Prediction of Hazard Identification and Characterization of Several Compounds used as Food Additives Applying *In Silico* Methods

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Abstract:
In frame of risk analysis, hazard identification and characterization are parts of risk assessment that should be performed to assure the safety aspect of a substance that will be used as food additive. According to WHO guidance, the two steps can be performed experimentally, based on epidemiological data or based on chemical structure calculation. In line with this guidance, the aim of this research was to obtain data describe the hazardous properties of several compounds used as food additive applying predictive *in silico* methods. Hazardous properties were limited to mutagenicity and carcinogenicity effects. The software applied including Toxtree, T.E.S.T., VEGA Benigni/Bossa, VEGA CAESAR and VEGA SARpy. A positive result can be accepted if all the prediction models give the same results and alerts on the compound for carcinogenic and mutagenic potential. If only one or more, but not all those prediction models, showing alert on a compound or give positive result, than it is assumed that the results are still not enough to predict carcinogenic or mutagenic effects of that compound unless experimental data supporting those effects are available. Fifty four compounds of flavour functional class were included in our study. Following our criteria, 20 compounds of them show negative results on mutagenicity prediction; however the rests 34 compounds give different prediction results and hence need experimental data for confirmation. In the case of carcinogenicity predictions, 14 compounds of them were predicted to show no carcinogenic potential, 10 compounds of them were predicted to show carcinogenic potential, while the rests 30 compounds show different prediction results and hence need experimental data for confirmation. Based on overall results, it was concluded that this *in silico* approaches can be used to perform preliminary evaluation of hazardous properties, especially those of mutagenicity and carcinogenicity effects.

Key words: *Food additives, in silico methods, hazardous properties, risk analysis*

Introduction

Hazard identification and characterization are parts of risk assessment that should be performed to assure the safety aspect of a substance that will be used as food additive. When a risk assessment is performed, there are often insufficient data available to complete the assignment. Scientific information can be used to support many food safety risk assessments and it is available from a variety of sources, both national and international. According to WHO guidance, hazard identification and characterization can be performed experimentally, based on epidemiological data, or based on chemical structure calculation / prediction [1]. Mutagenicity and carcinogenicity tests are important part of the hazard assessment for food additives.

Flavour is one of the food additives functional classes that can give consumer perception about the food they consume. Food having attractive flavour will be the one that a consumer chooses. In Indonesia, 1828 compounds are permitted as flavouring substances [2]. Most of them are included in the lists of JECFA, EC and FEMA. However some compounds are also included in the list of IARC as 2A or 2B groups. Some listed flavours belong to compound group with high chemical reactivities but despite these reactivities, they are usually lack of toxicity data. These very limited toxicity data makes difficult to an agreement on safety level concern and consequently the development of management options was challenging [3].

Currently, the interest to apply *in silico* methods for toxicity screening of a compound has increased. These methods were considered useful only for providing a rough and initial estimation, but now it has attracted interest as they can guide the sciencetist to investigate toxic potential [4]. The application of *in silico* approaches for predicting preclinical toxicological endpoints, clinical adverse effects, and metabolism of pharmaceutical substances has become of high interest to the scientific community and the public [5]. *In silico* / computational approaches can be used to study toxicity of a compound based on its molecular structure and properties predicted from similar compounds whose biological activities are known [6]. The balances between experimental methods and *in silico* / computational approaches are expected to reduce the number of safety issues [7]. Computational toxicological prediction is needed to reduce cost and time, animal testing and to develop legislation related with the use of

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new substances [8]. Several *in silico* tools are available for toxicity prediction of a substance, such as Toxtree, T.E.S.T., VEGA Benigni/Bossa, VEGA CAESAR etc. They have been tuned to predict global toxicity endpoints, such as mutagenicity and carcinogenicity.

The aim of this study was to obtain data described the hazardous properties of several compounds used as food additive, especially of flavour functional class, applying predictive *in silico* methods. Hazardous properties were limited to mutagenicity and carcinogenicity effects.

Experimental

Computational Software

Computational analysis was performed applying the following softwares: TOXTREE (version 2.6.0), T.E.S.T. (version 4.1), VEGA Benigni/Bossa (version 1.0.0-DEV), VEGA CAESAR (version 2.1.12 for mutagenicity and 2.1.8 for carcinogenicity) and VEGA SARpy (version 1.0.6-DEV). All these softwares can be used to predict mutagenicity and/or carcinogenicity end point. Toxtree is rule based software, while T.E.S.T. and CAESAR are statistical based softwares and SARpy is rule/statistical based software [9, 10]. VEGA Benigni/Bossa uses similar prediction model as Toxtree.

Tested compound

Thirty seven aromatic aldehydes and 17 furan derivatives are included in the study. The 37 aromatic aldehydes are p-tolylacetaldehyde, p-isopropyl phenylacetaldehyde, cinnamaldehyde, 3-(p-isopropylphenyl) propionaldehyde, alpha-amyl cinnamaldehydedimethyl-acetal, p-methyl cinnamaldehyde, alpha-methyl cinnamaldehyde, alpha-buthylcinnamaldehyde, alpha-amyl cinnamaldehyde, alpha-hexyl cinnamaldehyde, p-methoxycinnamaldehyde, o-methoxy-cinnamaldehyde, p-methoxy-alphamethyl-cinnamaldehyde, 2-methyl-4-phenylbutyraldehyde, 3-methyl-2-phenylbutyraldehyde, 2-methyl-3-(p-isopropylphenyl) propionaldehyde, 2-methyl-3-tolyl propionaldehyde, 2-phenyl propionaldehyde, 2-phenyl propionaldehyde dimethyl acetal, 2-(p-tolyl) propionaldehyde, benzaldehyde, benzaldehyde dime-thyl acetal, benzaldehydeglycerylacetal, benzaldehyde propylene glycol acetal, 4-ethylbenzaldehyde, tolualdehyde, tolualdehydeglycerylacetal, cuminaldehyde, 2,4-dimethyl-benzaldehyde, veratraldehyde, p-methoxybenzaldehyde, p-ethoxybenzaldehyde, salicylaldehyde, 2-hydroxy-4-methyl-benzaldehyde, 4-hydroxy benzaldehyde, o-methoxybenzaldehyde and 4-hydroxy-3,5-dimethoxy-benzaldehyde.

Seventeen furan derivates are 2-benzofurancarboxaldehyde, 2-furfurylidenebutyraldehyde, 2-methylfuran, 2,5-dimethylfuran, 2-ethylfuran, 2-butylfuran, 2-pentylfuran, 2-heptylfuran, 2-decylfuran, 2-butyrylfuran, 2-pentanoylfuran, 3-methyl-2-(3-methylbut-2-enyl)-furan, 2,3-dimethylbenzofuran, 2,4-difurfurylfuran, 2-acetyl-5-methylfuran, 2-acetyl-3,5-dimethylfuran and 2-acetyl-2,5-dimethylfuran.

Nine compounds with known mutagenicity and carcinogenicity properties from experimental results are used as positive control. They are 2-naphthyl-amine, 4-(N-nitrosomethyl-amino)-1-(3-pyridyl)-1-butano-ne, 4-amino biphenyl, benzidine, benzo[a]pyrene, bis (chloromethyl) ether, chloromethyl methyl ether, ethylene oxide and N-nitrosonor-nicotine [11].

Methodology

Prior application of computational softwares, all compounds were drawn and their geometry structures were optimized using Hyperchem (version 8.0.10), except for Toxtree and VEGA. These softwares need no geometry optimization but the structure of a compound must be first converted into SMILE notation prior calculation. The toxicity predictions were then carried out by means of existing rules available in those software. The following incusion and exclusion criteria are applied: If all softwares give negative results on carcinogenicity and mutagenicity predictions, then a compound is classified as show no carcinogenicity and / or mutagenicity potential. On the contrary if all softwares give positive results on carcinogenicity and mutagenicity predictions, then a compound is classified as show carcinogenicity and/or mutagenicity potential. If these softwares give different / inconsistent results, then no classification of carcinogenicity and / or mutagenicity potential can be made. The last two cases need experimental data for confirmation.

Results and Discussion

Mutagenicity and Carcinogenicity

All positive control compounds show mutagenic potential / structural alert for their mutagenicity prediction when predicted using all softwares, except bis(chloromethyl)-ether and chloromethylmethylether for SARPy model as well as N-nitrosonornicotine for VEGA Benigni/Bossa test model. The results of positive control compounds are displayed in Table 1. In the case of tested compounds, 20 compounds of them show no mutagenicity prediction / no structural alert for mutagenicity when predicted using all softwares. However 34 compounds of them show different results when predicted using the same softwares and hence need experimental data for confirmation. Mutagenicity prediction results of tested compounds are summarized in Table 2.

Carcinogenicity predictions of all compounds were performed applying Toxtree, VEGA Benigni/Bossa, and CAESAR. All positive control compounds show carcinogenic/have structural alert for their carcinogenicity when predicted using all the software, except N-nitrosonornicotine when tested with VEGA Benigni/Bossa test model. The results of positive control compounds are displayed in Table 3.

In the case of tested compounds, 14 compounds of them show no carcinogenicity prediction / no structural alert for carcinogenicity, 10 compounds of them show carcinogenicity prediction / structural alert for carcinogenicity when predicted using all softwares, while the rests (30 compounds) show different results when predicted using the same softwares and hence need experimental data for confirmation. Furthermore confirmation from experimental data is also necessary for the 10 positive compounds. Mutagenicity prediction results of tested compounds are summarized in Table 4.

Test models of mutagenicity and carcinogenicity available in the softwares applied were actually already compared and tested by other researchers. It is already count for its specificity, selectivity and accuracy. It is stated that the best prediction result was obtained by software using statistical model [9,10,12].

In our study, three of five softwares are statistical based software. However, due to differences in results among these softwares, it was still difficult to make a final decision.

Following that criteria, 20 compounds showed negative results on mutagenicity prediction; however 34 compounds need experimental data for confirmation. 10 compounds were predicted to have carcinogenic potential, while 14 compounds showed negative results on carcinogenicity prediction; however 40 compounds need experimental data for confirmation.

The application and acceptability of this *in silico* assessment can be used in other food additives and to perform preliminary evaluation of hazardous properties, especially those of mutagenicity and carcinogenicity effects.

The main problem of *in silico* prediction application in the risk assessment of food additives, especially for hazard identification and characterization of a food additive is difficulties in making decision. For this reasons, application and acceptability of *in silico* result in this field is still limited only for performing preliminary evaluation of hazardous properties, especially those of mutagenicity and carcinogenicity effects. Despite these limitations and difficulties, *in silico* methods can be very useful when the experimental data are not available or when the experimental tests are very difficult or practically impossible to be carried out.

Conclusions

Following our criteria, from total 54 tested compounds, 20 compounds of them show negative results on mutagenicity predictions; the rests 34 compounds give different prediction results and hence need experimental data for confirmation. In the case of carcinogenicity predictions, 14 compounds of them were predicted to show no carcinogenic potential, 10 compounds of them were predicted to show carcinogenic potential, while the rests 30 compounds show different prediction results. Consequently, 40 tested compounds still need experimental data on their carcinogenicities for confirmation. Based on overall results, it was concluded that despite the need of experimental data, *in silico* approaches can be used to perform preliminary evaluation of hazardous properties, especially those of mutagenicity and carcinogenicity effects.

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Table 1. Mutagenicity Prediction for Control Chemical

No.	Compound	Toxtree	T.E.S.T.	VEGA Benigni/Bossa	CAESAR	SARpy
1	Benzo[a]pyrene	Structural alert for *S. typhimurium* mutagenicity	Mutagenicity Positive	Mutagen	Mutagen	Mutagen
2	Benzidine	Structural alert for *S. typhimurium* mutagenicity; Potensial *S. typhimurium* TA 100 mutagen	Mutagenicity Positive	Mutagen	Mutagen	Mutagen
3	Bis (chloromethyl) ether	Structural alert for *S. typhimurium* mutagenicity	Mutagenicity Positive	Mutagen	Mutagen	Non Mutagen
4	Chloromethyl methyl ether	Structural alert for *S. typhimurium* mutagenicity	Mutagenicity Positive	Mutagen	Mutagen	Non Mutagen
5	Ethylene oxide	Structural alert for *S. typhimurium* mutagenicity	Mutagenicity Positive	Mutagen	Mutagen	Mutagen
6	N'-Nitrosonornicotine	Structural alert for *S. typhimurium* mutagenicity	Mutagenicity Positive	Non Mutagen	Mutagen	Mutagen
7	2-Naphthylamine	Structural alert for *S. typhimurium* mutagenicity; Potensial *S. typhimurium* TA 100 mutagen	Mutagenicity Positive	Mutagen	Mutagen	Mutagen
8	4-Aminobiphenyl	Structural alert for *S. typhimurium* mutagenicity; Potensial *S. typhimurium* TA 100 mutagen	Mutagenicity Positive	Mutagen	Mutagen	Mutagen
9	4-(N-Nitrosomethyl-amino)-1-(3-pyridyl)-1-butanone	Structural alert for *S. typhimurium* mutagenicity	Mutagenicity Positive	Mutagen	Mutagen	Mutagen

Table 2. Mutagenicity Prediction for Test Chemical

No.	Compound	Toxtree	T.E.S.T.	VEGA Benigni/Bossa	CAESAR	SARPy
1	p-Tolylacetaldehyde	Structural alert for *S. typhimurium* mutagenicity	Mutagenicity Positive	Mutagen	Non Mutagen	Non Mutagen
2	p-Isopropylphenyl-acetaldehyde	Structural alert for *S. typhimurium* mutagenicity	Mutagenicity Negative	Mutagen	Non Mutagen	Non Mutagen
3	Cinnamaldehyde	No alert for S.typhimurium mutagenicity; Unlikely to be a *S. typhimurium* TA100 mutagen	Mutagenicity Positive	Non Mutagen	Non Mutagen	Non Mutagen
4	3-(p-isopropylphenyl) propionaldehyde	No alert for *S. typhimurium* mutagenicity; Unlikely to be a *S. typhimurium* TA100 mutagen	Mutagenicity Negative	Mutagen	Non Mutagen	Non Mutagen
5	alpha-Amylcinnamal-dehyde dimethyl acetal	No alert for *S. typhimurium* mutagenicity	Mutagenicity Negative	Non Mutagen	Non Mutagen	Non Mutagen
6	p-Methylcinnamal-dehyde	No alert for *S. typhimurium* mutagenicity; Unlikely to be a *S. typhimurium* TA100 mutagen	Mutagenicity Positive	Mutagen	Non Mutagen	Non Mutagen
7	alpha-Methylcinnamaldehyde	No alert for *S. typhimurium* mutagenicity; Unlikely to be a *S. typhimurium* TA100 mutagen	Mutagenicity Positive	Mutagen	Non Mutagen	Non Mutagen
8	alpha-Buthylcinnamal-dehyde	No alert for *S. typhimurium* mutagenicity; Unlikely to be a *S. typhimurium* TA100 mutagen	Mutagenicity Negative	Non Mutagen	Non Mutagen	Non Mutagen
9	alpha-Amylcinnamal-dehyde	No alert for *S. typhimurium* mutagenicity; Unlikely to be a *S. typhimurium* TA100 mutagen	Mutagenicity Negative	Non Mutagen	Non Mutagen	Non Mutagen
10	alpha-Hexylcinnamal-dehyde	No alert for *S. typhimurium* mutagenicity; Unlikely to be a *S. typhimurium* TA100 mutagen	Mutagenicity Negative	Non Mutagen	Non Mutagen	Non Mutagen
11	p-Methoxycinnamal-dehyde	No alert for *S. typhimurium* mutagenicity; Unlikely to be a *S. typhimurium* TA100 mutagen	Mutagenicity Positive	Mutagen	Non Mutagen	Non Mutagen
12	o-Methoxycinnamal-dehyde	No alert for *S. typhimurium* mutagenicity; Unlikely to be a *S. typhimurium* TA100 mutagen	Mutagenicity Positive	Mutagen	Non Mutagen	Non Mutagen
13	p-Methoxy-alpha-methylcinnamaldehyde	No alert for *S. typhimurium* mutagenicity; Unlikely to be a *S. typhimurium* TA100 mutagen	Mutagenicity Positive	Mutagen	Non Mutagen	Non Mutagen
14	2-Methyl-4-phenylbu-tyraldehyde	Structural alert for *S. typhimurium* mutagenicity	Mutagenicity Negative	Mutagen	Non Mutagen	Non Mutagen
15	3-Methyl-2-phenylbu-tyraldehyde	Structural alert for *S. typhimurium* mutagenicity	Mutagenicity Negative	Mutagen	Non Mutagen	Non Mutagen
16	2-Methyl-3-(p-isopropylphenyl) propionaldehyde	Structural alert for *S. typhimurium* mutagenicity	Mutagenicity Negative	Mutagen	Non Mutagen	Non Mutagen

Table 2. (continued)

17	2-Methyl-3-tolyl-propionaldehyde	Structural alert for *S. typhimurium* mutagenicity	Mutagenicity Negative	Mutagen	Non Mutagen	Non Mutagen
18	2-Phenylpropional-dehyde	Structural alert for *S. typhimurium* mutagenicity	Mutagenicity Negative	Mutagen	Non Mutagen	Non Mutagen
19	2-Phenylpropional-dehyde dimethyl acetal	No alert for *S. typhimurium* mutagenicity	Mutagenicity Negative	Non Mutagen	Non Mutagen	Non Mutagen
20	2-(p-Tolyl)propional-dehyde	Structural alert for *S. typhimurium* mutagenicity	Mutagenicity Negative	Mutagen	Non Mutagen	Non Mutagen
21	Benzaldehyde	Structural alert for *S. typhimurium* mutagenicity	Mutagenicity Negative	Mutagen	Non Mutagen	Non Mutagen
22	Benzaldehyde dimethyl acetal	No alert for *S. typhimurium* mutagenicity	Mutagenicity Negative	Non Mutagen	Non Mutagen	Non Mutagen
23	Benzaldehydeglyceryl-acetal	No alert for *S. typhimurium* mutagenicity	Mutagenicity Negative	Non Mutagen	Mutagen	Non Mutagen
24	Benzaldehyde propylene glycol acetal	No alert for *S. typhimurium* mutagenicity	Mutagenicity Negative	Non Mutagen	Mutagen	Non Mutagen
25	4-Ethylbenzaldehyde	Structural alert for *S. typhimurium* mutagenicity	Mutagenicity Negative	Mutagen	Non Mutagen	Non Mutagen
26	Tolualdehyde	Structural alert for *S. typhimurium* mutagenicity	Mutagenicity Negative	Mutagen	Non Mutagen	Non Mutagen
27	Tolualdehydeglyceryl-acetal	No alert for *S. typhimurium* mutagenicity	Mutagenicity Negative	Non Mutagen	Non Mutagen	Non Mutagen
28	Cuminaldehyde	Structural alert for *S. typhimurium* mutagenicity	Mutagenicity Negative	Mutagen	Non Mutagen	Non Mutagen
29	2,4-Dimethylbenzal-dehyde	Structural alert for *S. typhimurium* mutagenicity	Mutagenicity Negative	Mutagen	Non Mutagen	Non Mutagen
30	Veratraldehyde	Structural alert for *S. typhimurium* mutagenicity	Mutagenicity Negative	Mutagen	Non Mutagen	Non Mutagen
31	p-Methoxybenzal-dehyde	Structural alert for *S. typhimurium* mutagenicity	Mutagenicity Negative	Mutagen	Non Mutagen	Non Mutagen
32	p-Ethoxybenzaldehyde	Structural alert for *S. typhimurium* mutagenicity	Mutagenicity Negative	Mutagen	Non Mutagen	Non Mutagen
33	Salicylaldehyde	Structural alert for *S. typhimurium* mutagenicity	Mutagenicity Negative	Mutagen	Non Mutagen	Non Mutagen
34	2-Hydroxy-4-methyl-benzaldehyde	Structural alert for *S. typhimurium* mutagenicity	Mutagenicity Negative	Mutagen	Non Mutagen	Non Mutagen
35	4-Hydroxybenzal-dehyde	Structural alert for *S. typhimurium* mutagenicity	Mutagenicity Negative	Mutagen	Non Mutagen	Non Mutagen
36	o-Methoxybenzal-dehyde	Structural alert for *S. typhimurium* mutagenicity	Mutagenicity Negative	Mutagen	Non Mutagen	Non Mutagen
37	4-Hydroxy-3,5-dime-thoxybenzaldehyde	Structural alert for *S. typhimurium* mutagenicity	Mutagenicity Negative	Mutagen	Non Mutagen	Non Mutagen
38	2-Benzofurancarboxal-dehyde	No alert for *S. typhimurium* mutagenicity	Mutagenicity Positive	Non Mutagen	Mutagen	Non Mutagen
39	2-Furfurylidenebu-tyraldehyde	No alert for *S. typhimurium* mutagenicity; Unlikely to be a *S. typhimurium* TA100 mutagen	Mutagenicity Negative	Mutagen	Non Mutagen	Non Mutagen

Table 2. (continued)

40	2-Methylfuran	No alert for *S. typhimurium* mutagenicity	Mutagenicity Negative	Non Mutagen	Non Mutagen	Non Mutagen
41	2,5-Dimethylfuran	No alert for *S. typhimurium* mutagenicity	Mutagenicity Negative	Non Mutagen	Non Mutagen	Non Mutagen
42	2-Ethylfuran	No alert for *S. typhimurium* mutagenicity	Mutagenicity Negative	Non Mutagen	Non Mutagen	Non Mutagen
43	2-Butylfuran	No alert for *S. typhimurium* mutagenicity	Mutagenicity Negative	Non Mutagen	Non Mutagen	Non Mutagen
44	2-Pentylfuran	No alert for *S. typhimurium* mutagenicity	Mutagenicity Negative	Non Mutagen	Non Mutagen	Non Mutagen
45	2-Heptylfuran	No alert for *S. typhimurium* mutagenicity	Mutagenicity Negative	Non Mutagen	Non Mutagen	Non Mutagen
46	2-Decylfuran	No alert for *S. typhimurium* mutagenicity	Mutagenicity Negative	Non Mutagen	Non Mutagen	Non Mutagen
47	2-Butyrylfuran	No alert for *S. typhimurium* mutagenicity	Mutagenicity Negative	Non Mutagen	Non Mutagen	Non Mutagen
48	2-Pentanoylfuran	No alert for *S. typhimurium* mutagenicity	Mutagenicity Negative	Non Mutagen	Non Mutagen	Non Mutagen
49	3-Methyl-2-(3-methylbut-2-enyl)-furan	No alert for *S. typhimurium* mutagenicity	Mutagenicity Negative	Non Mutagen	Non Mutagen	Non Mutagen
50	2,3-Dimethylbenzofuran	No alert for *S. typhimurium* mutagenicity	Mutagenicity Negative	Non Mutagen	Non Mutagen	Non Mutagen
51	2,4-Difurfurylfuran	No alert for *S. typhimurium* mutagenicity	Mutagenicity Positive	Non Mutagen	Non Mutagen	Non Mutagen
52	2-Acetyl-5-methylfuran	No alert for *S. typhimurium* mutagenicity	Mutagenicity Positive	Non Mutagen	Mutagen	Non Mutagen
53	2-Acetyl-3,5-dimethylfuran	No alert for *S. typhimurium* mutagenicity	Mutagenicity Negative	Non Mutagen	Non Mutagen	Non Mutagen
54	3-Acetyl-2,5-dimethylfuran	No alert for *S. typhimurium* mutagenicity	Mutagenicity Negative	Non Mutagen	Non Mutagen	Non Mutagen

Table 3. Carcinogenicity Prediction for Control Chemical

No.	Compound	Toxtree	VEGA Benigni/Bossa	CAESAR
1	Benzo[a]pyrene	Structural alert for genotoxic carcinogenicity	Carcinogen	Carcinogen
2	Benzidine	Structural alert for genotoxic carcinogenicity	Carcinogen	Carcinogen
3	Bis (chloromethyl) ether	Structural alert for genotoxic carcinogenicity	Carcinogen	Carcinogen
4	Chloromethyl methyl ether	Structural alert for genotoxic carcinogenicity	Carcinogen	Carcinogen
5	Ethylene oxide	Structural alert for genotoxic carcinogenicity	Carcinogen	Carcinogen
6	N'-Nitrosonornicotine	Structural alert for genotoxic carcinogenicity	Non Carcinogen*	Carcinogen
7	2-Naphthylamine	Structural alert for genotoxic carcinogenicity; Potential carcinogen	Carcinogen	Carcinogen
8	4-Aminobiphenyl	Structural alert for genotoxic carcinogenicity; Potential carcinogen	Carcinogen	Carcinogen
9	4-(N-Nitrosomethylamino)-1-(3-pyridyl)-1-butanone	Structural alert for genotoxic carcinogenicity	Carcinogen	Carcinogen

Table 4. Carcinogenicity Prediction for Test Chemical

No.	Compound	TOXTREE	VEGA Benigni/Bossa	CAESAR
1	p-Tolylacetaldehyde	Structural alert for genotoxic carcinogenicity	Carcinogenic	Non Carcinogenic
2	p-Isopropylphenylacetaldehyde	Structural alert for genotoxic carcinogenicity	Carcinogenic	Carcinogenic
3	Cinnamaldehyde	Negative for genotoxic carcinogenicity and non genotoxic carcinogenicity	Non Carcinogenic	Non Carcinogenic
4	3-(p-isopropylphenyl) propionaldehyde	Structural alert for genotoxic carcinogenicity	Carcinogenic	Carcinogenic
5	alpha-Amylcinnamaldehyde dimethyl acetal	Negative for genotoxic carcinogenicity and non genotoxic carcinogenicity	Non Carcinogenic	Non Carcinogenic
6	p-Methylcinnamaldehyde	Negative for genotoxic carcinogenicity and non genotoxic carcinogenicity	Carcinogenic	Non Carcinogenic
7	alpha-Methylcinnamaldehyde	Negative for genotoxic carcinogenicity and non genotoxic carcinogenicity	Carcinogenic	Non Carcinogenic
8	alpha-Buthylcinnamaldehyde	Negative for genotoxic carcinogenicity and non genotoxic carcinogenicity	Non Carcinogenic	Non Carcinogenic
9	alpha-Amylcinnamaldehyde	Negative for genotoxic carcinogenicity and non genotoxic carcinogenicity	Carcinogenic	Non Carcinogenic
10	alpha-Hexylcinnamaldehyde	Negative for genotoxic carcinogenicity and non genotoxic carcinogenicity	Non Carcinogenic	Non Carcinogenic
11	p-Methoxycinnamaldehyde	Negative for genotoxic carcinogenicity and non genotoxic carcinogenicity	Carcinogenic	Non Carcinogenic
12	o-Methoxycinnamaldehyde	Negative for genotoxic carcinogenicity and non genotoxic carcinogenicity	Carcinogenic	Carcinogenic
13	p-Methoxy-alpha-methylcinnamaldehyde	Negative for genotoxic carcinogenicity and non genotoxic carcinogenicity	Carcinogenic	Non Carcinogenic
14	2-Methyl-4-phenylbutyraldehyde	Structural alert for genotoxic carcinogenicity	Carcinogenic	Non Carcinogenic
15	3-Methyl-2-phenylbutyraldehyde	Structural alert for genotoxic carcinogenicity	Carcinogenic	Carcinogenic
16	2-Methyl-3-(p-isopropylphenyl) propionaldehyde	Structural alert for genotoxic carcinogenicity	Carcinogenic	Carcinogenic
17	2-Methyl-3-tolylpropionaldehyde	Structural alert for genotoxic carcinogenicity	Carcinogenic	Non Carcinogenic
18	2-Phenylpropionaldehyde	Structural alert for genotoxic carcinogenicity	Carcinogenic	Non Carcinogenic
19	2-Phenylpropionaldehyde dimethyl acetal	Negative for genotoxic carcinogenicity and non genotoxic carcinogenicity	Non Carcinogenic	Carcinogenic
20	2-(p-Tolyl)propionaldehyde	Structural alert for genotoxic carcinogenicity	Carcinogenic	Carcinogenic
21	Benzaldehyde	Structural alert for genotoxic carcinogenicity	Carcinogenic	Non Carcinogenic
22	Benzaldehyde dimethyl acetal	Negative for genotoxic carcinogenicity and non genotoxic carcinogenicity	Non Carcinogenic	Carcinogenic
23	Benzaldehydeglycerylacetal	Negative for genotoxic carcinogenicity and non genotoxic carcinogenicity	Non Carcinogenic	Non Carcinogenic

Table 4. (continued)

24	Benzaldehyde propylene glycol acetal	Negative for genotoxic carcinogenicity and non genotoxic carcinogenicity	Non Carcinogenic	Carcinogenic
25	4-Ethylbenzaldehyde	Structural alert for genotoxic carcinogenicity	Carcinogenic	Non Carcinogenic
26	Tolualdehyde	Structural alert for genotoxic carcinogenicity	Carcinogenic	Non Carcinogenic
27	Tolualdehydeglycerylacetal	Negative for genotoxic carcinogenicity and non genotoxic carcinogenicity	Non Carcinogenic	Carcinogenic
28	Cuminaldehyde	Structural alert for genotoxic carcinogenicity	Carcinogenic	Carcinogenic
29	2,4-Dimethylbenzaldehyde	Structural alert for genotoxic carcinogenicity	Carcinogenic	Carcinogenic
30	Veratraldehyde	Structural alert for genotoxic carcinogenicity	Carcinogenic	Non Carcinogenic
31	p-Methoxybenzaldehyde	Structural alert for genotoxic carcinogenicity	Carcinogenic	Non Carcinogenic
32	p-Ethoxybenzaldehyde	Structural alert for genotoxic carcinogenicity	Carcinogenic	Non Carcinogenic
33	Salicylaldehyde	Structural alert for genotoxic carcinogenicity	Carcinogenic	Carcinogenic
34	2-Hydroxy-4-methylbenzaldehyde	Structural alert for genotoxic carcinogenicity	Carcinogenic	Carcinogenic
35	4-Hydroxybenzaldehyde	Structural alert for genotoxic carcinogenicity	Carcinogenic	Non Carcinogenic
36	o-Methoxybenzaldehyde	Structural alert for genotoxic carcinogenicity	Carcinogenic	Carcinogenic
37	4-Hydroxy-3,5-dimethoxybenzaldehyde	Structural alert for genotoxic carcinogenicity	Carcinogenic	Non Carcinogenic
38	2-Benzofurancarboxaldehyde	Negative for genotoxic carcinogenicity and non genotoxic carcinogenicity	Non Carcinogenic	Non Carcinogenic
39	2-Furfurylidenebutyraldehyde	Negative for genotoxic carcinogenicity and non genotoxic carcinogenicity	Carcinogenic	Non Carcinogenic
40	2-Methylfuran	Negative for genotoxic carcinogenicity and non genotoxic carcinogenicity	Non Carcinogenic	Carcinogenic
41	2,5-Dimethylfuran	Negative for genotoxic carcinogenicity and non genotoxic carcinogenicity	Non Carcinogenic	Non Carcinogenic
42	2-Ethylfuran	Negative for genotoxic carcinogenicity and non genotoxic carcinogenicity	Non Carcinogenic	Non Carcinogenic
43	2-Butylfuran	Negative for genotoxic carcinogenicity and non genotoxic carcinogenicity	Non Carcinogenic	Non Carcinogenic
44	2-Pentylfuran	Negative for genotoxic carcinogenicity and non genotoxic carcinogenicity	Non Carcinogenic	Non Carcinogenic
45	2-Heptylfuran	Negative for genotoxic carcinogenicity and non genotoxic carcinogenicity	Non Carcinogenic	Non Carcinogenic
46	2-Decylfuran	Negative for genotoxic carcinogenicity and non genotoxic carcinogenicity	Non Carcinogenic	Non Carcinogenic
47	2-Butyrylfuran	Negative for genotoxic carcinogenicity and non genotoxic carcinogenicity	Non Carcinogenic	Non Carcinogenic
48	2-Pentanoylfuran	Negative for genotoxic carcinogenicity and non genotoxic carcinogenicity	Non Carcinogenic	Carcinogenic

Table 4. (continued)

49	3-Methyl-2-(3-methylbut-2-enyl)-furan	Negative for genotoxic carcinogenicity and non genotoxic carcinogenicity	Non Carcinogenic	Non Carcinogenic
50	2,3-Dimethylbenzofuran	Negative for genotoxic carcinogenicity and non genotoxic carcinogenicity	Non Carcinogenic	Carcinogenic
51	2,4-Difurfurylfuran	Negative for genotoxic carcinogenicity and non genotoxic carcinogenicity	Non Carcinogenic	Carcinogenic
52	2-Acetyl-5-methylfuran	Negative for genotoxic carcinogenicity and non genotoxic carcinogenicity	Non Carcinogenic	Carcinogenic
53	2-Acetyl-3,5-dimethylfuran	Negative for genotoxic carcinogenicity and non genotoxic carcinogenicity	Non Carcinogenic	Carcinogenic
54	3-Acetyl-2,5-dimethylfuran	Negative for genotoxic carcinogenicity and non genotoxic carcinogenicity	Non Carcinogenic	Carcinogenic