Sanco D3/AS D, p. 2005.	22/11/2022
Swiss Ordinance Annex 10 (previously Annex 6)	Annex 6 of the Ordinance of the FDHA on articles and materials of 23 November 2005 (RS 817.023.21)	22/11/2022
ESCO Reports	European Food Safety Authority; Report of ESCO WG on non-plastic Food Contact Materials. Supporting Publications 2012:139 [63 pp.]. Available online: www.efsa.europa.eu	22/11/2022
Council of Europe (CoE) Resolutions	Council of Europe Resolutions https://www.edqm.eu/en/food-contact-materials-and-articles	22/11/2022
EFSA OpenFoodTox database	https://www.efsa.europa.eu/en/data-report/chemical-hazards-database-openfoodtox (December 14, 2021).	25/10/2022
Annex VI of the CLP Regulation (EC) No 1272/2008	https://echa.europa.eu/en/information-on-chemicals/annex-vi-to-clp (May 11,2022)	10/01/2023
CoRAP List	https://echa.europa.eu/en/information-on-chemicals/evaluation/community-rolling-action-plan/corap-table	15/12/2022
Biocidal Active substances list (ECHA)	https://echa.europa.eu/en/regulations/biocidal-products-regulation/approval-of-active-substances/list-of-approved-active-substances (February 23, 2022)	10/01/2023
Candidate list (ECHA) or List of Substance of Very High Concern (SVHC)	https://echa.europa.eu/substances-of-very-high-concern-identification-explained (June 14, 2022)	10/01/2023
SIN list	https://sinlist.chemsec.org/ (October 14, 2021).	19/12/2022
Endocrine Disruptor Lists	https://edlists.org/ (July 07, 2023).	11/10/2023