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# Potential toxicity, physicochemical properties and environmental behavior of L-carvone

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The monoterpene ketone carvone [6,8(9)-p-pentadien-2-one; 1-methyl-4-isopropenyl-6-cyclohexene-2-one] is a colorless to pale yellowish liquid with a characteristic cumin-spiced odor and a sharp, warm, sweet taste. It is found as a main component in essential oils of cumin, fennel, spearmint, *etc*., from which it is isolated, but for industrial purposes it is synthesized. It is stable in soaps, detergents and cosmetics. Its uses include the preparation of perfume compositions with spicy and fantasy notes, fern, clove, chypre, *etc*., aromatic compositions for the food industry - mainly mint-carvone type, spices, liqueurs, *etc*. It is used in significant quantities in gums and oral hygiene preparations (toothpastes, mouthwashes, *etc*.). The goal of the present work is to predict the physical, toxicological properties and environmental fate of L-carvone by the CompTox Chemistry Dashboard.

**Keywords:** L-carvone, toxicity, physicochemical properties, environmental behavior

## INTRODUCTION

The volatile monocyclic ketone carvone [6,8(9)-*p*-pentadien-2-one; 1-methyl-4-isopropenyl-6-cyclohexene-2-one] is a colorless to pale yellowish liquid. Two enantiomers of carvone, (R)-(-)-carvone (L-carvone) and (S)-(+)-carvone (D-carvone), are found in various essential oils. Both enantiomers are with different properties: (S)-(+)-carvone has an optical activity of $\alpha_D^{20}$ +64.3° and is the main constituent of caraway (*ca*. 60%) and dill essential oils (*ca.* 50%). (S)-(+)-carvone possesses the typical caraway aroma., Therefore, D-carvone and the essential oils that contain it are mainly used as a taste enhancer for the food industry (in aromatic compositions - mainly mint-carvone type, spices, liqueurs, *etc*.), and fragrance industry (in perfume compositions with spicy and fantasy notes, fern, clove, chypre, *etc*.) (R)-(-)-carvone has an optical activity $\alpha_D^{20}$ -62.5°, occurs in spearmint oil at a concentration of 70-80%. L-carvone and the essential oils that contain it are frequently added to toothpastes, mouth washes, chewing gums, *etc*. [1–3].

Both enantiomers are isolated by fractional distillation of essential oils, but for industrial purposes they are synthesized [1].

Some physicochemical (density, surface tension and refractive index), kinetic and thermodynamic parameters (surface energy, surface heat capacity, reaction rate constant) of carvone were measured at a temperature range between 6 and 30 °C [4].

It has been found that treating cedar wood with carvone affects its physicochemical properties, which may allow to reduce or inhibit the adhesion of microorganisms responsible for its biodegradation [5].

Carvone also exhibits a variety of biological properties, e.g., antimicrobial [4, 6–12], antioxidant [13, 14], antidiabetic [15, 16], anti-inflammatory [17–19], neurological [20–22], *etc.* [23, 24].

Carvone is among the 80 aromatic substances, described in Regulation 2023/1554 [25] that can cause hypersensitivity of skin and allergy contact dermatitis [26–30]. Its presence in the essential oils used in perfumery and cosmetic preparations should be defined on the label at a concentration above 0.01% in rinse-off products for skin and hair (shampoos, shower gels, masks, *etc*.) and over 0.001%, in those that remain in contact with skin (creams, toilet milks, lotions, *etc*.).

The goal of the present work is to predict the physical, toxicological properties and environmental fate of L-carvone by the CompTox Chemistry Dashboard.

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## MATERIALS AND METHODS

### *CompTox Chemistry Dashboard*

The free and accessible web-based application Dashboard was used, which has access to nine databases of chemical compounds [31, 32].

### *Environmental fate and transport*

The data were obtained from online databases or predicted using different models such as: EPI Suite, NICEATM, TEST and OPERA. Various properties were included in the study, for example the adsorption coefficient, atmospheric hydroxylation rate, biodegradation half-life, fish biotransformation half-life, as well as parameters to assess bioaccumulation potential, such as bioaccumulation factors and bioconcentration factors. The obtained predicted values were derived using different models [31, 32].

### *Chemical properties*

Experimental and predicted physical and chemical properties, such as log octanol-water partition coefficient (logP), water solubility (S), melting point (MP) and others, were used. The data is presented in two separate tables, as experimental and predicted [31, 32].

## RESULTS AND DISCUSSION

Knowledge of the physicochemical properties of potential chemical alternatives is a requirement of the alternatives assessment process for two reasons. First, the inherent hazard of a chemical, such as its capacity to interfere with normal biological processes, and its physical hazards and environmental fate (degradation, persistence) are determined by its intrinsic physicochemical properties and the system with which it is interacting. Second, physicochemical properties can be used to eliminate from consideration chemicals that are likely to exhibit particular physical or toxicological hazards. As important as this data is, obtaining it is relatively fast and inexpensive, and can be readily done at the initial stages of the alternatives assessment [33].

Physical properties include freezing point, boiling point, melting point, infrared spectrum, electronic parameters, viscosity, and density. Some of them (e.g., electronic parameters, molecular weight, boiling/freezing point) are directly associated with environmental fate and health effects. A number of different software packages and algorithms are available for predicting physicochemical properties, and predictions are often in excellent agreement with experimentally-derived values [33].

In the present work, the CompTox Chemistry Dashboard was used to predict the physicochemical properties (environmental fate) of L-carvone.

Data of prediction environmental fate (Bioconcentration factor, Atmospheric hydroxylation rate, Biodegradation half-life, Fish biotransformation half-life (Km) and Soil adsorption coefficient (Koc) and physicochemical properties) of L-carvone are presented in Table 1.

**Table 1.** Predicted properties for L-carvone.

| Property | Experimental average | Predicted average | Experimental median | Predicted median | Experimental range | Predicted range |
|---|---|---|---|---|---|---|
| Bioconcentration factor, L/kg | - | 7.55 | - | 7.55 | - | 4.62 to 10.5 |
| Atmospheric hydroxylation rate, ml/molecule.s | - | 1.66 e-10 | - | 1.66 e-10 | - | 1.66 e-10 |
| Biodegradation half-life, days | - | 3.47 | - | 3.47 | - | 3.47 |
| Fish biotrans formation half-life (Km), days | - | 9.12e-2 | - | 9.12e-2 | - | 9.12e-2 to 0.237 |
| Soil adsorption coefficient (Koc), L/kg | - | 214 | - | 214 | - | 214 |
| Polarizability, Å$^3$ | - | 18.0 | - | 18.0 | - | 18.0 |
| Henry's law, atm-m$^3$/mol | - | 3.31e-4 | - | 3.31e-4 | - | 3.31e-4 |
| ReadyBiodeg, Binary 0/1 | - | 0.00 | - | 0.00 | - | 0.00 |
| Boiling point, °C | 230 | 227 | 230 | 227 | 229 to 230 | 224 to 231 |
| Flash point, °C | - | 86.5 | - | 86.5 | - | 84.1 to 88.9 |
| Melting point, °C | - | 18.1 | - | 9.86 | - | 9.00 to 35.4 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Molar refractivity, ml | - | 45.5 | - | 45.5 | - | 45.5 |
| Molar volume, ml | - | 160 | - | 160 | - | 160 |
| Surface tension, dyn/ml | - | 32.0 | - | 32.0 | - | 29.8 to 34.2 |
| Density, g/ml | - | 0.947 | - | 0.947 | - | 0.940 to 0.954 |
| logD5.5, Log10 unitless | - | 2.36 | - | 2.36 | - | 2.36 |
| logD7.4, Log10 unitless | - | 2.36 | - | 2.36 | - | 2.36 |
| Liquid chromatography Ret., min | - | 9.34 | - | 9.34 | - | 9.34 |
| Vapor pressure, mm Hg | 0.103 | 9.86e-2 | 0.103 | 0.102 | 0.103 | 6.56e-2 to 0.1… |
| Water solubility, mol/L | 8.70e-3 | 6.75e-3 | 8.71e-3 | 6.79e-3 | 8.67e-3 to 8.71e-3 | 2.44e-3 to 1.10e-2 |
| Index of refraction, | - | 1.48 | - | 1.48 | - | 1.48 |
| LogKoa: Octanol-Air | - | 5.06 | - | 5.06 | - | 5.06 |
| LogKow: Octanol-Water | 2.71 | 2.62 | 2.71 | 2.58 | 2.71 | 2.27 to 3.07 |

**Table 2**. Predicted results of physicochemical properties for L-carvone using software TEST.

| Properties | Experimental value | Consensus | Hierarchical clustering | Single model | Group contribution | Nearest neighbor |
|---|---|---|---|---|---|---|
| Normal boiling point, °C | 231.0 | 224.3 | 230.7 | | 213.0 | 229.3 |
| Melting point, °C | | 35.4 | 46.7 | | 9.4 | 50.0 |
| Flash point, °C | 88.9 | 83.1 | 89.0 | | 73.6 | 86.8 |
| Vapor pressure, mm Hg | 0.103 | 0.156 | 0.122 | | 0.175 | 0.177 |
| Density, g/ml | 0.940 | 0.954 | 0.957 | | 0.957 | 0.948 |
| Surface tension, dyn/ml | 34.239 | 34.201 | 34.301 | | 35.331 | 32.970 |
| Water solubility, mg/L | 1299.528 | 731.267 | 1272.750 | | 216.953 | 1416.181 |

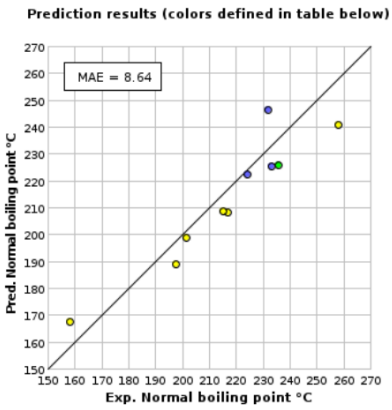
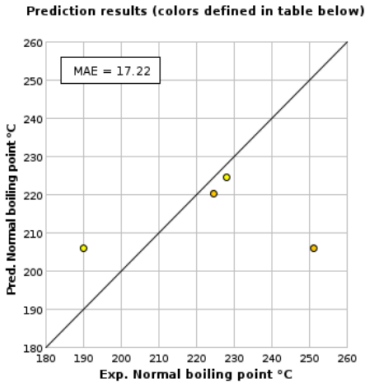
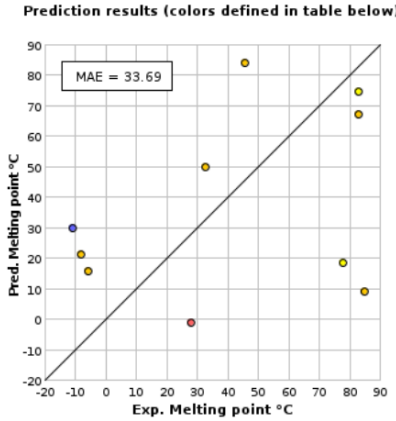
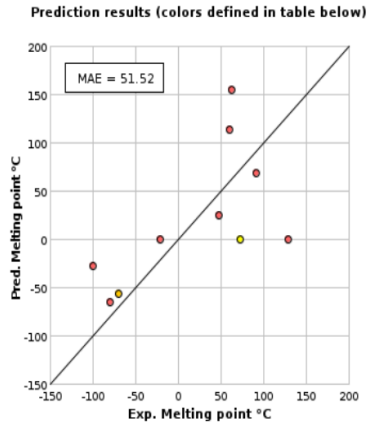
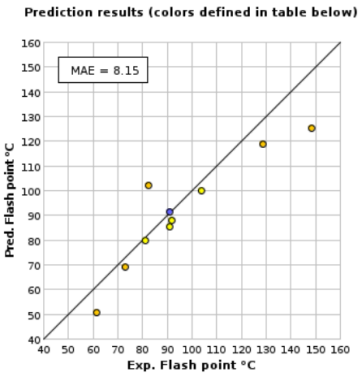
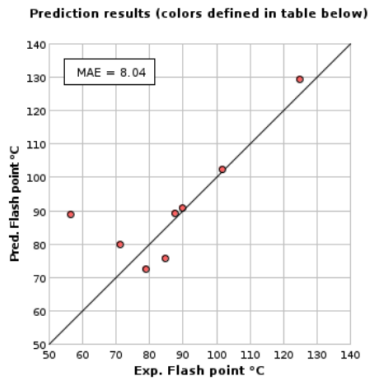
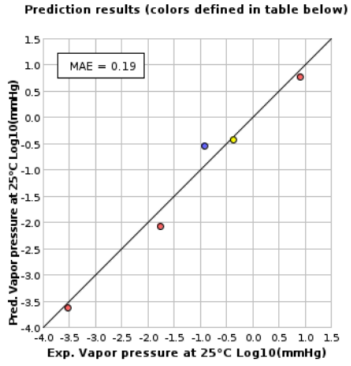
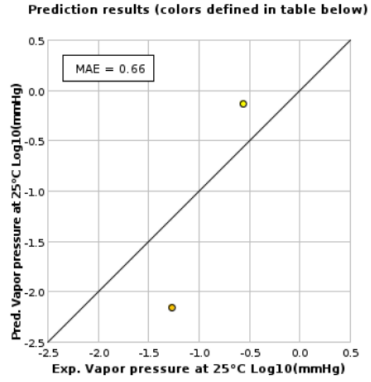
There are four experimental data values of L-carvone. The physicochemical properties are estimated by OPERA.

An EPA software application (the Toxicity Evaluation Software Tool (TEST)) was implemented for evaluating the physicochemical properties of L-carvone using quantitative structure-activity relationship (QSAR) methodologies. Data predicting the properties of L-carvone by the software TEST (CompTox Chemistry Dashboard) is presented in Table 2.

There are six experimental values of L-carvone – normal boiling point, flash point, vapor pressure, density, surface tension and water solubility. Prediction results (Consensus method) for all properties are obtained of individual predictions with the following methods (Hierarchical clustering, Single model, Group contribution, Nearest neighbor).

For each property a training and external test set are defined. Predictions for the test chemical and for the most similar chemicals in the sets have been made (Table 3).

**Table 3**. Predicted physicochemical properties of L-carvone by Consensus method.

| Physical and chemical properties | Predictions for the test chemical and for the most similar chemicals in the training set | MAE* | Predictions for the test chemical and for the most similar chemicals in the external test set | MAE* |
|---|---|---|---|---|
| Normal boiling point, °C |  | Entire set-10.36<br>Similarity coefficient ≥ 0.5 (8.64) |  | Entire set-11.46<br>Similarity coefficient ≥ 0.5 (17.22) |
| Melting point, °C |  | Entire set-26.64<br>Similarity coefficient ≥ 0.5 (33.69) |  | Entire set-30.21<br>Similarity coefficient ≥ 0.5 (51.52) |
| Flash point, °C |  | Entire set-14.53<br>Similarity coefficient ≥ 0.5 (8.15) |  | Entire set-16.91<br>Similarity coefficient ≥ 0.5 (8.04) |
| Vapor pressure, mm Hg |  | Entire set-0.40<br>Similarity coefficient ≥ 0.5 (0.19) |  | Entire set-0.47<br>Similarity coefficient ≥ 0.5 (0.66) |

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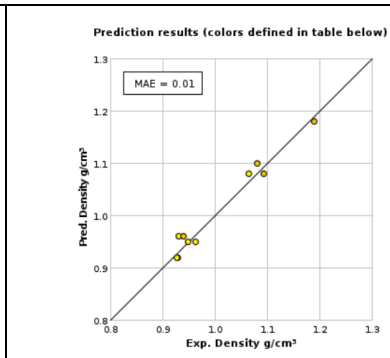
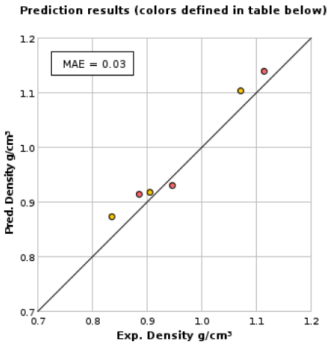
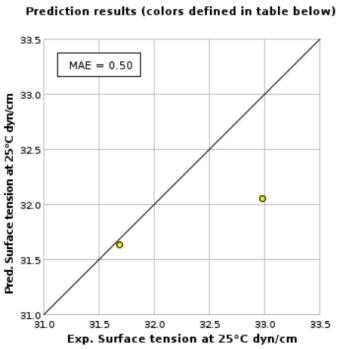
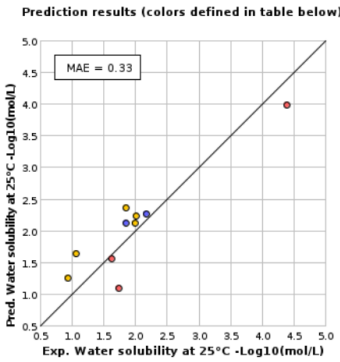
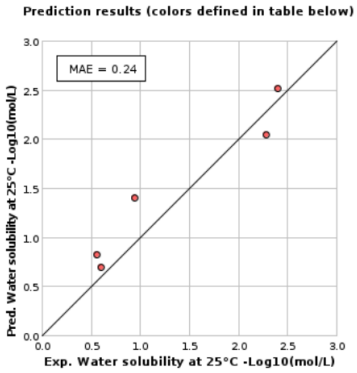
|---|---|---|---|---|
| Density, g/ml |  | Entire set-0.04<br>Similarity coefficient ≥ 0.5 (0.01) |  | Entire set-0.04<br>Similarity coefficient ≥ 0.5 (0.03) |
| Surface tension, dyn/ml |  | Entire set-1.16<br>Similarity coefficient ≥ 0.5 (0.50) | No chemicals in the test set exceed a minimum similarity coefficient of 0.5 for comparison purposes | Entire set-1.32<br>Similarity coefficient ≥ 0.5 (1.38) |
| Water solubility, mg/L; |  | Entire set-0.50;<br>Similarity coefficient ≥ 0.5 (0.33) |  | Entire set-0.58<br>Similarity coefficient ≥ 0.5 (0.24) |

*Mean absolute error in g/ml

A toxic endpoint is the result of a study conducted to determine how dangerous a substance is. The data collected from such studies are used to report the relative toxicity of the compound to various regulatory agencies and environmental compliance groups. Toxic endpoints can include mortality, behavior, reproductive status or physiological and biochemical changes [34].

Toxic endpoints are acute or chronic. Acute studies generally last no longer than a week and examine endpoints such as mortality and behavior. With acute studies, a common endpoint is an $LD_{50}$, which is the dose of a compound required to kill half the organisms in the study. Chronic studies are longer in duration (more than a week) and include endpoints such as reproduction, long-term survival and growth. Chronic studies are valuable because they examine the effects of extremely low concentrations of compounds that may persist in the environment for long periods of time [34].

There are two experimental values (toxicological endpoints) for L-carvone – Ames mutagenicity and oral rat $LD_{50}$. Prediction results (Consensus method) for all toxicological endpoints for L-carvone are obtained of individual predictions with the following methods (Hierarchical clustering, Single model, Group contribution, Nearest neighbor).

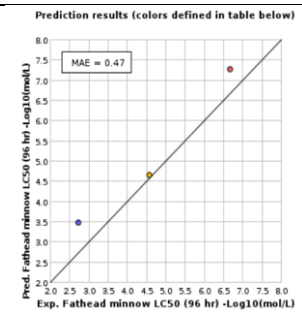
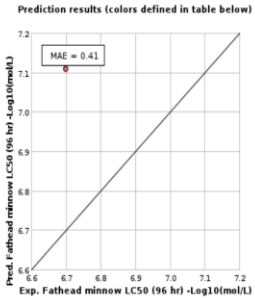
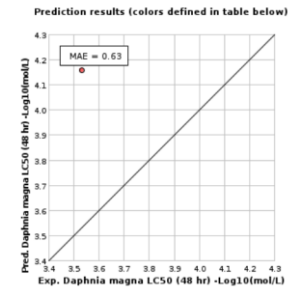
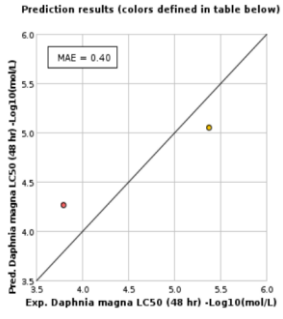
Data predicting the toxicological endpoints of L-carvone by the CompTox Chemistry Dashboard are presented in Table 4.

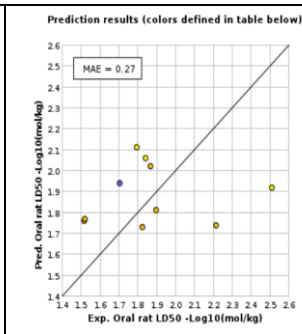
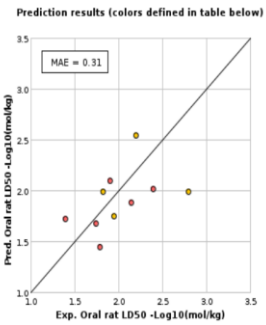
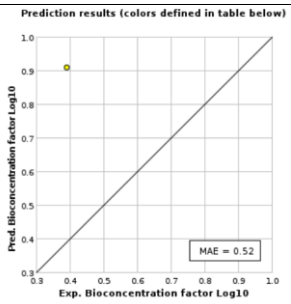
For each toxicological endpoint a training and an external test set are defined. Predictions for the test chemical and for the most similar chemicals in the sets were made (Table 5).

**Table 4**. Predicted results of toxicological endpoints for L-carvone.

| Properties | Experimental value | Consensus | Hierarchical clustering | Single model | Group contribution | Nearest neighbor |
|---|---|---|---|---|---|---|
| 96 h fathead minnow $LC_{50}$, mg/L | | 5.573 | 10.113 | 5.630 | 5.157 | 3.284 |
| 48 h *Daphnia magna* $LC_{50}$, mg/L | | 1.802 | 1.768 | 1.768 | 1.870 | |
| 48 h *Tetrahymena pyriformis* $IGC_{50}$, mg/L | | | | | 1.857 | |
| Oral rat LD50, mk/kg | 1639.780 | 1635.355 | 2039.099 | | | 1311.552 |
| Bioconcentration factor | | 4.628 | 2.120 | 24.353 | 1.920 | |
| Developmental toxicity | | true | true | false | | true |
| Ames mutagenicity | false | false | false | | | false |
| Estrogen Receptor RBA | | | | | | $1.289\times10^{-4}$ |
| Estrogen Receptor Binding | | false | false | false | false | true |

**Table 5**. Predicted toxicological properties of L-carvone from Consensus method.

| Toxicological properties | Predictions for the test chemical and for the most similar chemicals in the training set | MAE* | Predictions for the test chemical and for the most similar chemicals in the external test set | MAE* |
|---|---|---|---|---|
| 96 h fathead minnow $LC_{50}$, mg/L |  | Entire set-0.48 Similarity coefficient ≥ 0.5 (0.47) |  | Entire set-0.55 Similarity coefficient ≥ 0.5 (0.41) |
| 48 h *Daphnia magna* $LC_{50}$, mg/L |  | Entire set-0.50 Similarity coefficient ≥ 0.5 (0.63) |  | Entire set-0.74 Similarity coefficient ≥ 0.5 (0.40) |
| 48 h *Tetrahymena pyriformis* $IGC_{50}$, mg/L | No similar chemicals could be predicted | | No similar chemicals could be predicted | |

| | | | | |
|---|---|---|---|---|
| Oral rat $LD_{50}$, mk/kg |  | Entire set-0.34 Similarity coefficient ≥ 0.5 (0.27) |  | Entire set-0.43 Similarity coefficient ≥ 0.5 (0.31) |
| Bioconcentration factor |  | Entire set-0.42 Similarity coefficient ≥ 0.5 (0.52) | No chemicals in the test set exceed a minimum similarity coefficient of 0.5 for comparison purposes | Entire set-0.51 Similarity coefficient ≥ 0.5 (0.80) |
| Developmental toxicity | | Concordance -1.00 Sensitivity-1.00 Specificity-1.00 | | Concordance-0.80 Sensitivity-0.75 Specificity-1.00 |
| Ames Mutageni-city | | Concordance -0.90 Sensitivity-0 Specificity-1 | | Concordance-0.80 Sensitivity-N/A Specificity-0.80 |
| Estrogen Receptor RBA | No similar chemicals could be predicted | | No similar chemicals could be predicted | |
| Estrogen Receptor Binding | | Concordance -N/A Sensitivity-N/A Specificity-N/A | | Concordance-0.75 Sensitivity-1.00 Specificity-0.50 |

*Mean absolute error in g/ml

## CONCLUSION

Prediction and investigation of the physicochemical properties of L-carvone can be used as a screening for its potential to cause human and environmental toxicity. Experimental data is limited, forcing decisions about the potential of a compound now and in the future to be made on the basis of limited data and information. Therefore, alternative *in silico* methods, such as the CompTox Chemistry Dashboard, are used to assess their behavior (potential toxicity, physicochemical properties and environmental fate and transport).

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