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Development of a new quantitative structure–activity relationship model for predicting Ames mutagenicity of food flavor chemicals using StarDrop™ auto-Modeller™

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Abstract

Background: Food flavors are relatively low molecular weight chemicals with unique odor-related functional groups that may also be associated with mutagenicity. These chemicals are often difficult to test for mutagenicity by the Ames test because of their low production and peculiar odor. Therefore, application of the quantitative structure–activity relationship (QSAR) approach is being considered. We used the StarDrop™ Auto-Modeller™ to develop a new QSAR model.

Results: In the first step, we developed a new robust Ames database of 406 food flavor chemicals consisting of existing Ames flavor chemical data and newly acquired Ames test data. Ames results for some existing flavor chemicals have been revised by expert reviews. We also collected 428 Ames test datasets for industrial chemicals from other databases that are structurally similar to flavor chemicals. A total of 834 chemicals' Ames test datasets were used to develop the new QSAR models. We repeated the development and verification of prototypes by selecting appropriate modeling methods and descriptors and developed a local QSAR model. A new QSAR model "StarDrop NIHS 834_67" showed excellent performance (sensitivity: 79.5%, specificity: 96.4%, accuracy: 94.6%) for predicting Ames mutagenicity of 406 food flavors and was better than other commercial QSAR tools.

Conclusions: A local QSAR model, StarDrop NIHS 834_67, was customized to predict the Ames mutagenicity of food flavor chemicals and other low molecular weight chemicals. The model can be used to assess the mutagenicity of food flavors without actual testing.

Keywords: Quantitative structure–activity relationship (QSAR), Food flavors, Mutagenicity Ames test, StarDrop™ auto-Modeller™, Machine learning

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Introduction

Food flavor chemicals are used and/or present in foods at very low level. Human exposure to these flavor chemicals through foods is too low to raise concerns about general toxicity. Regarding mutagenicity, however, there are health concerns even with trace amounts because there is no threshold for mutagenicity, and even very low levels of exposure of mutagenic chemicals do not result in zero carcinogenic risk [1]. Therefore, the presence or absence of mutagenicity is an important point for risk assessment of flavor chemicals.

The bacterial reverse mutation test (Ames test) is an important mutagenicity test, but it requires approximately 2 g of sample for a dose-finding study and main study [2]. On the other hand, the amount of flavor produced industrially is extremely small, which often means that testing is impossible. Additionally, the peculiar odor of some flavors sometimes makes it difficult to perform the test in the laboratory. Recently, quantitative structure–activity relationship (QSAR) approaches instead of the Ames test have been frequently used for assessing the mutagenicity of chemicals [3]. Ono et al. assessed the viability of QSAR tools by using three QSAR tools to calculate the Ames mutagenicity of 367 flavor chemicals (for which Ames test results were available) [4]. Consequently, the highest sensitivity (the ability of a QSAR tool to detect Ames positives chemicals correctly) was 38.9% with the single tool and 47.2% even with the combination of three tools, which indicated that application of QSAR tools to assess the Ames mutagenicity of flavor chemicals was still premature. Therefore, it is necessary to improve or develop QSAR tools for predicting Ames mutagenicity of flavor chemicals.

Flavor chemicals are relatively low molecular weight chemical substances mainly composed of carbon, hydrogen, oxygen, nitrogen, and sulfur that often have specific functional groups. In Japan, most food flavors are classified into 18 types according to their chemical structure [5]. Therefore, with a focus on their characteristic chemical space, we thought that there was potential to increase the predictive performance by developing a local QSAR model customized for flavor chemicals. In recent years, computational software has been provided to assist with development of QSAR models by machine learning. We have tried to develop a QSAR model specialized for flavor chemicals using StarDrop™ software, which has a module (Auto-Modeller™) that can generate predictive models automatically.

Before developing the QSAR model, we developed a new robust Ames database of 406 food flavor chemicals that is based on Ono's database [4]. We re-evaluated ambiguous data judged as "equivocal" in Ono's database via literature review and incorporated Ames test data of flavor chemicals from other publicly available databases. In parallel, we performed the Ames test with key flavor chemicals of which Ames data is unknown and incorporated their results into the new database. This benchmark food flavor chemical database is useful for development of QSAR models and evaluation of QSAR model performance.

Materials & methods

Ames test database of food flavor chemicals

We utilized the Ames test database of food flavor chemicals reported by Ono et al. [4], but because the database includes 14 "equivocal" judgments (Table 1), we re-evaluated by reviewing the reference literature and re-classified them as positive, negative, or inconclusive. Ames test data of the "inconclusive" chemicals were excluded from the database. If there were any other flavor chemicals from publicly available Ames test database (Hansen database [6]), they were also added.

Ames test

Ames tests were performed for 45 flavor chemicals. The purities and suppliers of the test chemicals are shown in Table 2. The Ames tests were conducted by contract research organizations following Good Laboratory Practice compliance according to the Industrial Safety and Health Act test guideline with preincubation method [7]. The test guideline requires five strains (*Salmonella thyphimurium* TA100, TA98, TA1535, TA1537, and *Escherichia coli* WP2 *uvrA*) under both the presence and absence of metabolic activation (rat S9 mix prepared from phenobarbital and 5,6-benzoflavone-induced rat liver), which is similar to the Organization of Economic Co-operation and Development guideline TG471 [8]. The positive criterion is when the number of revertant colonies increased more than twice as much as the control in at least one Ames test strain in the presence or absence of S9 mix. Dose dependency and reproducibility were also considered in the final judgment. The relative activity value (RAV), which is defined as the number of induced revertant colonies per mg, was calculated for the positive result.

Commercial QSAR tools

DEREK Nexus™ is a knowledge-based commercial software developed by Lhasa Limited, UK [9, 10]. The software includes knowledge rules created by considering insights related to structural alert, chemical compound examples, and metabolic activations and mechanisms. We used DEREK Nexus™ version 6.1.0 in this study. DEREK Nexus™ ranks the possibility of mutagenicity (certain, probable, plausible, equivocal, doubted, improbable, impossible, open, contradicted, nothing to report) by applying a "reasoning rule." When it is "certain,"

Table 1 Re-evaluation of Ames test data, which were categorized as "equivocal" by Ono et al. [4]

No.	JECFA No.	Chemical Name	CAS No.	Judgement after review	Key reference*	Comments
1	252	isobutanal	78–84-2	Negative	[13]	The study condition did not meet current standard. Other available data indicative of negative.
2	690	phenol	108–95-2	Negative	[14]	Only one positive report of which response was weak. Other available data indicative of negative.
3	738	furfuryl alcohol	98–00-0	Negative	[15]	Only one report was positive among 6 reports reviewed in the key reference. Although no detail was available, the study conditon is unlikely meet current standard.
4	744	furfural	98–01-1	Negative	[15]	Among 14 reports reviewed in the key reference, 4 reports indicative of positive were questionable. Other 10 reports were negative.
5	836	2-hydroxy-1,2-diphenylethanone	119–53-9	Inconclusive	[16]	Weak positive. Other available data are a mixture of positives/negatives. No conclusion drawn.
6	1168	3-propylidenephthalide	17,369–59-4	Inconclusive	[17]	One positive report reviewed in the key reference raised a question about purity. Other available data were also unclear.
7	1172	6-methylcoumarin	92–48-8	Negative	[18]	Ambiguous response. Other available data indicative of negative.
8	1342	delta-3-carene	13,466–78-9	Inconclusive	[19]	Positve though not meeting current standard. Recent other data (Saverni, 2012) indicative of negative. No conclusion drawn.
9	1450	4-hydroxy-5-methyl-3(2H)-furanone	19,322–27-1	Positive	[20]	Confirmed positive response. No other data negate the conclusion was available.
10	1481	ethyl maltol	4940-11-8	Inconclusive	[21]	Two conflicting reports reviewed in the key reference. No conclusion drawn.
11	1560	allyl isothiocyanate	57–06-7	Positive	[22]	Weak positive. Other available data are a mixture of positives/negatives. "Isothiocyanate" structure adopted as "positve alert" in representative QSAR tools.
12	1561	butyl isothiocyanate	592–82-5	Positive	[23]	Confirmed positive response. No other data negate the conclusion was available.
13	1563	phenethyl isothiocyanate	2257–09-2	Positive	[22]	Weak positive. Other available data also indicate positive.
14	1776	ethyl 2-[(5-methyl-2-propan-2-yl cyclohexanecarbonyl)amino]acetate	68,489–14-5	Negative	[15]	Since the study report indicative of weak positive reviewed in the key reference was unpublished, no reliability confirmed. Recent GLP data submitted to MHLW under ANEI-HOU was negative (undisclosed).

* Reference that was considered as a basis to draw a conclusion of "equivocal".

"probable," "plausible," or "equivocal," the query chemical is predicted to be positive in the Ames test.

CASE Ultra is a QSAR-based toxicity prediction software developed by MultiCASE Inc. (USA). CASE Ultra uses a statistical method to automatically extract alerts based on training data by using machine learning technology [11, 12]. The structural characteristics of the alert surroundings are called the "modulator," and these are also learned automatically from the training data. In this algorithm, to construct a QSAR model with continuous toxicity endpoints, various physical chemistry parameters and descriptors are used. We used CASE Ultra version 1.8.0.2 with the GT1_BMUT module in this study. The prediction result of each module is ranked as "known positive," "positive," "negative," "known negative," "inconclusive," or "out of domain." A query chemical ranked "known positive," "positive" or "inconclusive" is predicted to be positive in the Ames test.

Table 2 Flavor chemicals in which Ames test was newly conducted

No.	JECFA No.	Chemical Name	CAS No	Purity (%)	Supplier	Category*	Ames test result	Comments for Ames test
1	128	hexyl acetate	142–92-7	99.7	Inoue Perfumery MFG. Co.,Ltd.	Esters	Negative	
2	236	delta-dodecalactone	713–95-1	98.5	SODA AROMATIC Co., Ltd.	Lactones	Negative	
3	255	2-methylbutyric acid	116–53-0	99.9	Inoue Perfumery MFG. Co.,Ltd.	Fatty acids	Negative	
4	256	2-ethylbutanal	97–96-1	99.4	SODA AROMATIC Co., Ltd.	Aliphatic higher aldehydes	Negative	
5	327	(5or6)-decenoic acid	72,881–27-7	83.8	SODA AROMATIC Co., Ltd.	Fatty acids	Negative	
6	410	2,3-pentanedione	600–14-6	99.7	Frutarom Ltd	Ketones	Positive**	-S9mix: positive in TA100, TA98 +S9mix: positive in TA100 Maximum RAV; 323 (−S9, TA100)
7	452	dimethyl sulfide	75–18-3	25	Inoue Perfumery MFG. Co.,Ltd.	Thioethers	Negative	
8	470	2-[(methylthio)methyl]-2-butenal	40,878–72-6	98.1	T. HASEGAWA CO., LTD.	Aliphatic higher aldehydes	Positive	-S9mix: positive in TA100 +S9mix: positive in TA100, WP2uvrA Maximum RAV; 225 (−S9, TA100)
9	520	2-mercaptopinane	23,832–18-0	98.0	SIGMA ALDRICH	Thiols	Negative	
10	687	4′-methoxycinnamaldehyde	1963-36-6	98	Alfa Aesar	Aromatic aldehydes	Positive	+S9mix: weak positive in TA100
11	725	4-ethenyl-2-methoxyphenol	7786–61-0	99.8	T. HASEGAWA CO., LTD.	Phenols	Negative	
12	728	raspberry ketone	5471-51-2	99.9	Jiangxi Zhangshu Crown Capital Fragrance Limited	Ketones	Positive	+S9mix: positive in TA1535 Maximum RAV; 10 (+S9, TA1535)
13	745	5-methylfurfural	620–02-0	99.8	R.C. Treatt & Co. Ltd	Furfurals and its derivatives	Negative	
14	866	4-methylbenzaldehyde	104–87-0	99.6	Penta International Corporation	Aromatic aldehydes	Negative	
15	928	hexanal propyleneglycol acetal	1599–49-1	99.9	San-Ei Gen F.F.I.,Inc.	Ethers	Negative	
16	941	acetaldehyde diethyl acetal	105–57-7	99.4	Ogawa & Co., Ltd.	Ethers	Negative	
17	1031	2-(4-methyl-5-thiazolyl)ethanol	137–00-8	99.9	Inoue Perfumery MFG. Co.,Ltd.	Aromatic alcohols	Negative	
18	1072	2-furanmethanethiol	98–02-2	99.5	SIGMA ALDRICH	Thiols	Negative	
19	1208	4-methyl-2-pentenal	5362-56-1	99.2	T. HASEGAWA CO., LTD.	Aliphatic higher aldehydes	Positive	-S9mix: positive in TA100 +S9mix: positive in TA100 Maximum RAV; 1340 (−S9, TA100)
20	1256	isoeugenyl methyl ether	93–16-3	99.4	Inoue Perfumery MFG. Co.,Ltd.	Phenol ethers	Negative	
21	1301	indole	120–72-9	99.7	SIGMA ALDRICH	Indoles and its derivatives	Negative	
22	1304	skatole	83–34-1	98	SIGMA ALDRICH	Indoles and its derivatives	Negative	
23	1340	gamma-terpinene (p-Mentha-1,4-diene)	99–85-4	98.7	Takata Koryo Co., Ltd.	Terpene hydrocarbons	Negative	
24	1341	1,3,5-undecatriene	16,356–11-9	96.6	Givaudan Japan K.K.	Aliphatic higher hydrocarbons	Negative	
25	1354	2-hexenol	2305-21-7	96	SODA AROMATIC Co., Ltd.	Aliphatic higher alcohols	Negative	
26	1451	4-methoxy-2,5-dimethyl-3(2H)-furanone	4077-47-8	97	Tokyo Chemical Industry Co., Ltd.	Ketones	Negative	
27	1454	linalool oxide (furanoid)	1365-19-1	99.5	T. HASEGAWA CO., LTD.	Aliphatic higher alcohols	Negative	
28	1456	2,5-dimethyl-4-oxo-3(5H)-furyl acetate	4166–20-5	> 95	Takata Koryo Co., Ltd.	Esters	Positive	-S9mix: positive in TA100 Maxmum RAV; 77 (−S9, TA100)
29	1472	5-methyl-2-phenyl-2-hexenal	21,834–92-4	96.5	Frutarom Ltd	Aromatic aldehydes	Negative	

Table 2 Flavor chemicals in which Ames test was newly conducted *(Continued)*

No.	JECFA No.	Chemical Name	CAS No	Purity (%)	Supplier	Category*	Ames test result	Comments for Ames test
30	1506	3-acetyl-2,5-dimethylfuran	10,599–70-9	98	Tokyo Chemical Industry Co., Ltd.	Ketones	Positive	-S9mix: positive in TA100, WP2uvrA, TA98 +S9mix: positive in TA100 Maximum RAV; 1281 (–S9, TA100)
31	1519	4,5-dihydro-2,5-dimethyl-4-oxofuran-3-yl butyrate	114,099–96-6	97.0	Tokyo Chemical Industry Co., Ltd.	Esters	Positive	+S9mix: positive in TA100 Maximum RAV; 38 (+S9, TA100)
32	1560	allyl isothiocyanate	57–06-7	> 97	Nippon Terpene Chemicals, Inc.	Isothiocyanates	Positive	-S9mix: weak positive in TA100, TA1535, TA98 +S9mix: weak positive in TA100, TA1535
33	1853	2-(l-menthoxy)ethanol	38,618–23-4	98.7	Takasago International Corporation	Aliphatic higher alcohols	Negative	
34	1882	vanillin propyleneglycol acetal	68,527–74-2	98.8	Inoue Perfumery MFG. Co.,Ltd.	Phenols	Negative	
35	1894	5-hexenyl isothiocyanate	49,776–81-0	95.8	T. HASEGAWA CO., LTD.	Isothiocyanates	Negative	
36	2100	furfural propyleneglycol acetal	4359-54-0	99.7	Inoue Perfumery MFG. Co.,Ltd.	Furfurals and its derivatives	Positive	-S9mix: positive in TA100 Maxmum RAV; 302 (–S9, TA100)
37	2101	furfuryl formate	13,493–97-5	> 98.9	T. HASEGAWA CO., LTD.	Esters	Positive	-S9mix: positive in TA100, WP2uvrA, TA98 +S9mix: positive in TA100, TA98 Maximum RAV; 396 (–S9, TA100)
38	2141	butyl 2-naphthyl ether	10,484–56-7	99.9	Koyo Chemical	Phenol ethers	Negative	
39	2144	methyl beta-phenylglycidate	37,161–74-3	99.8	T. HASEGAWA CO., LTD.	Esters	Positive	-S9mix: positive in TA100, WP2uvrA +S9mix: positive in WP2uvrA Maximum RAV; 84 (–S9, TA100)
40	2157	6-methoxyquinoline	5263–87-6	98.9	Tokyo Chemical Industry Co., Ltd.	Ethers	Positive	-S9mix: positive in all strains +S9mix: positive in all strains Maximum RAV; 51,177 (–S9, TA100)
41	–	2,4-dimethyl-4-phenyltetrahydrofuran	82,461–14-1	99.2	Seikodo Ishida Co., ltd.	Ethers	Negative	
42	–	2-butoxyethyl acetate	112–07-2	99.4	Tokyo Chemical Industry Co., Ltd.	Esters	Negative	
43	–	2-methyl-2-butanethiol	1679-09-0	95	Tronto Research Chemicals Inc.	Thiols	Negative	
44	–	2-methylquinoline	91–63-4	98	Tokyo Chemical Industry Co., Ltd.	Not classified ***	Positive	+S9mix: positive in TA100 Maximum RAV; 604 (+S9, TA100)
45	–	S-methyl methanethiosulfonate	2949-92-0	98.3	Tokyo Chemical Industry Co., Ltd.	Esters	Positive	-S9mix: positive in TA100, WP2uvrA Maximum RAV: 2913

* Eighteen categories (and other than specified else) classified according to their substructures defined in the Japanese Food Sanitation Law
** Contradictory result to the exisiting data
*** Not categorized as "flavorchemical" in Japan

Table 3 2 × 2 contingency matrix for Ames mutagenicity classification

		QSAR prediction	
Ames test result		positive	negative
	positive	true positive (TP)	false negative (FN)
	negative	false positve (FP)	true negative (TN)

Software for developing a new QSAR model

StarDrop™ developed by Optibrium Ltd. (UK) is an integrated software for drug discovery that includes the statistics-based QSAR model generation tool, Auto-Modeller™. Using multiple modeling techniques and a suite of built-in descriptors, Auto-Modeller™ automatically generates tailored predictive models based on the study dataset for the domain that needs to be predicted.

Analysis of QSAR tool performance

Because the Ames test results are binary, positive, or negative, their predictive power can be objectively quantified and assessed from their coincidence from the QSAR calculation results. The 2 × 2 prediction matrix comprising true positive (TP), false positive (FP), false negative (FN), and true negative (TN) is given in Table 3. Sensitivity (ability to detect positive substances) is calculated as TP / (TP + FN), specificity (ability to detect negative substances) is calculated as TN / (TN + FP), and accuracy (prediction rate of positive and negative) is calculated as (TP + TN) / (TP + TN + FP + FN). Applicability is provided by (TP + TN + FP + FN) / total number.

Results

Development of a new Ames test database of food flavor chemicals

We developed a new Ames test database consisting of 406 food flavor chemicals (Table 4). The data source is described as follows.

Ono et al. reported an Ames test database consisting of 367 food flavor chemicals (positive: 24, equivocal: 12, negative: 331) [4]. However, it actually contained 369 chemicals (positive: 24, equivocal: 14, negative: 331). Table 1 shows the 14 equivocal chemicals. We reviewed key references that led to "equivocal" and re-evaluated to determine if there was evidence of positivity or negativity in view of current testing criteria. Our final judgment and the supporting reasons are described in Table 1 [13–23]. If there was insufficient evidence or no detailed information available for the judgment, we concluded that they were "inconclusive." Among 14 equivocal flavoring chemicals, four were positive, six were negative, and four were inconclusive. In total, 365 flavor chemicals (positive: 28, negative: 337), excluding four inconclusive chemicals, were added to the new database.

Two flavor chemicals, quinoline (91–22–5) and 4-methylquinoline (491–35–0) have been added to the new database. Their Ames test data were found in the Hansen data set [6].

We newly performed Ames tests for 45 flavor chemicals. The information of tested samples and the Ames test results are shown in Table 2. Ten of the 45 Ames test results were previously reported [24]. The raw Ames test data are available in the Additional files. Among 45 flavor chemicals, 15 were positive and 30 were negative. Six chemicals, indole (120–72–9), 5-methylfurfural (620–02–0), 2,3-pentanedione (600–14–6), allyl isothiocyanate (57–06–7), skatole (83–34–1), and gamma-terpinene (p-Mentha-1,4-diene) (99–85–4), are also present in Ono's database. In Ono's database [4], 2,3-pentanedione was judged as negative, but it clearly increased the mutant frequency in TA100 in the absence of S9 mix (Additional file (6)). The results of these Ames tests are reflected in the new database. Finally, 39 new food flavor chemicals were added to the database.

Development of a new QSAR model for predicting Ames mutagenicity

We developed a new QSAR model for predicting Ames mutagenicity by using StarDrop™ Auto-Modeller™. To develop the QSAR model, the available Ames test study dataset is essential. We used 406 datasets of flavor chemicals in the new Ames test database to develop the model. To further increase the size of the dataset (especially positive data), we added Ames test data of chemicals structurally similar to flavor chemicals. We previously developed a large Ames test database consisting of > 12,000 industrial chemicals [25]. We selected 428 chemicals (positive: 255; negative: 173) from the database that have molecular weights < 500 and possess a characteristic substructure of flavor chemicals defined in the Food Sanitation Law in Japan [5]. The Ames test data of 834 chemicals (positive: 299, negative: 535) were integrated as the study dataset for the development of the QSAR model.

Prototypes of predictive models were built by using an automatic process. The study dataset was divided into training (70%) and validation (30%) data by using the cluster method, which uses an unsupervised non-hierarchical clustering algorithm developed by Butina [26]. Auto-Modeller™ has three modeling methods (Gaussian process, random forest, and decision tree) for the category model. In a pretest, the random forest model gave the best performance for our target. The descriptors were automatically generated, including whole molecule descriptors (e.g., molecular weight, logP, and polar surface area) and 2D structural descriptors from

Table 4 406 food flavor chemicals assessed by Ames test and QSARs

Table 4 406 food flavor chemicals assessed by Ames test and QSARs

No.	JECFA No.	Chemical name	CAS#	Ames Result	StarDrop NIHS 834_67	Derek Nexus 6.1.0	CASE Ultra 1.8.0.2 GT1_BMUT	Note*
1	217	trans-anethole	4180-23-8	Positive	Positive	INACTIVE	Known Negative	
2	408	diacetyl	431-03-8	Positive	Positive	PLAUSIBLE	Known Negative	
3	410	2,3-pentadione	600-14-6	Positive	Positive	PLAUSIBLE	Known Negative	Ames test was newly conducted (Table 2). Negative in Ono's data was revised.
4	429	menthone	89-80-5	Positive	Negative	INACTIVE	Known Negative	
5	470	2-[(methylthio)methyl]-2-butenal	40878-72-6	Positive	Positive	PLAUSIBLE	Positive	Ames test was newly conducted (Table 2).
6	507	methylsulfinylmethane	67-68-5	Positive	Positive	INACTIVE	Known Positive	
7	656	trans-cinnamaldehyde	104-55-2	Positive	Negative	PLAUSIBLE	Known Positive	
8	687	4'-methoxycinnamaldehyde	1963-36-6	Positive	Positive	PLAUSIBLE	Positive	Ames test was newly conducted (Table 2).
9	712	resorcinol	108-46-3	Positive	Positive	INACTIVE	Known Negative	
10	728	raspberry ketone	5471-51-2	Positive	Negative	INACTIVE	Negative	Ames test was newly conducted (Table 2).
11	735	2-phenylphenol	90-43-7	Positive	Positive	INACTIVE	Known Positive	
12	739	furfuryl acetate	623-17-6	Positive	Positive	PROBABLE	Known Positive	
13	767	2,6-dimethylpyrazine	108-50-9	Positive	Negative	INACTIVE	Known Positive	
14	820	4-phenyl-3-buten-2-one	122-57-6	Positive	Negative	INACTIVE	Known Positive	
15	937	pyruvaldehyde	78-98-8	Positive	Positive	PLAUSIBLE	Known Positive	
16	1032	thiazole	288-47-1	Positive	Positive	INACTIVE	Known Positive	
17	1147	1-penten-3-one	1629-58-9	Positive	Positive	PROBABLE	Known Positive	
18	1175	trans, trans-2,4-hexadienal	142-83-6	Positive	Positive	PLAUSIBLE	Known Positive	
19	1208	4-methyl-2-pentenal	5362-56-1	Positive	Negative	PLAUSIBLE	Positive	Ames test was newly conducted (Table 2).
20	1302	6-methylquinoline	91-62-3	Positive	Positive	PLAUSIBLE	Known Positive	
21	1307	methyl 2-pyrrolyl ketone	1072-83-9	Positive	Positive	INACTIVE	Known Positive	
22	1346	cadinene (mixture of isomers)	29350-73-0	Positive	Negative	INACTIVE	Known Negative	
23	1353	2-hexenal	6728-26-3	Positive	Positive	PLAUSIBLE	Known Positive	
24	1364	2-pentenal	764-39-6	Positive	Positive	PLAUSIBLE	Known Positive	
25	1446	4-hydroxy-2,5-dimethyl-3(2H)-furanone	3658-77-3	Positive	Positive	PROBABLE	Known Positive	
26	1449	2-ethyl-4-hydroxy-5-methyl-3(2H)-furanone	27538-09-6	Positive	Positive	PLAUSIBLE	Negative	
27	1450	4-hydroxy-5-methyl-3(2H)-furanone	19322-27-1	Positive	Positive	PROBABLE	Known Positive	Equivocal in Ono's data was revised (Table 1).
28	1456	2,5-dimethyl-4-oxo-3(5H)-furyl acetate	4166-20-5	Positive	Positive	PLAUSIBLE	Negative	Ames test was newly conducted (Table 2).
29	1480	maltol	118-71-8	Positive	Positive	EQUIVOCAL	Known Positive	Ames test was newly conducted (Table 2).
30	1503	2-furyl methyl ketone	1192-62-7	Positive	Negative	EQUIVOCAL	Known Positive	
31	1506	3-acetyl-2,5-dimethylfuran	10599-70-9	Positive	Positive	EQUIVOCAL	Known Positive	Ames test was newly conducted (Table 2).
32	1519	4,5-dihydro-2,5-dimethyl-4-oxofuran-3-yl butyrate	114099-96-6	Positive	Positive	PLAUSIBLE	Negative	Ames test was newly conducted (Table 2).
33	1560	allyl isothiocyanate	57-06-7	Positive	Positive	PLAUSIBLE	Known Positive	Equivocal in Ono's data was revised (Table 1). Ames test was newly conducted (Table 2).
34	1561	butyl isothiocyanate	592-82-5	Positive	Positive	PLAUSIBLE	Known Positive	Equivocal in Ono's data was revised (Table 1).
35	1563	phenethyl isothiocyanate	2257-09-2	Positive	Positive	PLAUSIBLE	Known Positive	Equivocal in Ono's data was revised (Table 1).
36	1576	ethyl 3-phenylglycidate	121-39-1	Positive	Positive	PLAUSIBLE	Known Negative	
37	2100	furfural propyleneglycol acetal	4359-54-0	Positive	Positive	INACTIVE	Negative	Ames test was newly conducted (Table 2).
38	2101	furfuryl formate	13493-97-5	Positive	Positive	EQUIVOCAL	Inconclusive	Ames test was newly conducted (Table 2).
39	2144	methyl beta-phenylglycidate	37161-74-3	Positive	Positive	PLAUSIBLE	Known Positive	Ames test was newly conducted (Table 2).
40	2157	6-methoxyquinoline	5263-87-6	Positive	Positive	PROBABLE	Known Positive	Ames test was newly conducted (Table 2).
41	-	2-methylquinoline	91-63-4	Positive	Positive	PLAUSIBLE	Known Positive	Ames test was newly conducted (Table 2).
42	-	4-methylquinoline	491-35-0	Positive	Positive	PROBABLE	Known Positive	Hansen database [6]
43	-	quinoline	91-22-5	Positive	Positive	PROBABLE	Known Positive	Hansen database [6]
44	-	S-methyl methanethiosulfonate	2949-92-0	Positive	Negative	INACTIVE	Out of Domain	Ames test was newly conducted (Table 2).
45	3	allyl hexanoate	123-68-2	Negative	Negative	INACTIVE	Known Negative	
46	7	allyl isovalerate	2835-39-4	Negative	Negative	INACTIVE	Known Negative	
47	19	allyl cinnamate	1866-31-5	Negative	Negative	INACTIVE	Inconclusive	
48	22	benzaldehyde	100-52-7	Negative	Negative	INACTIVE	Known Negative	
49	23	benzyl acetate	140-11-4	Negative	Negative	INACTIVE	Known Negative	
50	24	benzyl benzoate	120-51-4	Negative	Negative	INACTIVE	Negative	
51	25	benzyl alcohol	100-51-6	Negative	Negative	INACTIVE	Known Negative	
52	42	isoamyl formate	110-45-2	Negative	Negative	INACTIVE	Known Negative	
53	52	isoamyl alcohol	123-51-3	Negative	Negative	INACTIVE	Known Negative	
54	58	geranyl acetate	105-87-3	Negative	Negative	INACTIVE	Known Negative	
55	79	formic acid	64-18-6	Negative	Negative	INACTIVE	Known Negative	
56	80	acetaldehyde	75-07-0	Negative	Negative	INACTIVE	Known Negative	
57	81	acetic acid	64-19-7	Negative	Negative	INACTIVE	Known Negative	
58	82	propyl alcohol	71-23-8	Negative	Negative	INACTIVE	Known Negative	
59	83	propionaldehyde	123-38-6	Negative	Negative	INACTIVE	Known Negative	
60	84	propionic acid	79-09-4	Negative	Negative	INACTIVE	Known Negative	
61	85	butyl alcohol	71-36-3	Negative	Negative	INACTIVE	Known Negative	
62	86	butyraldehyde	123-72-8	Negative	Negative	INACTIVE	Known Negative	
63	87	butyric acid	107-92-6	Negative	Negative	INACTIVE	Known Negative	
64	88	amyl alcohol	71-41-0	Negative	Negative	INACTIVE	Known Negative	
65	92	hexanal	66-25-1	Negative	Negative	INACTIVE	Negative	
66	93	hexanoic acid	142-62-1	Negative	Negative	INACTIVE	Known Negative	
67	95	heptanal	111-71-7	Negative	Negative	INACTIVE	Known Negative	
68	96	heptanoic acid	111-14-8	Negative	Negative	INACTIVE	Known Negative	
69	97	1-octanol	111-87-5	Negative	Negative	INACTIVE	Known Negative	

Table 4 406 food flavor chemicals assessed by Ames test and QSARs *(Continued)*

70	98	octanal	124-13-0	Negative	Negative	INACTIVE	Negative	
71	99	octanoic acid	124-07-2	Negative	Negative	INACTIVE	Known Negative	
72	101	nonanal	124-19-6	Negative	Negative	INACTIVE	Known Negative	
73	104	decanal	112-31-2	Negative	Negative	INACTIVE	Known Negative	
74	105	decanoic acid	334-48-5	Negative	Negative	INACTIVE	Known Negative	
75	107	undecanal	112-44-7	Negative	Negative	INACTIVE	Negative	
76	109	lauryl alcohol	112-53-8	Negative	Negative	INACTIVE	Known Negative	
77	111	lauric acid	143-07-7	Negative	Negative	INACTIVE	Known Negative	
78	113	myristic acid	544-63-8	Negative	Negative	INACTIVE	Known Negative	
79	114	1-hexadecanol	36653-82-4	Negative	Negative	INACTIVE	Known Negative	
80	116	stearic acid	57-11-4	Negative	Negative	INACTIVE	Known Negative	
81	125	methyl acetate	79-20-9	Negative	Negative	INACTIVE	Known Negative	
82	127	butyl acetate	123-86-4	Negative	Negative	INACTIVE	Known Negative	
83	128	hexyl acetate	142-92-7	Negative	Negative	INACTIVE	Negative	Ames test was newly conducted (Table 2).
84	139	acetone	67-64-1	Negative	Negative	INACTIVE	Known Negative	
85	184	butyl stearate	123-95-5	Negative	Negative	INACTIVE	Negative	
86	196	ethyl isovalerate	108-64-5	Negative	Negative	INACTIVE	Known Negative	
87	219	4-hydroxybutyric acid lactone	96-48-0	Negative	Negative	INACTIVE	Known Negative	
88	225	gamma-heptalactone	105-21-5	Negative	Negative	INACTIVE	Negative	
89	229	gamma-nonalactone	104-61-0	Negative	Negative	INACTIVE	Negative	
90	233	gamma-undecalactone	104-67-6	Negative	Negative	INACTIVE	Known Negative	
91	236	delta-dodecalactone	713-95-1	Negative	Negative	INACTIVE	Negative	Ames test was newly conducted (Table 2).
92	239	omega-pentadecalactone	106-02-5	Negative	Negative	INACTIVE	Known Negative	
93	249	cis-4-hydroxy-6-dodecenoic acid lactone; 1,4-dodec-6-enolactone	18679-18-0	Negative	Negative	INACTIVE	Negative	
94	251	isobutyl alcohol	78-83-1	Negative	Negative	INACTIVE	Known Negative	
95	252	isobutyraldehyde	78-84-2	Negative	Negative	INACTIVE	Known Negative	Equivocal in Ono's data was revised (Table 1).
96	253	isobutyric acid	79-31-2	Negative	Negative	INACTIVE	Negative	
97	254	2-methylbutyraldehyde	96-17-3	Negative	Negative	INACTIVE	Known Negative	
98	255	2-methylbutyric acid	116-53-0	Negative	Negative	INACTIVE	Known Negative	Ames test was newly conducted (Table 2).
99	256	2-ethylbutanal	97-96-1	Negative	Negative	INACTIVE	Negative	Ames test was newly conducted (Table 2).
100	258	3-methylbutyraldehyde	590-86-3	Negative	Negative	INACTIVE	Known Negative	
101	260	2-methylpentanal	123-15-9	Negative	Negative	INACTIVE	Negative	
102	267	2-ethyl-1-hexanol	104-76-7	Negative	Negative	INACTIVE	Known Negative	
103	273	2,6-dimethyloctanal	7779-07-9	Negative	Negative	INACTIVE	Negative	
104	277	isopropyl alcohol	67-63-0	Negative	Negative	INACTIVE	Known Negative	
105	278	2-butanone	78-93-3	Negative	Negative	INACTIVE	Known Negative	
106	301	4-methyl-2-pentanone	108-10-1	Negative	Negative	INACTIVE	Known Negative	
107	302	2,6-dimethyl-4-heptanone	108-83-8	Negative	Negative	INACTIVE	Known Negative	
108	305	isopropyl acetate	108-21-4	Negative	Negative	INACTIVE	Known Negative	
109	311	isopropyl myristate	110-27-0	Negative	Negative	INACTIVE	Known Negative	
110	327	(5or6)-decenoic acid	72881-27-7	Negative	Negative	INACTIVE	Negative	Ames test was newly conducted (Table 2).
111	333	oleic Acid	112-80-1	Negative	Negative	INACTIVE	Known Negative	
112	346	methyl linoleate	112-63-0	Negative	Negative	INACTIVE	Known Positive	
113	349	2,6-dimethyl-5-heptenal	106-72-9	Negative	Negative	INACTIVE	Negative	
114	356	linalool	78-70-6	Negative	Negative	INACTIVE	Known Negative	
115	359	linalyl acetate	115-95-7	Negative	Negative	INACTIVE	Known Positive	
116	366	alpha-terpineol	98-55-5	Negative	Negative	INACTIVE	Known Negative	
117	374	p-menth-8-en-1-ol; β-terpineol	138-87-4	Negative	Negative	INACTIVE	Negative	
118	380	carvone	99-49-0	Negative	Negative	INACTIVE	Known Negative	
119	381	carveol	99-48-9	Negative	Negative	INACTIVE	Known Negative	
120	382	carvyl acetate	97-42-7	Negative	Negative	INACTIVE	Known Negative	
121	388	alpha-ionone	127-41-3	Negative	Negative	INACTIVE	Known Negative	
122	389	beta-ionone	79-77-6	Negative	Negative	INACTIVE	Known Negative	
123	398	methyl-alpha-ionone	127-42-4	Negative	Negative	INACTIVE	Known Negative	
124	400	methyl-delta-ionone	7784-98-7	Negative	Negative	INACTIVE	Known Negative	
125	405	acetoin	513-86-0	Negative	Negative	INACTIVE	Known Negative	
126	413	3,4-hexanedione	4437-51-8	Negative	Positive	PLAUSIBLE	Known Positive	
127	418	methylcyclo- pentenolone	80-71-7	Negative	Negative	INACTIVE	Known Negative	
128	424	2-hydroxy-2-cyclohexen-1-one	10316-66-2	Negative	Negative	PROBABLE	Negative	
129	427	menthol	89-78-1	Negative	Negative	INACTIVE	Known Negative	
130	443	l-menthol ethylene glycol carbonate	156324-78-6	Negative	Negative	INACTIVE	Negative	
131	444	(-)-menthol 1- and 2-propylene glycol carbonate	156329-82-2	Negative	Negative	INACTIVE	Negative	
132	446	dl-menthone 1,2-glycerol ketal	63187-91-7	Negative	Negative	INACTIVE	Negative	
133	452	dimethyl sulfide	75-18-3	Negative	Negative	INACTIVE	Out of Domain	Ames test was newly conducted (Table 2).
134	458	allyl sulfide	592-88-1	Negative	Negative	INACTIVE	Negative	
135	492	methylthio 2-(acetyloxy)propionate	74586-09-7	Negative	Negative	INACTIVE	Negative	
136	493	methylthio 2-(propionyloxy) propionate	827024-53-3	Negative	Negative	INACTIVE	Negative	
137	520	2-mercaptopinane	23832-18-0	Negative	Negative	INACTIVE	Out of Domain	Ames test was newly conducted (Table 2).
138	521	allyl mercaptan	870-23-5	Negative	Negative	INACTIVE	Out of Domain	
139	525	benzenethiol	108-98-5	Negative	Negative	INACTIVE	Known Positive	
140	526	benzyl mercaptan	100-53-8	Negative	Negative	INACTIVE	Known Negative	
141	532	1,2-ethanedithiol	540-63-6	Negative	Negative	INACTIVE	Known Negative	

Table 4 406 food flavor chemicals assessed by Ames test and QSARs *(Continued)*

142	551	2-mercaptopropionic acid	79-42-5	Negative	Negative	INACTIVE	Negative	
143	564	dimethyl disulfide	624-92-0	Negative	Negative	INACTIVE	Known Negative	
144	572	allyl disulfide	2179-57-9	Negative	Negative	INACTIVE	Negative	
145	578	phenyl disulfide	882-33-7	Negative	Negative	INACTIVE	Known Negative	
146	579	benzyl disulfide	150-60-7	Negative	Negative	INACTIVE	Known Positive	
147	595	ethyl acetoacetate	141-97-9	Negative	Positive	INACTIVE	Known Negative	
148	610	hydroxycitronellol	107-74-4	Negative	Negative	INACTIVE	Negative	
149	611	hydroxycitronellal	107-75-5	Negative	Negative	INACTIVE	Negative	
150	612	hydroxycitronellal dimethyl acetal	141-92-4	Negative	Negative	INACTIVE	Negative	
151	614	diethyl malonate	105-53-3	Negative	Negative	INACTIVE	Known Negative	
152	616	dimethyl succinate	106-65-0	Negative	Negative	INACTIVE	Known Negative	
153	618	fumaric acid	110-17-8	Negative	Negative	INACTIVE	Known Negative	
154	619	l-malic acid	97-67-6	Negative	Negative	INACTIVE	Known Negative	
155	623	adipic acid	124-04-9	Negative	Negative	INACTIVE	Known Negative	
156	625	dibutyl sebacate	109-43-3	Negative	Negative	INACTIVE	Known Negative	
157	626	ethylene brassylate	105-95-3	Negative	Negative	INACTIVE	Known Negative	
158	627	aconitic acid	499-12-7	Negative	Negative	INACTIVE	Known Negative	
159	645	3-phenylpropionaldehyde	104-53-0	Negative	Negative	INACTIVE	Negative	
160	647	cinnamyl alcohol	104-54-1	Negative	Negative	INACTIVE	Known Negative	
161	657	cinnamic acid	621-82-9	Negative	Negative	INACTIVE	Known Negative	
162	659	ethyl cinnamate	103-36-6	Negative	Negative	INACTIVE	Known Negative	
163	667	cyclohexyl cinnamate	7779-17-1	Negative	Negative	INACTIVE	Negative	
164	670	benzyl cinnamate	103-41-3	Negative	Negative	INACTIVE	Negative	
165	674	alpha-amylcinnamyl alcohol	101-85-9	Negative	Negative	INACTIVE	Negative	
166	683	alpha-methylcinnamaldehyde	101-39-3	Negative	Negative	INACTIVE	Known Negative	
167	685	alpha-amylcinnamaldehyde	122-40-7	Negative	Negative	INACTIVE	Known Negative	
168	686	alpha-hexylcinnamaldehyde	101-86-0	Negative	Negative	INACTIVE	Positive	
169	688	o-methoxycinnamaldehyde	1504-74-1	Negative	Negative	PLAUSIBLE	Known Positive	
170	689	p-methoxy-alpha-methyl-cinnamaldehyde	65405-67-6	Negative	Negative	INACTIVE	Positive	
171	690	phenol	108-95-2	Negative	Negative	INACTIVE	Known Negative	Equivocal in Ono's data was revised (Table 1).
172	691	o-cresol	95-48-7	Negative	Negative	INACTIVE	Known Negative	
173	692	m-cresol	108-39-4	Negative	Negative	INACTIVE	Known Negative	
174	693	p-cresol	106-44-5	Negative	Negative	INACTIVE	Known Negative	
175	694	p-ethylphenol	123-07-9	Negative	Negative	INACTIVE	Known Negative	
176	706	2,5-xylenol	95-87-4	Negative	Negative	INACTIVE	Known Negative	
177	707	2,6-xylenol	576-26-1	Negative	Negative	INACTIVE	Known Negative	
178	708	3,4-xylenol	95-65-8	Negative	Negative	INACTIVE	Known Negative	
179	709	thymol	89-83-8	Negative	Negative	INACTIVE	Known Negative	
180	713	guaiacol	90-05-1	Negative	Negative	INACTIVE	Known Negative	
181	721	2,6-dimethoxyphenol	91-10-1	Negative	Negative	INACTIVE	Known Negative	
182	725	4-ethenyl-2-methoxyphenol	7786-61-0	Negative	Negative	INACTIVE	Known Negative	Ames test was newly conducted (Table 2).
183	727	2-hydroxyacetophenone	118-93-4	Negative	Negative	INACTIVE	Negative	
184	733	4-(1,1-dimethylethyl) phenol	98-54-4	Negative	Negative	INACTIVE	Known Negative	
185	736	phenyl salicylate	118-55-8	Negative	Positive	INACTIVE	Known Negative	
186	738	furfuryl alcohol	98-00-0	Negative	Negative	INACTIVE	Known Negative	Equivocal in Ono's data was revised (Table 1).
187	744	furfural	98-01-1	Negative	Negative	EQUIVOCAL	Known Negative	Equivocal in Ono's data was revised (Table 1).
188	745	5-methylfurfural	620-02-0	Negative	Negative	EQUIVOCAL	Known Negative	Ames test was newly conducted (Table 2).
189	753	pulegone	89-82-7	Negative	Negative	INACTIVE	Known Positive	
190	758	menthofuran	494-90-6	Negative	Negative	EQUIVOCAL	Known Negative	
191	761	2-methylpyrazine	109-08-0	Negative	Negative	INACTIVE	Known Negative	
192	762	2-ethylpyrazine	13925-00-3	Negative	Negative	INACTIVE	Known Negative	
193	765	2,3-dimethylpyrazine	5910-89-4	Negative	Negative	INACTIVE	Known Negative	
194	766	2,5-dimethylpyrazine	123-32-0	Negative	Negative	INACTIVE	Known Negative	
195	768	2-ethyl-3-methylpyrazine	15707-23-0	Negative	Negative	INACTIVE	Negative	
196	774	2,3,5-trimethylpyrazine	14667-55-1	Negative	Negative	INACTIVE	Known Negative	
197	775	2-ethyl-3 (5 or 6)-dimethylpyrazine	13925-07-0	Negative	Negative	INACTIVE	Negative	
198	780	2,3,5,6-tetramethylpyrazine	1124-11-4	Negative	Negative	INACTIVE	Negative	
199	788	2-methoxy-3-methylpyrazine	2847-30-5	Negative	Negative	INACTIVE	Negative	
200	798	5-methylquinoxaline	13708-12-8	Negative	Negative	INACTIVE	Negative	
201	799	alpha-methylbenzyl alcohol	98-85-1	Negative	Negative	INACTIVE	Known Negative	
202	806	acetophenone	98-86-2	Negative	Negative	INACTIVE	Known Negative	
203	811	methyl beta-naphthyl ketone	93-08-3	Negative	Negative	INACTIVE	Known Negative	
204	812	4-acetyl-6-t-butyl-1,1-dimethylindan	13171-00-1	Negative	Negative	INACTIVE	Known Negative	
205	818	4-(p-methoxyphenyl)-2-butanone	104-20-1	Negative	Negative	INACTIVE	Negative	
206	819	4-phenyl-3-buten-2-ol	17488-65-2	Negative	Negative	INACTIVE	Negative	
207	824	propiophenone	93-55-0	Negative	Negative	INACTIVE	Negative	
208	825	alpha-propylphenethyl alcohol	705-73-7	Negative	Negative	INACTIVE	Negative	
209	826	1-(p-methoxyphenyl)-1-penten-3-one	104-27-8	Negative	Negative	INACTIVE	Negative	
210	831	benzophenone	119-61-9	Negative	Negative	INACTIVE	Known Negative	
211	833	1-Phenyl-1,2-propanedione	579-07-7	Negative	Negative	INACTIVE	Known Negative	
212	834	ethyl benzoylacetate	94-02-0	Negative	Negative	INACTIVE	Negative	
213	841	benzyl formate	104-57-4	Negative	Negative	INACTIVE	Inconclusive	
214	850	benzoic acid	65-85-0	Negative	Negative	INACTIVE	Known Negative	

Table 4 406 food flavor chemicals assessed by Ames test and QSARs *(Continued)*

215	851	methyl benzoate	93-58-3	Negative	Negative	INACTIVE	Known Negative	
216	857	isoamyl benzoate	94-46-2	Negative	Negative	INACTIVE	Negative	
217	864	p-isopropylbenzyl alcohol	536-60-7	Negative	Negative	INACTIVE	Negative	
218	866	4-methylbenzaldehyde	104-87-0	Negative	Negative	INACTIVE	Known Negative	Ames test was newly conducted (Table 2).
219	867	tolualdehydes (mixed ortho, meta, para)	529-20-4	Negative	Negative	INACTIVE	Known Negative	
220	868	cuminaldehyde	122-03-2	Negative	Negative	INACTIVE	Negative	
221	870	butyl 4-hydroxybenzoate	94-26-8	Negative	Negative	INACTIVE	Known Negative	
222	871	anisyl alcohol	105-13-5	Negative	Negative	INACTIVE	Known Negative	
223	877	veratraldehyde	120-14-9	Negative	Negative	INACTIVE	Known Negative	
224	878	p-methoxybenzaldehyde	123-11-5	Negative	Negative	INACTIVE	Known Negative	
225	879	p-ethoxybenzaldehyde	10031-82-0	Negative	Negative	INACTIVE	Negative	
226	884	methyl anisate	121-98-2	Negative	Negative	INACTIVE	Negative	
227	888	vanillyl butyl ether	82654-98-6	Negative	Negative	INACTIVE	Known Negative	
228	889	vanillin	121-33-5	Negative	Negative	INACTIVE	Known Negative	
229	893	ethyl vanillin	121-32-4	Negative	Negative	INACTIVE	Known Negative	
230	894	piperonyl acetate	326-61-4	Negative	Negative	INACTIVE	Known Negative	
231	896	piperonal	120-57-0	Negative	Negative	INACTIVE	Known Positive	
232	897	salicyladehyde	90-02-8	Negative	Negative	INACTIVE	Known Negative	
233	899	methyl salicylate	119-36-8	Negative	Negative	INACTIVE	Known Negative	
234	909	glycerol	56-81-5	Negative	Negative	INACTIVE	Known Negative	
235	918	glyceryl monostearate	123-94-4	Negative	Negative	INACTIVE	Known Negative	
236	925	propylene glycol	57-55-6	Negative	Negative	INACTIVE	Known Negative	
237	928	hexanal propyleneglycol acetal	1599-49-1	Negative	Negative	INACTIVE	Negative	Ames test was newly conducted (Table 2).
238	930	lactic acid	598-82-3	Negative	Negative	INACTIVE	Known Negative	
239	931	ethyl lactate	97-64-3	Negative	Negative	INACTIVE	Known Negative	
240	935	butyl butyryllactate	7492-70-8	Negative	Negative	INACTIVE	Negative	
241	936	pyruvic acid	127-17-3	Negative	Negative	INACTIVE	Known Negative	
242	938	ethyl pyruvate	617-35-6	Negative	Positive	INACTIVE	Known Negative	
243	941	acetaldehyde diethyl acetal	105-57-7	Negative	Negative	INACTIVE	Known Positive	Ames test was newly conducted (Table 2).
244	951	pyrazine	290-37-9	Negative	Negative	INACTIVE	Known Negative	
245	953	ethyl vanillin isobutyrate	188417-26-7	Negative	Negative	INACTIVE	Negative	
246	973	p-mentha-1,8-dien-7-al	2111-75-3	Negative	Negative	INACTIVE	Known Negative	
247	977	2,6,6-trimethylcyclohexa-1,3-dienyl methanal	116-26-7	Negative	Negative	INACTIVE	Inconclusive	
248	987	phenethyl alcohol	60-12-8	Negative	Negative	INACTIVE	Known Negative	
249	1002	phenylacetaldehyde	122-78-1	Negative	Negative	INACTIVE	Negative	
250	1007	phenylacetic acid	103-82-2	Negative	Negative	INACTIVE	Known Negative	
251	1009	ethyl phenylacetate	101-97-3	Negative	Negative	INACTIVE	Negative	
252	1013	isobutyl phenylacetate	102-13-6	Negative	Negative	INACTIVE	Known Negative	
253	1014	isoamyl phenylacetate	102-19-2	Negative	Negative	INACTIVE	Negative	
254	1023	p-tolylacetaldehyde	104-09-6	Negative	Negative	INACTIVE	Negative	
255	1027	ethyl (p-tolyloxy)acetate	67028-40-4	Negative	Negative	INACTIVE	Negative	
256	1028	2-phenoxyethyl isobutyrate	103-60-6	Negative	Negative	INACTIVE	Negative	
257	1029	sodium 2-(4-methoxyphenoxy)propanoate	13794-15-5	Negative	Negative	INACTIVE	Negative	
258	1031	2-(4-methyl-5-thiazolyl)ethanol	137-00-8	Negative	Negative	INACTIVE	Negative	Ames test was newly conducted (Table 2).
259	1035	4,5-dimethylthiazole	3581-91-7	Negative	Negative	INACTIVE	Out of Domain	
260	1043	4-methylthiazole	693-95-8	Negative	Negative	INACTIVE	Known Negative	
261	1050	5-methyl-2-thiophenecarboxaldehyde	13679-70-4	Negative	Negative	INACTIVE	Negative	
262	1072	2-furanmethanethiol	98-02-2	Negative	Negative	INACTIVE	Negative	Ames test was newly conducted (Table 2).
263	1094	cyclohexyl butyrate	1551-44-6	Negative	Negative	INACTIVE	Known Negative	
264	1100	cyclohexanone	108-94-1	Negative	Negative	INACTIVE	Known Negative	
265	1101	cyclopentanone	120-92-3	Negative	Negative	INACTIVE	Negative	
266	1106	2-hexylidene cyclopentanone	17373-89-6	Negative	Negative	INACTIVE	Negative	
267	1108	2,2,6-trimethylcyclohexanone	2408-37-9	Negative	Negative	INACTIVE	Negative	
268	1111	tetramethyl ethylcyclohexenone (mixture of isomers)	17369-60-7	Negative	Negative	INACTIVE	Negative	
269	1112	isophorone	78-59-1	Negative	Negative	INACTIVE	Known Negative	
270	1120	6-methyl-5-hepten-2-one	110-93-0	Negative	Negative	INACTIVE	Negative	
271	1124	3-penten-2-one	625-33-2	Negative	Positive	INACTIVE	Negative	
272	1131	4-methyl-3-penten-2-one	141-79-7	Negative	Negative	INACTIVE	Known Negative	
273	1134	6-methyl-3,5-heptadien-2-one	1604-28-0	Negative	Negative	INACTIVE	Inconclusive	
274	1135	(E)-7-methyl-3-octen-2-one	33046-81-0	Negative	Negative	INACTIVE	Negative	
275	1153	1-decen-3-ol	51100-54-0	Negative	Negative	INACTIVE	Negative	
276	1164	(+/-)-(2,6,6,-trimethyl-2-hydroxycyclohexylidene)acetic acid gamma-lactone	15356-74-8	Negative	Negative	INACTIVE	Negative	
277	1166	octahydrocoumarin	4430-31-3	Negative	Negative	INACTIVE	Negative	
278	1171	dihydrocoumarin	119-84-6	Negative	Negative	INACTIVE	Known Negative	
279	1172	6-methylcoumarin	92-48-8	Negative	Negative	INACTIVE	Known Negative	Equivocal in Ono's data was revised (Table 1).
280	1185	2,4-nonadienal	6750-03-4	Negative	Negative	INACTIVE	Positive	
281	1186	nona-2-trans-6-cis-dienal	557-48-2	Negative	Negative	INACTIVE	Known Negative	
282	1190	2-trans,4-trans-decadienal	25152-84-5	Negative	Negative	INACTIVE	Known Negative	
283	1193	ethyl 2,4,7-decatrienoate	78417-28-4	Negative	Negative	INACTIVE	Negative	
284	1199	(+/-)-2-methyl-1-butanol	137-32-6	Negative	Negative	INACTIVE	Negative	
285	1209	2-methyl-2-pentenal	623-36-9	Negative	Negative	PLAUSIBLE	Positive	
286	1219	dl-citronellol	106-22-9	Negative	Negative	INACTIVE	Known Negative	

Table 4 406 food flavor chemicals assessed by Ames test and QSARs *(Continued)*

287	1220	citronellal	106-23-0	Negative	Negative	INACTIVE	Known Negative	
288	1223	geraniol	106-24-1	Negative	Negative	INACTIVE	Known Negative	
289	1225	citral	5392-40-5	Negative	Negative	INACTIVE	Known Negative	
290	1230	farnesol	4602-84-0	Negative	Negative	INACTIVE	Known Negative	
291	1234	eucalyptol	470-82-6	Negative	Negative	INACTIVE	Known Negative	
292	1241	anisole	100-66-3	Negative	Negative	INACTIVE	Negative	
293	1243	p-methylanisole	104-93-8	Negative	Negative	INACTIVE	Negative	
294	1244	p-propylanisole	104-45-0	Negative	Negative	INACTIVE	Negative	
295	1248	1,2-dimethoxybenzene	91-16-7	Negative	Negative	INACTIVE	Negative	
296	1249	m-dimethoxybenzene	151-10-0	Negative	Negative	INACTIVE	Negative	
297	1250	p-dimethoxybenzene	150-78-7	Negative	Negative	INACTIVE	Known Negative	
298	1255	diphenyl ether	101-84-8	Negative	Negative	INACTIVE	Known Negative	
299	1256	dibenzyl ether	103-50-4	Negative	Negative	INACTIVE	Known Negative	
300	1257	beta-naphthyl methyl ether	93-04-9	Negative	Negative	INACTIVE	Inconclusive	
301	1258	beta-naphthyl ethyl ether	93-18-5	Negative	Negative	INACTIVE	Known Negative	
302	1259	beta-naphthyl isobutyl ether	2173-57-1	Negative	Negative	INACTIVE	Negative	
303	1260	isoeugenol	97-54-1	Negative	Negative	INACTIVE	Known Negative	
304	1263	isoeugenyl phenylacetate	120-24-1	Negative	Negative	INACTIVE	Negative	
305	1264	propenylguaethol	94-86-0	Negative	Negative	INACTIVE	Negative	
306	1266	isoeugenyl methyl ether	93-16-3	Negative	Negative	INACTIVE	Negative	Ames test was newly conducted (Table 2).
307	1268	isoeugenyl benzyl ether	120-11-6	Negative	Negative	INACTIVE	Negative	
308	1289	3-mercapto-2-methylbutan-1-ol	227456-33-9	Negative	Negative	INACTIVE	Negative	
309	1301	indole	120-72-9	Negative	Negative	INACTIVE	Known Positive	Ames test was newly conducted (Table 2).
310	1303	isoquinoline	119-65-3	Negative	Positive	INACTIVE	Known Negative	
311	1304	skatole	83-34-1	Negative	Negative	INACTIVE	Known Negative	Ames test was newly conducted (Table 2).
312	1314	pyrrole	109-97-7	Negative	Negative	INACTIVE	Known Negative	
313	1315	3-ethylpyridine	536-78-7	Negative	Negative	INACTIVE	Negative	
314	1316	3-acetylpyridine	350-03-8	Negative	Negative	INACTIVE	Negative	
315	1323	camphene	79-92-5	Negative	Negative	INACTIVE	Known Negative	
316	1324	beta-caryophyllene	87-44-5	Negative	Negative	INACTIVE	Known Negative	
317	1325	p-cymene	99-87-6	Negative	Negative	INACTIVE	Negative	
318	1326	d-limonene	5989-27-5	Negative	Negative	INACTIVE	Known Negative	
319	1327	myrcene	123-35-3	Negative	Negative	INACTIVE	Negative	
320	1329	alpha-pinene	80-56-8	Negative	Negative	INACTIVE	Known Negative	
321	1330	beta-pinene	127-91-3	Negative	Negative	INACTIVE	Known Negative	
322	1332	biphenyl	92-52-4	Negative	Negative	INACTIVE	Known Negative	
323	1334	4-methylbiphenyl	644-08-6	Negative	Negative	INACTIVE	Negative	
324	1335	`	90-12-0	Negative	Negative	INACTIVE	Known Negative	
325	1340	p-mentha-1,4-diene	99-85-4	Negative	Negative	INACTIVE	Negative	Ames test was newly conducted (Table 2).
326	1341	1,3,5-undecatriene	16356-11-9	Negative	Negative	INACTIVE	Negative	Ames test was newly conducted (Table 2).
327	1351	ethyl acrylate	140-88-5	Negative	Negative	INACTIVE	Known Negative	
328	1354	2-hexenol	2305-21-7	Negative	Negative	INACTIVE	Negative	Ames test was newly conducted (Table 2).
329	1356	methyl 2-nonynoate	111-80-8	Negative	Negative	INACTIVE	Out of Domain	
330	1357	methyl 2-octynoate	111-12-6	Negative	Negative	INACTIVE	Out of Domain	
331	1360	2-heptenal	18829-55-5	Negative	Negative	PLAUSIBLE	Known Negative	
332	1362	2-nonenal	2463-53-8	Negative	Negative	INACTIVE	Inconclusive	
333	1363	2-octenal	2363-89-5	Negative	Negative	INACTIVE	Inconclusive	
334	1371	(E)-2-butenoic acid	107-93-7	Negative	Negative	INACTIVE	Negative	
335	1385	borneol	507-70-0	Negative	Negative	INACTIVE	Known Negative	
336	1391	isobornyl propionate	2756-56-1	Negative	Negative	INACTIVE	Negative	
337	1395	d-camphor	464-49-3	Negative	Negative	INACTIVE	Known Negative	
338	1408	3-L-menthoxypropane-1,2-diol	87061-04-9	Negative	Negative	INACTIVE	Known Negative	
339	1411	3-(l-menthoxy)-2-methylpropane-1,2-diol	195863-84-4	Negative	Negative	INACTIVE	Negative	
340	1413	d,l-menthol 1- and 2-propylene glycol carbonate	30304-82-6	Negative	Negative	INACTIVE	Negative	
341	1416	p-menthane-3,8-diol	42822-86-6	Negative	Negative	INACTIVE	Negative	
342	1441	2-(3-phenylpropyl)tetrahydrofuran	3208-40-0	Negative	Negative	INACTIVE	Negative	
343	1443	tetrahydrofurfuryl alcohol	97-99-4	Negative	Negative	INACTIVE	Known Negative	
344	1445	tetrahydrofurfuryl propionate	637-65-0	Negative	Positive	INACTIVE	Negative	
345	1451	4-methoxy-2,5-dimethyl-3(2H)-furanone	4077-47-8	Negative	Negative	PLAUSIBLE	Negative	Ames test was newly conducted (Table 2).
346	1454	linalool oxide (furanoid)	1365-19-1	Negative	Negative	INACTIVE	Negative	Ames test was newly conducted (Table 2).
347	1459	beta-methylphenethyl alcohol	1123-85-9	Negative	Negative	INACTIVE	Negative	
348	1467	2-phenylpropionaldehyde	93-53-8	Negative	Negative	INACTIVE	Negative	
349	1468	2-phenylpropionaldehyde dimethyl acetal	90-87-9	Negative	Negative	INACTIVE	Negative	
350	1470	2-phenylpropyl isobutyrate	65813-53-8	Negative	Negative	INACTIVE	Negative	
351	1472	5-methyl-2-phenyl-2-hexenal	21834-92-4	Negative	Negative	INACTIVE	Inconclusive	Ames test was newly conducted (Table 2).
352	1487	2-methylfuran	534-22-5	Negative	Negative	EQUIVOCAL	Known Negative	
353	1488	2,5-dimethylfuran	625-86-5	Negative	Negative	EQUIVOCAL	Known Negative	
354	1494	3-methyl-2-(3-methylbut-2-enyl)-furan	15186-51-3	Negative	Negative	INACTIVE	Negative	
355	1497	3-(2-furyl)acrolein	623-30-3	Negative	Negative	PLAUSIBLE	Known Negative	
356	1511	4-(2-furyl)-3-buten-2-one	623-15-4	Negative	Negative	INACTIVE	Known Negative	
357	1513	ethyl 3-(2-furyl)propanoate	10031-90-0	Negative	Positive	INACTIVE	Negative	
358	1526	O-ethyl S-(2-furylmethyl)thiocarbonate	376595-42-5	Negative	Positive	INACTIVE	Negative	
359	1529	eugenol	97-53-0	Negative	Negative	INACTIVE	Known Negative	

Table 4 406 food flavor chemicals assessed by Ames test and QSARs *(Continued)*

360	1534	methyl anthranilate	134-20-3	Negative	Negative	INACTIVE	Known Negative	
361	1535	ethyl anthranilate	87-25-2	Negative	Negative	INACTIVE	Known Negative	
362	1536	butyl anthranilate	7756-96-9	Negative	Negative	INACTIVE	Known Negative	
363	1537	isobutyl anthranilate	7779-77-3	Negative	Negative	INACTIVE	Known Negative	
364	1540	linalyl anthranilate	7149-26-0	Negative	Negative	INACTIVE	Known Negative	
365	1541	cyclohexyl anthranilate	7779-16-0	Negative	Negative	INACTIVE	Known Negative	
366	1543	phenylethyl anthranilate	133-18-6	Negative	Negative	INACTIVE	Known Negative	
367	1545	methyl N-methylanthranilate	85-91-6	Negative	Negative	INACTIVE	Known Negative	
368	1549	methyl N-formylanthranilate	41270-80-8	Negative	Negative	INACTIVE	Negative	
369	1552	N-benzoylanthranilic acid	579-93-1	Negative	Negative	INACTIVE	Negative	
370	1562	benzyl isothiocyanate	622-78-6	Negative	Negative	PLAUSIBLE	Known Positive	
371	1575	beta-caryophyllene oxide	1139-30-6	Negative	Negative	INACTIVE	Negative	
372	1577	ethyl methylphenylglycidate	77-83-8	Negative	Negative	INACTIVE	Known Negative	
373	1579	ethylamine	75-04-7	Negative	Negative	INACTIVE	Known Negative	
374	1581	isopropylamine	75-31-0	Negative	Negative	INACTIVE	Known Negative	
375	1582	butylamine	109-73-9	Negative	Negative	INACTIVE	Known Negative	
376	1583	isobutylamine	78-81-9	Negative	Negative	INACTIVE	Known Negative	
377	1584	sec-butylamine	13952-84-6	Negative	Negative	INACTIVE	Known Negative	
378	1585	pentylamine	110-58-7	Negative	Negative	INACTIVE	Known Negative	
379	1592	acetamide	60-35-5	Negative	Positive	INACTIVE	Known Negative	
380	1595	2-isopropyl-N,2,3-trimethylbutyramide	51115-67-4	Negative	Negative	INACTIVE	Negative	
381	1598	N-isobutyl (E,E)-2,4-decadienamide	18836-52-7	Negative	Negative	INACTIVE	Negative	
382	1600	piperine	94-62-2	Negative	Negative	INACTIVE	Known Negative	
383	1607	piperidine	110-89-4	Negative	Negative	INACTIVE	Negative	
384	1609	pyrrolidine	123-75-1	Negative	Negative	INACTIVE	Known Positive	
385	1610	trimethylamine	75-50-3	Negative	Negative	INACTIVE	Known Negative	
386	1611	triethylamine	121-44-8	Negative	Negative	INACTIVE	Known Negative	
387	1615	piperazine	110-85-0	Negative	Negative	INACTIVE	Known Negative	
388	1649	1-phenyl-3-methyl-3-pentanol	10415-87-9	Negative	Negative	INACTIVE	Negative	
389	1654	alpha,alpha-dimethylphenethyl formate	10058-43-2	Negative	Negative	INACTIVE	Inconclusive	
390	1681	allyl thiohexanoate	156420-69-8	Negative	Negative	INACTIVE	Negative	
391	1687	3,6-diethyl-1,2,4,5-tetrathiane	54717-12-3	Negative	Negative	INACTIVE	Negative	
392	1700	allyl propyl disulfide	2179-59-1	Negative	Negative	INACTIVE	Known Negative	
393	1716	dihydroxyacetone dimer	62147-49-3	Negative	Positive	INACTIVE	Known Positive	
394	1767	N-(heptan-4-yl)benzo[d][1,3]dioxole-5-carboxamide	745047-51-2	Negative	Negative	INACTIVE	Negative	
395	1768	N1-(2,4-dimethoxybenzyl)-N2-(2-(pyridin-2-yl)ethyl)oxalamide	745047-53-4	Negative	Negative	INACTIVE	Negative	
396	1772	N-gluconyl ethanolamine	686298-93-1	Negative	Positive	INACTIVE	Negative	
397	1774	N-lactoyl ethanolamine	5422-34-4	Negative	Negative	INACTIVE	Known Positive	
398	1776	N-[(ethoxycarbonyl)methyl)-p-menthane-3-carboxamide	68489-14-5	Negative	Negative	INACTIVE	Negative	Equivocal in Ono's data was revised (Table 1).
399	1777	N-[2-(3,4-dimethoxyphenyl)ethyl]-3,4-dimethoxycinnamic acid amide	69444-90-2	Negative	Negative	INACTIVE	Negative	
400	1853	2-(l-menthoxy)ethanol	38618-23-4	Negative	Negative	INACTIVE	Negative	Ames test was newly conducted (Table 2).
401	1882	vanillin propyleneglycol acetal	68527-74-2	Negative	Negative	INACTIVE	Negative	Ames test was newly conducted (Table 2).
402	1894	5-hexenyl isothiocyanate	49776-81-0	Negative	Negative	PLAUSIBLE	Inconclusive	Ames test was newly conducted (Table 2).
403	2141	butyl 2-naphthyl ether	10484-56-7	Negative	Negative	INACTIVE	Negative	Ames test was newly conducted (Table 2).
404	-	2-butoxyethyl acetate	112-07-2	Negative	Negative	INACTIVE	Negative	Ames test was newly conducted (Table 2).
405	-	2,4-dimethyl-4-phenyltetrahydrofuran	82461-14-1	Negative	Positive	INACTIVE	Negative	Ames test was newly conducted (Table 2).
406	-	2-methyl-2-butanethiol	1679-09-0	Negative	Negative	INACTIVE	Out of Domain	Ames test was newly conducted (Table 2).

* Note: No description means that Ono's Ames data was unrevised.

the training set. Because the accuracy of the prototype depends on the training data set and the data splitting process is not replicable, 80 prototypes were built to search for the best model. The prototypes that earned favorable prediction scores were selected for further performance evaluation by using the Ames test data of flavoring chemicals, and their performances were compared with those of the benchmarks. Finally, a new QSAR model "StarDrop NIHS 834_67" was developed. The prediction result is ranked as "positive" or "negative."

Performance of the QSAR model

We evaluated the performance of StarDrop NIHS834_67 to predict the Ames mutagenicity. We calculated the Ames mutagenicity of 406 food flavors listed in the new

Table 5 Results of QSAR calculation of 406 flavor chemicals in 2X2 contingency matrix

		StarDrop NIHS 834_67		Derek Nexus 6.1.0		CASE Ultra 1.8.0.2 GT1_BMUT		
		P	N	P	N	P	N	OOD
Ames test result	P	35	9	31	13	31	12	1
	N	13	349	14	348	28	327	7

P positive, *N* negative, *OOD* out of domain

Table 6 Performance of three QSARs for predicting Ames mutagenicity of 406 flavor chemicals

	Sensitivity (%)	Specificity (%)	Accuracy (%)	Applicability (%)
StarDrop NIHS 834_67	79.5	96.4	94.6	100.0
Derek Nexus 6.1.0	70.5	96.1	93.3	100.0
CASE Ultra 1.8.0.2 GT1_BMUT	70.5	90.3	88.2	98.0

Table 7 Ames positive chemicals, but predicted as negative by StarDrop NIHS 834_67 (False negative)

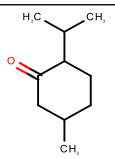
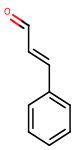
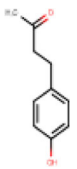
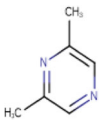
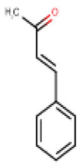
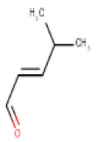
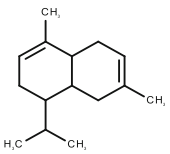
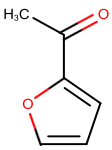
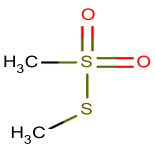
No.	JECFA No.	Chemical Name	CAS No.	Structure	Substructure Class	Note
1	429	menthone	89–80-5		Ketones	DEREK: INACTIVE CASE Ultra: Known Negative
2	656	trans-cinnamaldehyde	104–55-2		Aromatic aldehydes	DEREK: PLAUSIBLE CASE Ultra: Known Positive
3	728	raspberry ketone	5471-51-2		Ketones	DEREK: INACTIVE CASE Ultra: Negative
4	767	2,6-dimethylpyrazine	108–50-9		Newly designated flavors	DEREK: INACTIVE CASE Ultra: Known Positive
5	820	4-phenyl-3-buten-2-one	122–57-6		Ketones	DEREK: INACTIVE CASE Ultra: Known Positive
6	1208	4-methyl-2-pentenal	5362-56-1		Aliphatic higher aldehydes	DEREK: PLAUSIBLE CASE Ultra: Positive
7	1346	cadinene (mixture of isomers)	29,350–73-0		Terpene hydrocarbons	DEREK: INACTIVE CASE Ultra: Known Negative
8	1503	2-Furyl methyl ketone	1192–62-7		Ketones	DEREK: EQUIVOCAL CASE Ultra: Known Positive
9	–	S-methyl methanethiosulfonate	2949-92-0		Esters	DEREK: INACTIVE CASE Ultra: Out of Domain

Table 8 Ames negative chemicals, but predicted as positive by StarDrop NIHS 834_67 (False positive)

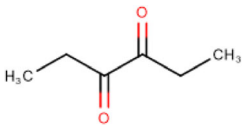
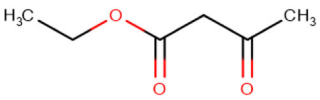
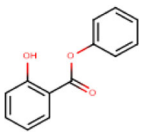
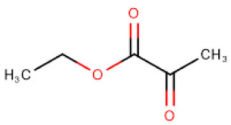
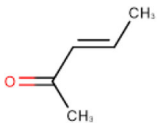
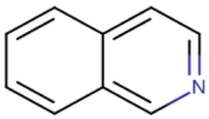
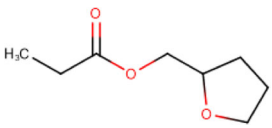
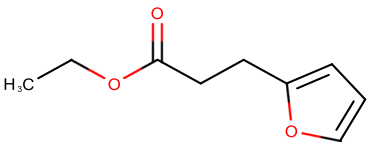
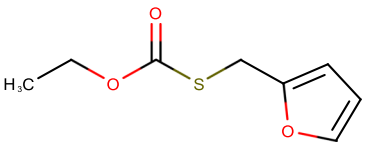
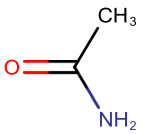
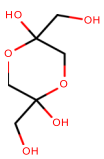
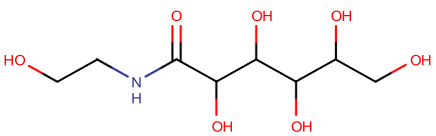
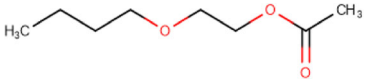
No.	JECFA No.	Chemical Name	CAS No.	Structure	Substructure Class	Note
1	413	3,4-hexanedione	4437-51-8		Ketones	DEREK: PLAUSIBLE CASE Ultra: Known Positive
2	595	ethyl acetoacetate	141–97-9		Esters	DEREK: INACTIVE CASE Ultra: Known Negative
3	736	phenyl salicylate	118–55-8		Esters	DEREK: INACTIVE CASE Ultra: Known Negative
4	938	ethyl pyruvate	617–35-6		Esters	DEREK: INACTIVE CASE Ultra: Known Negative
5	1124	3-penten-2-one	625–33-2		Ketones	DEREK: INACTIVE CASE Ultra: Negative
6	1303	isoquinoline	119–65-3		Newly designated flavors	DEREK: INACTIVE CASE Ultra: Known Negative
7	1445	tetrahydrofurfuryl propionate	637–65-0		Esters	DEREK: INACTIVE CASE Ultra: Negative
8	1513	ethyl 3-(2-furyl)propanoate	10,031–90-0		Esters	DEREK: INACTIVE CASE Ultra: Negative
9	1526	O-ethyl S-(2-furylmethyl)thiocarbonate	376,595–42-5		Esters	DEREK: INACTIVE CASE Ultra: Negative
10	1592	acetamide	60–35-5		Not classified	DEREK: INACTIVE CASE Ultra: Known Negative
11	1716	dihydroxyacetone dimer	62,147–49-3		Ketones	DEREK: INACTIVE CASE Ultra: Known Positive
12	1772	N-gluconyl ethanolamine	686,298–93-1		Not classified	DEREK: INACTIVE CASE Ultra: Negative

Table 8 Ames negative chemicals, but predicted as positive by StarDrop NIHS 834_67 (False positive) *(Continued)*

No.	JECFA No.	Chemical Name	CAS No.	Structure	Substructure Class	Note
13	–	2-butoxyethyl acetate	112–07-2		Esters	DEREK: INACTIVE CASE Ultra: Negative

Ames test database by using StarDrop NIHS 834_67, DEREK Nexus™, and CASE Ultra. Table 4 shows the results of the QSAR calculation. Table 5 is a 2 × 2 prediction matrix, and Table 6 shows the performance (sensitivity, specificity, accuracy, and applicability) of the three (Q) SARs. StarDrop NIHS 834_67 showed the best performance. Table 7 shows nine FN chemicals that were positive in the Ames test but were negatively predicted by NIHS834_67. Table 8 shows 13 FP chemicals that were negative in the Ames test but were positively predicted by NIHS834_67.

Discussion

We have developed new Ames database consisting of 406 types of food flavor chemicals. This benchmark food flavor chemicals database is open to the public and useful for risk assessment of food additives and developing QSAR models for predicting Ames mutagenicity of food flavor chemicals and other low molecular weight chemicals. The main body of the database is derived from the database reported by Ono et al. [4]. We re-assessed 14 "equivocal" chemicals and classified them as negative, positive, or inconclusive. However, the positive and negative chemicals remaining in Ono's database were not re-assessed. Some of these chemicals may also be misjudged. In fact, 2,3-pentanedione (600–14–6), which was negative in Ono's database, was clearly positive in the present Ames test (Additional file (6)). To ensure database robustness, it is necessary to re-assess the test results reported as positive and negative. As will be described later, especially, the results of the Ames test that differ from the QSAR prediction results could be questioned.

In 2012, Ono et al. reported the performance of three commercial QSAR tools (Derek for Windows, MultiCASE, and ADMEWorks) for predicting Ames mutagenicity of 367 food flavor chemicals [4]. Derek for Windows and MultiCASE are earlier models of DEREK Nexus™ and CASE Ultra, respectively. As a result, the sensitivity, specificity, and accuracy were 38.9, 93.4, and 88.0% (Derek for Windows), 25.0, 94.3, and 87.5% (MultiCASE), respectively. In this study, we evaluated the performance of DEREK Nexus™ and CASE Ultra for 406 food flavors in the new Ames database. As a result, the sensitivity, specificity, and accuracy were 70.5, 96.1, and 93.3% (DEREK Nexus™) and 70.5, 90.3, and 88.2% (CASE Ultra), respectively. These results indicate that the performance of the QSAR prediction has improved significantly over the last decade. The improvement in sensitivity was particularly remarkable. Improvement of the QSAR models and accumulation of newly acquired Ames test training data may have contributed to the high performance. In particular, the NIHS-sponsored Ames/QSAR International Challenge Project has contributed significantly to improving the performance of commercial QSAR tools, such as DEREK Nexus™ and CASE Ultra, which have acquired over 12,000 unique chemical Ames datasets [24]. The newly developed StarDrop NIHS 834_67 outperformed DEREK Nexus™ and CASE Ultra. StarDrop NIHS 834_67 also acquired 428 chemicals (positive: 255, negative: 173) selected from the 12,000 unique chemical Ames datasets. Despite incorporating the same training data, StarDrop NIHS 834_67 provided higher prediction, probably due to differences in the target chemical space. Flavor chemicals are relatively low molecular weight and have unique functional groups that allow them to focus on the chemical space of interest and develop highly predictable models with relatively small size training data. Our attempt to develop a local QSAR model that focused on flavor chemicals has been somewhat successful. However, it is not surprising that that StarDrop NIHS 834_67 showed higher performance than other QSAR tools. It may be because StarDrop NIHS 834_67 used the results of 39 new flavor chemical datasets and revised existing flavor chemical data for training and validation data.

Considering that the estimated interlaboratory reproducibility of the Ames test has been reported to be approximately 85% [27, 28], the performance of the prediction may be approaching the upper limit. Nonetheless, FN and FP analysis points to improvements in the database and QSAR models. Of the nine FN flavor chemicals by StarDrop NIHS 834_67, menthone (89–80–5), raspberry ketone (54–51–2), and cadinene (29350–73–0) were also predicted as negative by DEREK Nexus™ and CASE Ultra (Table 7). The Ames mutagenicity of these chemicals, which were predicted to be negative by the three QSARs, may actually be negative chemicals. We need to perform actual Ames tests to confirm.

In this study, we examined the Ames tests for raspberry ketone (54–51–2) and the result was positive (Table 4). However, the mutagenic activity was very weak (RAV: 10) (Additional file (12)). Structural features

found in FN chemicals include the α, β-unsaturated carbonyl structures, trans-cinnamaldehyde (104–55–2), 4-phenyl-3-buten-2-one (122–57–6), 4-methyl-2-pentenal (5362–56–1), and 2- furyl methyl ketone (1192–62–7), which were predicted to be positive by DEREK Nexus™ and/or CASE Ultra. The α, β-unsaturated carbonyl structure is a typical alert for Ames mutagenicity [29–31]. These predictions indicate that the alert is incorporated in DEREK Nexus™ and CASE Ultra but not in StarDrop NIHS 834_67. By incorporating α and β-unsaturated carbonyl chemicals as training data, it is expected that the FN rate of StarDrop NIHS 834_67 will be reduced and the predictability will be improved.

On the other hand, of the 13 FP chemicals, 3,4-hexanedione (4437–51–8) was also predicted as positive by DEREK Nexus™ and CASE Ultra. The Ames mutagenicity of this chemical may actually be positive. Interestingly, 12 other FP flavor chemicals were correctly predicted as negative by DEREK Nexus™ and CASE Ultra, which highlights the different characteristics between StarDrop NIHS 834_67 and other QSAR tools and indicates the potential for further improvement.

Conclusions

We developed a new Ames database of 406 food flavor chemicals. Using this database and other Ames datasets of chemicals that are structurally similar to flavor chemicals, we also developed a new QSAR model for predicting Ames mutagenicity. The local QSAR model, StarDrop NIHS 834_67, is customized to efficiently predict the mutagenicity of food flavors and other low molecular weight chemicals, delivering performance superior to that of other commercial QSAR tools. By further improving the model, it can be used to assess the mutagenicity of food flavors without actual testing.

Abbreviations

QSAR: Quantitative structure–activity relationship; TP: True positive; TN: True negative; FP: False positive; FN: False negative

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s41021-021-00182-6.

Additional file 1: Raw data for the Ames tests.

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Authors' contributions

Conceived and designed the studies: MH, QSAR model development: ST and MK, QSAR calculation: AK, Analyzed the data: TK, Ames test management: KS, MY, MY, KM, and KH, Wrote the paper: MH and TK. The authors read and approved the final manuscript.

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Availability of data and materials

All generated data are included in this manuscript. Raw data for the Ames tests are available in the Additional files.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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