Table 3
Performance of each combination of three (Q)SAR models.

Ames results	(Q)SAR				
	Combination-3(+)			Combination-3(-)	
	Combination-2(+)		Combination-2(-)		
	Combination-1(+)			Combination-1(-)	
	3+	2+, 1-	1+, 2-	3-	Total
Positive	3	3	5	13	24
Equivocal	1	0	5	6	12
Negative	1	5	56	269	331
Total	5	8	66	288	367

The highest sensitivity with the Ames results was provided by Derek for Windows (38.9%), followed by MultiCase (25.0%). ADME-Works provided the lowest sensitivity (13.9%), the specificities and concordances provided by three all models were more than 90% and 80%, respectively, and the applicability of all three (Q)SAR models was 100%. The applicability of each (Q)SAR model used depends on the system of the model; however, all compounds were evaluated by all three (Q)SAR models. The false positives and false negatives were 61–85% and 6–10%, respectively. In combinatorial (Q)SAR evaluation, sensitivity was 17.4% (combination-1) to 47.2% (combination-3), specificity 81.3% (combination-3) to 99.6% (combination-1), concordance 77.9% (combination-3) to 93.2% (combination-1), and applicability 79.8% (combination-1) to 100.0% (combination-2 and 3). For combination-1, some compounds could not be judged based on three (Q)SAR outcomes, such as two positives with one negative ("2+,1−") and one positive with two negatives ("1+,2−"), shown in Table 3, and so the applicability was less than 100% in this case.

4. Discussion

Our previous (Q)SAR models were developed especially to be customized for application to industrial chemicals, and the sensitivities of the previous combinatorial (Q)SAR systems were 73–99% (Hayashi et al., 2005). The sensitivities in the current study were lower, probably because the chemical structure domains in the data set specialized in flavors would be much different from those of the model training data set consisting of general industrial chemicals. The number of positives was very low compared with negatives, and the percentage of positive chemicals was about 7.3% (24/331). If a chemical had some positive results, most of the results indicated weak mutagenicity. This suggested that most of them are expected to not have genotoxicity, because the chemicals tested in the present study were evaluated as safe for use as food additive flavors by JECFA; however, according to our definition of Ames-positive in the present study, some flavors suspected as negative were judged as positive. For example, methylsulfinylmethane, phenol and eugenol, etc., were defined as Ames-positive based on only one positive result, while many other results for those chemicals indicated negative.

In combination-2, 325 Ames-negative chemicals were correctly judged as negative from 331 Ames-negatives and the specificity was 98.2%; however, only 7 Ames-positive chemicals were correctly judged as positive from 36 Ames-positives, including equivocal flavors, and the sensitivity was low (19.4%). In contrast, 79 chemicals were judged as positive in combination-3, and the sensitivity increased to 47.2%. The model with the highest sensitivity (38.9%) among three single models was Derek for Windows, as indicated in Table 2. The contribution of this increased sensitivity in combination-3 was mainly due to the result of the prediction by Derek

Table 5
False negative flavors in all three (Q)SAR models.

JECFA No.	Compound	CAS No.
217	*trans*-Anethole	4180-23-8
408	Diacetyl	431-03-8
429	Menthone	89-80-5
507	Methylsulfinylmethane (DMSO)	67-68-5
712	Resorcinol	108-46-3
735	2-Phenylphenol	90-43-7
767	2,6-Dimethylpyrazine	108-50-9
1032	Thiazole	228-47-1
1307	Methyl 2-pyrrolyl ketone	1072-83-9
1346	Cadinene	29350-73-0
1446	4-Hydroxy-2,5-dimethyl-3(2*H*)-furanone (DMHF)	3658-77-3
1449	4-Hydroxy-2-ethyl-5-methyl-3(2*H*)-furanone (HEMF)	27538-09-6
1480	Maltol	118-71-8

These compound names are used in JECFA. These 13 flavors have one or some report(s) that are Ames-positive, but they were negative with three (Q)SAR models (Derek for Windows, MultiCase, ADMEWorks) in our present study.

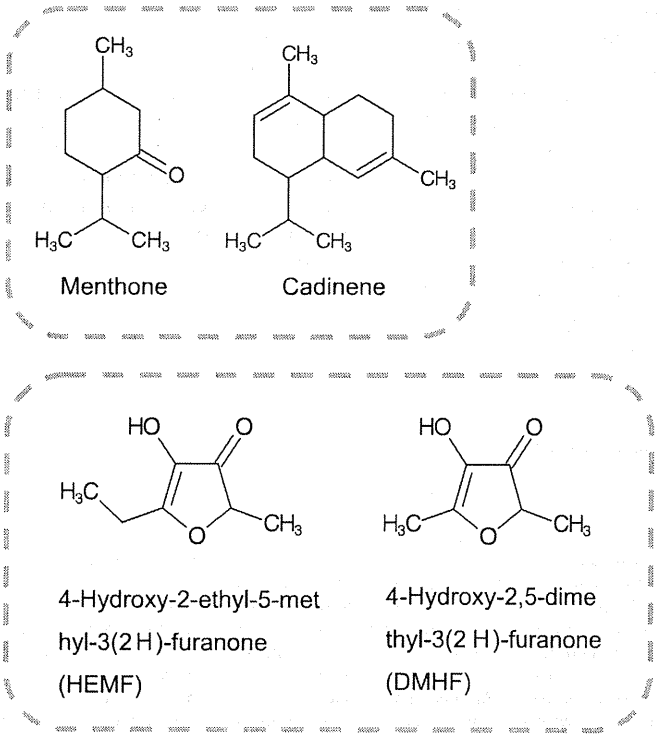

Fig. 1. Structures of false negative chemicals with a common sub-structure.

Table 4
Results of evaluation of each combination of three (Q)SAR models.

	Sensitivity (%)	Specificity (%)	Concordance	Applicability (%)	False positive (%)	False negative (%)
Combination-1	17.4	99.6	93.2	79.8	20.0	6.6
Combination-2	19.4	98.2	90.5	100.0	46.2	8.2
Combination-3	47.2	81.3	77.9	100.0	78.5	6.6

Appendix A
Flavoring compounds evaluated and their Ames and (Q)SAR results.

JECFA No.	Flavor chemicals	Ames result	Derek for Windows	MultiCASE	ADMEWorks
1175	*trans,trans*-2,4-Hexadienal	Positive	+	+	+
1302	6-Methylquinoline	Positive	+	+	+
937	Pyruvaldehyde	Positive	+	+	+
739	Furfuryl acetate	Positive	+	+	–
1147	1-Penten-3-one	Positive	+	+	–
1353	2-Hexenal	Positive	+	–	+
656	*trans*-cinnamaldehyde	Positive	+	–	–
1364	2-Pentenal	Positive	+	–	–
1503	2-Furyl methyl ketone	Positive	+	–	–
1576	Ethyl 3-phenylglycidate	Positive	+	–	–
820	4-Phenyl-3-buten-2-one	Positive	–	+	–
217	*trans*-Anethole	Positive	–	–	–
408	Diacetyl	Positive	–	–	–
429	Menthone	Positive	–	–	–
507	Methylsulfinylmethane (DMSO)	Positive	–	–	–
712	Resorcinol	Positive	–	–	–
735	2-Phenylphenol	Positive	–	–	–
767	2,6-Dimethylpyrazine	Positive	–	–	–
1032	Thiazole	Positive	–	–	–
1307	Methyl 2-pyrrolyl ketone	Positive	–	–	–
1346	Cadinene	Positive	–	–	–
1446	4-Hydroxy-2,5-dimethyl-3(2*H*)-furanone (DMHF)	Positive	–	–	–
1449	4-Hydroxy-2-ethyl-5-methyl-3(2*H*)-furanone (HEMF)	Positive	–	–	–
1480	Maltol	Positive	–	–	–
1560	Allyl isothiocyanate	Equivocal	+	+	+
738	Furfuryl alcohol	Equivocal	+	–	–
744	Furfural	Equivocal	+	–	–
1561	Butyl isothiocyanate	Equivocal	+	–	–
1563	Phenethyl isothiocyanate	Equivocal	+	–	–
1168	3-Propylidenephthalide	Equivocal	–	+	–
1450	4-Hydroxy-5-methyl-3(2*H*)-furanone	Equivocal	–	+	–
252	Isobutyraldehyde	Equivocal	–	–	+
690	Phenol	Equivocal	–	–	–
836	Benzoin	Equivocal	–	–	–
1172	6-Methylcoumarin	Equivocal	–	–	–
1342	δ-3-Carene	Equivocal	–	–	–
1481	Ethyl maltol	Equivocal	–	–	–
1776	*N*-[(Ethoxycarbonyl)methyl]-*p*-menthane-3-carboxamide	Equivocal	–	–	–
1209	2-Methyl-2-pentenal	Negative	+	+	+
686	alpha-Hexylcinnamaldehyde	Negative	+	+	–
689	*para*-Methoxy-alpha-methylcinnamaldehyde	Negative	+	+	–
973	*para*-Mentha-1,8-dien-7-al	Negative	+	+	–
977	2,6,6-Trimethylcyclohexa-1,3-dienyl methanal	Negative	+	+	–
1225	Citral	Negative	+	–	+
683	alpha-Methylcinnamaldehyde	Negative	+	–	–
685	alpha-Amylcinnamaldehyde	Negative	+	–	–
688	*ortho*-Methoxycinnamaldehyde	Negative	+	–	–
745	5-Methylfurfural	Negative	+	–	–
1185	2,4-Nonadienal	Negative	+	–	–
1186	Nona-2-*trans*-6-*cis*-dienal	Negative	+	–	–
1190	2-*trans*,4-*trans*-Decadienal	Negative	+	–	–
1360	2-Heptenal	Negative	+	–	–
1362	2-Nonenal	Negative	+	–	–
1363	2-Octenal	Negative	+	–	–
1487	2-Methylfuran	Negative	+	–	–
1488	2,5-Dimethylfuran	Negative	+	–	–
1497	3-(2-Furyl)acrolein	Negative	+	–	–
1562	Benzyl isothiocyanate	Negative	+	–	–
1577	Ethyl methylphenylglycidate	Negative	+	–	–
1716	Dihydroxyacetone	Negative	+	–	–
42	Isoamyl formate	Negative	–	+	–
413	3,4-Hexanedione	Negative	–	+	–
492	Methylthio 2-(acetyloxy)propionate	Negative	–	+	–
493	Methylthio 2-(propionyloxy)propionate	Negative	–	+	–
521	Allyl mercaptan	Negative	–	+	–
526	Benzyl mercaptan	Negative	–	+	–
841	Benzyl formate	Negative	–	+	–
1002	Phenylacetaldehyde	Negative	–	+	–
1023	*para*-Tolylacetaldehyde	Negative	–	+	–
1356	Methyl 2-nonynoate	Negative	–	+	–
1357	Methyl 2-octynoate	Negative	–	+	–
1681	Allyl thiohexanoate	Negative	–	+	–
1687	3,6-Diethyl-1,2,4,5-tetrathiane	Negative	–	+	–
1774	*N*-Lactoyl ethanolamine	Negative	–	+	–

(*continued on next page*)

Appendix A (*continued*)

JECFA No.	Flavor chemicals	Ames result	Derek for Windows	MultiCASE	ADMEWorks
83	Propionaldehyde	Negative	–	–	+
258	3-Methylbutyraldehyde	Negative	–	–	+
301	4-Methyl-2-pentanone	Negative	–	–	+
349	2,6-Dimethyl-5-heptenal	Negative	–	–	+
405	Acetoin	Negative	–	–	+
410	2,3-Pentanedione	Negative	–	–	+
532	1,2-Ethanedithiol	Negative	–	–	+
564	Dimethyl disulfide	Negative	–	–	+
761	2-Methylpyrazine	Negative	–	–	+
798	5-Methylquinoxaline	Negative	–	–	+
857	Isoamyl benzoate	Negative	–	–	+
884	Methyl anisate	Negative	–	–	+
899	Methyl salicylate	Negative	–	–	+
909	Glycerol	Negative	–	–	+
1013	Isobutyl phenylacetate	Negative	–	–	+
1120	6-Methyl-5-hepten-2-one	Negative	–	–	+
1131	4-Methyl-3-penten-2-one	Negative	–	–	+
1135	(*E*)-7-Methyl-3-octen-2-one	Negative	–	–	+
1268	Isoeugenyl benzyl ether	Negative	–	–	+
1534	Methyl anthranilate	Negative	–	–	+
1535	Ethyl anthranilate	Negative	–	–	+
1537	Isobutyl anthranilate	Negative	–	–	+
1543	Phenylethyl anthranilate	Negative	–	–	+
1545	Methyl *N*-methylanthranilate	Negative	–	–	+
1549	Methyl *N*-formylanthranilate	Negative	–	–	+
1654	α,α-Dimethylphenethyl formate	Negative	–	–	+
3	Allyl hexanoate	Negative	–	–	–
7	Allyl isovalerate	Negative	–	–	–
19	Allyl cinnamate	Negative	–	–	–
22	Benzaldehyde	Negative	–	–	–
23	Benzyl acetate	Negative	–	–	–
24	Benzyl benzoate	Negative	–	–	–
25	Benzyl alcohol	Negative	–	–	–
52	Isoamyl alcohol	Negative	–	–	–
58	Geranyl acetate	Negative	–	–	–
79	Formic acid	Negative	–	–	–
80	Acetaldehyde	Negative	–	–	–
81	Acetic acid	Negative	–	–	–
82	Propyl alcohol	Negative	–	–	–
84	Propionic acid	Negative	–	–	–
85	Butyl alcohol	Negative	–	–	–
86	Butyraldehyde	Negative	–	–	–
87	Butyric acid	Negative	–	–	–
88	Amyl alcohol	Negative	–	–	–
92	Hexanal	Negative	–	–	–
93	Hexanoic acid	Negative	–	–	–
95	Heptanal	Negative	–	–	–
96	Heptanoic acid	Negative	–	–	–
97	1-Octanol	Negative	–	–	–
98	Octanal	Negative	–	–	–
99	Octanoic acid	Negative	–	–	–
101	Nonanal	Negative	–	–	–
104	Decanal	Negative	–	–	–
105	Decanoic acid	Negative	–	–	–
107	Undecanal	Negative	–	–	–
109	Lauryl alcohol	Negative	–	–	–
111	Lauric acid	Negative	–	–	–
113	Myristic acid	Negative	–	–	–
114	1-Hexadecanol	Negative	–	–	–
116	Stearic acid	Negative	–	–	–
125	Methyl acetate	Negative	–	–	–
127	Butyl acetate	Negative	–	–	–
139	Acetone	Negative	–	–	–
184	Butyl stearate	Negative	–	–	–
196	Ethyl isovalerate	Negative	–	–	–
219	4-Hydroxybutyric acid lactone (gamma-Butyrolactone)	Negative	–	–	–
225	gamma-Heptalactone	Negative	–	–	–
229	gamma-Nonalactone	Negative	–	–	–
233	gamma-Undecalactone	Negative	–	–	–
239	omega-Pentadecalactone	Negative	–	–	–
249	1,4-Dodec-6-enolactone	Negative	–	–	–
251	Isobutyl alcohol	Negative	–	–	–
253	Isobutyric acid	Negative	–	–	–
254	2-Methylbutyraldehyde	Negative	–	–	–
260	2-Methylpentanal	Negative	–	–	–

Appendix A (*continued*)

JECFA No.	Flavor chemicals	Ames result	Derek for Windows	MultiCASE	ADMEWorks
267	2-Ethyl-1-hexanol	Negative	–	–	–
273	2,6-Dimethyloctanal	Negative	–	–	–
277	Isopropyl alcohol	Negative	–	–	–
278	2-Butanone	Negative	–	–	–
302	2,6-Dimethyl-4-heptanone	Negative	–	–	–
305	Isopropyl acetate	Negative	–	–	–
311	Isopropyl myristate	Negative	–	–	–
333	Oleic acid	Negative	–	–	–
346	Methyl linoleate	Negative	–	–	–
356	Linalool	Negative	–	–	–
359	Linalyl acetate	Negative	–	–	–
366	alpha-Terpineol	Negative	–	–	–
374	β-Teroineol	Negative	–	–	–
380	Carvone	Negative	–	–	–
381	Carveol	Negative	–	–	–
382	Carvyl acetate	Negative	–	–	–
388	alpha-Ionone	Negative	–	–	–
389	β-Ionone	Negative	–	–	–
398	Methyl-alpha-ionone	Negative	–	–	–
400	Methyl-delta-ionone	Negative	–	–	–
418	Methylcyclopentenolone	Negative	–	–	–
424	2-Hydroxy-2-cyclohexen-1-one	Negative	–	–	–
427	Menthol	Negative	–	–	–
443	(-)-Menthol ethyleneglycol	Negative	–	–	–
444	(-)-Menthol 1- and 2-propylene glycol carbonate	Negative	–	–	–
446	(±)-Menthone 1,2-glycerol ketal	Negative	–	–	–
458	Allyl sulfide	Negative	–	–	–
525	Benzenethiol	Negative	–	–	–
551	2-Mercaptopropionic acid	Negative	–	–	–
572	Allyl disulfide	Negative	–	–	–
578	Phenyl disulfide	Negative	–	–	–
579	Benzyl disulfide	Negative	–	–	–
595	Ethyl acetoacetate	Negative	–	–	–
610	Hydroxycitronellol	Negative	–	–	–
611	Hydroxycitronellal	Negative	–	–	–
612	Hydroxycitronellal dimethyl acetal	Negative	–	–	–
614	Diethyl malonate	Negative	–	–	–
616	Dimethyl succinate	Negative	–	–	–
618	Fumaric acid	Negative	–	–	–
619	*l*-Malic acid	Negative	–	–	–
623	Adipic acid	Negative	–	–	–
625	Dibutyl sebacate	Negative	–	–	–
626	Ethylene brassylate	Negative	–	–	–
627	Aconitic acid	Negative	–	–	–
645	3-Phenylpropionaldehyde	Negative	–	–	–
647	Cinnamyl alcohol	Negative	–	–	–
657	Cinnamic acid	Negative	–	–	–
659	Ethyl cinnamate	Negative	–	–	–
667	Cyclohexyl cinnamate	Negative	–	–	–
670	Benzyl cinnamate	Negative	–	–	–
674	alpha-Amylcinnamyl alcohol	Negative	–	–	–
691	*ortho*-Cresol	Negative	–	–	–
692	*meta*-Cresol	Negative	–	–	–
693	*para*-Cresol	Negative	–	–	–
694	*para*-Ethylphenol	Negative	–	–	–
706	2,5-Xylenol	Negative	–	–	–
707	2,6-Xylenol	Negative	–	–	–
708	3,4-Xylenol	Negative	–	–	–
709	Thymol	Negative	–	–	–
713	Guaiacol	Negative	–	–	–
721	2,6-Dimethoxyphenol	Negative	–	–	–
727	2-Hydroxyacetophenone	Negative	–	–	–
733	4-(1,1-Dimethyl)ethylphenol	Negative	–	–	–
736	Phenyl salicylate	Negative	–	–	–
753	Pulegone	Negative	–	–	–
758	Menthofuran	Negative	–	–	–
762	2-Ethylpyrazine	Negative	–	–	–
765	2,3-Dimethylpyrazine	Negative	–	–	–
766	2,5-Dimethylpyrazine	Negative	–	–	–
768	2-Ethyl-3-methylpyrazine	Negative	–	–	–
774	2,3,5-Trimethylpyrazine	Negative	–	–	–
775	2-Ethyl-3,5-dimethylpyrazine and 2-Ethyl-3,6-dimethylpyrazine	Negative	–	–	–
780	2,3,5,6-Tetramethylpyrazine	Negative	–	–	–
788	2-Methoxy-(3, 5 or 6)-methylpyrazine	Negative	–	–	–

(*continued on next page*)

Appendix A (*continued*)

JECFA No.	Flavor chemicals	Ames result	Derek for Windows	MultiCASE	ADMEWorks
799	alpha-Methylbenzyl alcohol	Negative	–	–	–
806	Acetophenone	Negative	–	–	–
811	Methyl beta-naphthyl ketone	Negative	–	–	–
812	4-Acetyl-6-*tert*-butyl-1,1-dimethylindan	Negative	–	–	–
818	4-(*para*-Methoxy-phenyl)-2-butanone	Negative	–	–	–
819	4-Phenyl-3-buten-2-ol	Negative	–	–	–
824	Propiophenone	Negative	–	–	–
825	alpha-Propylphenethyl alcohol	Negative	–	–	–
826	1-(*para*-Methoxyphenyl)-1-penten-3-one	Negative	–	–	–
831	Benzophenone	Negative	–	–	–
833	1-Phenyl-1,2- propanedione	Negative	–	–	–
834	Ethyl benzoylacetate	Negative	–	–	–
850	Benzoic acid	Negative	–	–	–
851	Methyl benzoate	Negative	–	–	–
864	Isopropylbenzyl alcohol	Negative	–	–	–
867	Tolualdehydes (mixed *ortho, meta, para*)	Negative	–	–	–
868	Cuminaldehyde	Negative	–	–	–
870	Butyl *para*-hydroxybenzoate	Negative	–	–	–
871	Anisyl alcohol	Negative	–	–	–
877	Veratraldehyde	Negative	–	–	–
878	*para*-Methoxybenzaldehyde	Negative	–	–	–
879	*para*-Ethoxybenzaldehyde	Negative	–	–	–
888	Vanillyl butyl ether	Negative	–	–	–
889	Vanillin	Negative	–	–	–
893	Ethyl vanillin	Negative	–	–	–
894	Piperonyl acetate	Negative	–	–	–
896	Piperonal	Negative	–	–	–
897	Salicylaldehyde	Negative	–	–	–
918	Glyceryl monostearate	Negative	–	–	–
925	Propylene glycol	Negative	–	–	–
930	Lactic acid	Negative	–	–	–
931	Ethyl lactate	Negative	–	–	–
935	Butyl butyryllactate	Negative	–	–	–
936	Pyruvic acid	Negative	–	–	–
938	Ethyl pyruvate	Negative	–	–	–
951	Pyrazine	Negative	–	–	–
953	Ethyl vanillin isobutyrate	Negative	–	–	–
987	Phenethyl alcohol	Negative	–	–	–
1007	Phenylacetic acid	Negative	–	–	–
1009	Ethyl phenylacetate	Negative	–	–	–
1014	Isoamyl phenylacetate	Negative	–	–	–
1027	Ethyl (*para*-tolyloxy)acetate	Negative	–	–	–
1028	2-Phenoxyethyl isobutyrate	Negative	–	–	–
1029	Sodium 2-(4-methoxyphenoxy)propanoate	Negative	–	–	–
1035	4,5-Dimethylthiazole	Negative	–	–	–
1043	4-Methylthiazole	Negative	–	–	–
1050	5-Methyl-2-thiophenecarboxyaldehyde	Negative	–	–	–
1094	Cyclohexyl butyrate	Negative	–	–	–
1100	Cyclohexanone	Negative	–	–	–
1101	Cycopentanone	Negative	–	–	–
1106	2-Hexylidene cyclopentanone	Negative	–	–	–
1108	2,2,6-Trimethylcyclohexanone	Negative	–	–	–
1111	Tetramethyethylcyclohexanone (mixure of isomers)	Negative	–	–	–
1112	Isophorone	Negative	–	–	–
1124	3-Penten-2-one	Negative	–	–	–
1134	6-Methyl-3,5-heptadien-2-one	Negative	–	–	–
1153	1-Decen-3-ol	Negative	–	–	–
1164	(+/-)-(2,6,6-Trimethyl-2-hydroxycyclohexylidene)acetic acid γ-lactone	Negative	–	–	–
1166	Octahydrocoumarin	Negative	–	–	–
1171	Dihydrocoumarin	Negative	–	–	–
1193	Ethyl 2,4,7-decatrienoate	Negative	–	–	–
1199	2-Methylbutanol	Negative	–	–	–
1219	*dl*-Citronellol	Negative	–	–	–
1220	Citronellal	Negative	–	–	–
1223	Geraniol	Negative	–	–	–
1230	Farnesol	Negative	–	–	–
1234	Eucalyptol	Negative	–	–	–
1241	Anisole	Negative	–	–	–
1243	*p*-Methylanisole	Negative	–	–	–
1244	*p*-Propylanisole	Negative	–	–	–
1248	1,2-Dimethoxybenzene	Negative	–	–	–
1249	*m*-Dimethoxybenzene	Negative	–	–	–
1250	*p*-Dimethoxybenzene	Negative	–	–	–
1255	Diphenyl ether	Negative	–	–	–
1256	Dibenzyl ether	Negative	–	–	–

Appendix A (*continued*)

JECFA No.	Flavor chemicals	Ames result	Derek for Windows	MultiCASE	ADMEWorks
1257	β-Naphthyl methyl ether	Negative	–	–	–
1258	β-Naphthyl ethyl ether	Negative	–	–	–
1259	β-Naphthyl isobutyl ether	Negative	–	–	–
1260	Isoeugenol	Negative	–	–	–
1263	Isoeugenyl phenylacetate	Negative	–	–	–
1264	Propenylguaethol	Negative	–	–	–
1289	*Erythro*- and *threo*-3-mercapto-2-methylbutanol	Negative	–	–	–
1301	Indole	Negative	–	–	–
1303	Isoquinoline	Negative	–	–	–
1304	Skatole	Negative	–	–	–
1314	Pyrrole	Negative	–	–	–
1315	3-Ethylpyridine	Negative	–	–	–
1316	3-Acetylpyridine	Negative	–	–	–
1323	Camphene	Negative	–	–	–
1324	β-Caryophyllene	Negative	–	–	–
1325	*p*-Cymene	Negative	–	–	–
1326	*d*-Limonene	Negative	–	–	–
1327	Myrcene	Negative	–	–	–
1329	α-Pinene	Negative	–	–	–
1330	β-Pinene	Negative	–	–	–
1332	Biphenyl	Negative	–	–	–
1334	4-Methylbiphenyl	Negative	–	–	–
1335	1-Methylnaphthalene	Negative	–	–	–
1340	*p*-Mentha-1,4-diene	Negative	–	–	–
1351	Ethyl acrylate	Negative	–	–	–
1371	(*E*)-2-Butenoic acid	Negative	–	–	–
1385	Borneol	Negative	–	–	–
1391	Isobornyl propionete	Negative	–	–	–
1395	*d*-Camphor	Negative	–	–	–
1408	3-*l*-Menthoxypropane-1,2-diol	Negative	–	–	–
1411	3-*l*-Menthoxy-2-methylpropan-1,2-diol	Negative	–	–	–
1413	*d,l*-Menthol 1- and 2-propylene glycol carbonate	Negative	–	–	–
1416	*p*-Menthan-3,8-diol	Negative	–	–	–
1441	2-(3-Phenylpropyl)tetrahydrofuran	Negative	–	–	–
1443	Tetrahydrofurfuryl alcohol	Negative	–	–	–
1445	Tetrahydrofurfuryl propionate	Negative	–	–	–
1459	β-Methylphenethyl alcohol	Negative	–	–	–
1467	2-Phenylpropionaldehyde	Negative	–	–	–
1468	2-Phenylpropionaldehyde dimethyl acetal	Negative	–	–	–
1470	2-Phenylpropyl isobutyrate	Negative	–	–	–
1494	3-Methyl-2-(3-methyl-2-butenyl)furan	Negative	–	–	–
1511	4-(2-Furyl)-3-buten-2-one	Negative	–	–	–
1513	Ethyl 3-(2-furyl)propanoate	Negative	–	–	–
1526	*O*-Ethyl *S*-(2-furylmethyl)thiocarbonate	Negative	–	–	–
1529	Eugenol	Negative	–	–	–
1536	Butyl anthranilate	Negative	–	–	–
1540	Linalyl anthranilate	Negative	–	–	–
1541	Cyclohexyl anthranilate	Negative	–	–	–
1552	*N*-Benzoylanthranilic acid	Negative	–	–	–
1575	beta-Caryophyllene oxide	Negative	–	–	–
1579	Ethylamine	Negative	–	–	–
1581	Isopropylamine	Negative	–	–	–
1582	Butylamine	Negative	–	–	–
1583	Isobutylamine	Negative	–	–	–
1584	*sec*-Butylamine	Negative	–	–	–
1585	Pentylamine	Negative	–	–	–
1592	Acetamide	Negative	–	–	–
1595	2-Isopropyl-*N*,2,3-trimethylbutyramide	Negative	–	–	–
1598	*N*-Isobutyl (*E,E*)-2,4-decadienamide	Negative	–	–	–
1600	Piperine	Negative	–	–	–
1607	Piperidine	Negative	–	–	–
1609	Pyrrolidine	Negative	–	–	–
1610	Trimethylamine	Negative	–	–	–
1611	Triethylamine	Negative	–	–	–
1615	Piperazine	Negative	–	–	–
1649	1-Phenyl-3-methyl-3-pentanol	Negative	–	–	–
1700	Allyl propyl disulfide	Negative	–	–	–
1767	*N*-(Heptan-4-yl)benzo[*d*][1,3]-dioxole-5-carboxamide	Negative	–	–	–
1768	N^1-(2,4-Dimethoxybenzyl)-N^2-(2-(pyridine-2-yl)ethyl)oxalamide	Negative	–	–	–
1772	*N*-Gluconyl ethanolamine	Negative	–	–	–
1777	*N*-[2-(3,4-Dimethoxyphenyl)ethyl]-3,4-dimethoxycinnamic acid amide	Negative	–	–	–

for Windows, which is a knowledge-based model. In the point of defining the priority of conducting Ames tests on many flavors, a model with higher sensitivity was better, and therefore combination-3 was the best among the current models. In this case, the percentage of false positive increases, we could confirm the actual results by conducting Ames tests for only limited numbers of flavors.

In the present study, 13/24 of chemicals reported as positive without Ames-equivocal were negative by all three (Q)SAR models. These 13 chemicals are shown in Table 5. On the other hand, one chemical, 2-methyl-2-pentenal, was negative in the Ames test but positive according to all three models. Detailed structural analysis of these 13 chemicals indicated that some of these chemicals possessed common sub-structures. The structures of false negatives with various common sub-structures are indicated in Fig. 1, and the chemicals enclosed within the dotted line in the figure have a common sub-structure. The applicability domain of each (Q)SAR model is basically limited within the chemical spaces of training chemical structures. The positive structural alerts for those sub-structures might not have been confirmed in our (Q)SAR models because of the lack of chemicals which have these sub-structures in our database used for the development of current (Q)SAR models. Expansion of the applicability domain of the (Q)SAR models by additional training including those sub-structures and development of sub-structural alerts could effectively contribute to increasing the predictability of mutagenicity for flavors, because many flavors possess categorically similar functional sub-structures or are composed of a series of derivatives.

There is an another possibility for the discrepancy between (Q)SAR prediction and experimental results. 2,5-Dimethyl-4-hydroxy-3(2H)-furanone and 4-hydroxy-2(or 5)-ethyl-5(or 2)-methyl-3(2H)-furanone may cause genotoxicity by indirect mechanisms, of action (in particular, generation of reactive oxygen species) (Hiramoto et al., 1996a,b) and for those such as trans-anethole, an Ames-positive result was reported only under the conditions with metabolic activation. The current (Q)SAR models were mainly developed based on information about typical genotoxic chemicals (Kirkland et al., 2005; Hayashi et al., 2005), and thus might be optimized for the direct mechanism rather than the indirect mechanism. Additional improvement of prediction might be achieved in combination with *in silico* tools which can predict indirect mechanisms.

In conclusion, the *in silico* prediction results from the combination of our (Q)SAR models were validated for priority setting to conduct Ames tests of many unevaluated flavors. The overall performance was lower than expected from the case of industrial chemicals; however, our combination (Q)SAR model approach was suitable for improving the *in silico* prediction and priority setting for Ames tests of flavors by raising the accuracy of each (Q)SAR model with a wider knowledge base for flavor-specific structures.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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Appendix A

See Appendix A.

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