重要な課題となっている。本研究においては、定量性という概念からよりヒトでの曝露状態に近いと考えられる *in vivo* での変異原性試験系をモデルに用い、発がん性との相関における定量的評価モデルを構築するとともに、低用量でのリスク評価に対して、定量的なアプローチを確立することを目的とした。これらにより、食品等に含まれて人が摂取する化学物質の発がんリスクに対して、定量的に評価し、必要な基準値等の設定に有効な理論的根拠を提供することが可能となる。

B．研究方法

低用量での定量的評価モデルの確立に当たっては、実験的アプローチに限界があるため、既存の変異原性試験データを活用した理論的なアプローチを採用した。発がん性試験と変異原性試験の定量的相関に関する検討においては、すでに報告しているMuta™Mouse およびBigBlue®トランスジェニックマウスを用いた変異原性試験データと、発がん性の強度の指標としての TD_{50} との相関図を基にして、新たなトランスジェニックマウスモデルである *gpt*-delta マウスにおけるデータの適応性に関して検討するとともに、*in vivo* 変異原性試験としての一般的応用を目指して、小核試験データへの適応を試みた。

以下にデータを用いた *in vivo* 変異原性試験の概略を示す。

・ トランスジェニックマウスを用いる遺伝子突然変異試験

突然変異検出のための標的遺伝子をラムダファージベクターに組み込んだのち、受精卵に導入することにより、全身に標的遺伝子を組み込んだトランスジェニックマウスを得る。マウスに被験物質を投与後、一定期間の後、各種臓器（組織）よりゲノムDNA を抽出し、ラムダパッケージングにより標的遺伝子をラムダファージへと回収し、適当な指示大腸菌に感染させてプラーク（またはコロニー）を形成させることにより標的遺伝子における突然変異を検出する系である。トランスジェニックマウスの系統により、用いる標的遺伝子が異なり、次のような種類がある。

Muta™Mouse－*lacZ* および *cII*

Big Blue®－*lacI* および *cII*（ラットもあり）

gpt-delta－*gpt* および Spi$^-$（*red*/*gam*）（ラットもあり）

・ 小核試験

主に骨髄を標的として、染色体異常誘発性を検討する試験。骨髄細胞および末梢血を用いる場合がある。骨髄を用いる場合には被験物質を投与1日後、末梢血を用いる場合には2日後に標的細胞を採取し、幼若赤血球中に出現する染色体の断片である小核を観察する。通常、マウスあたり2,000個の幼若赤血球中を観察し、小核を有する血球の出現頻度を求める。最近では、肝臓、皮膚など他の臓器を用いた小核試験も開発されている。

（倫理面への配慮）

本研究は遺伝毒性データベース作成に関するものであり、倫理上の問題はない。

C．研究結果

1. 低用量リスク評価に対する理論的アプローチ

低用量における反応性は当然小さくなるため、動物実験においてその変化を統計的に有意に検出するためには必要とされる動物数は大きくなる。過去に低用量での反応を調べるために動物数を増やしてその検出が試みられたこともあったが、これはあまり有効なアプローチではなく、さらに低用量での効果の検討には非現実的な量の動物を用いる必要もあり、動物愛護の観点からも望ましくない。よって動物実験における低用量域での真の用量反応性はブラックボックスのままである（図1）。

この低用量での反応性の検討を難しくしているもう一つの要因として、その試験系のコントロール値のばらつきが挙げられる。コントロール値のばらつきの程度が大きい試験系ほど低用量域での検出力が低くなり、用量反応は容易にコントロールのばらつきの範囲に埋もれてしまう。このコントロール値のばらつきの範囲こそがその試

験系の検出限界となり、用量反応がその範囲に含まれた用量以下では見かけ上の閾値となりいくら動物数を増やしても用量反応は検出できない（図 2）。

In vivo 変異原性試験のコントロール値のばらつきについては、比較的良く研究されている小核試験での報告例を図 3 に示す。観察対象となる小核を有する幼若赤血球数は 1,000 個あたり平均 2 個であり、実測値は 0 から 7 まで観察されている。これは、二項分布を仮定した場合の理論的な出現頻度とほぼ一致しており、統計学的に許容されるばらつきの範囲であるといえる。すなわち、実験が正しく行われている場合でも、コントロール値にこの程度のばらつきが生じるわけである。さらに、同一ラボでの長期間に渡るコントロール値のばらつき、いわゆるヒストリカルデータのばらつきを見ても、ほぼ平均値の 2 倍程度の範囲内にあり、経験的には平均値の約 2 倍である 1,000 個中 4 個を越えたときに初めて陽性であると判断する。

小核試験の場合には、比較的検出される事象の頻度が低いため、この程度のばらつきを許容せざるを得ないが、フローサイトメーターの導入により観察細胞数を増やすことによりこのばらつきを狭められるという期待もあるが、マウス個体ごとの生物学的なばらつきの可能性もあり、必ずしも観察細胞数を増やせばばらつきが減るとは限らない。

そこで、別の観点からのアプローチとして、高から中用量域での用量反応の詳細な解析から、外挿により低用量域での反応性を予測するモデルを提唱する。高用量では、得られる反応が十分であり、用量反応性に関するデータの定量性に関する信頼度が高い。よってより少ない数の動物個体数で信頼性のある用量反応データを取得できる。こうした、十分な用量反応の得られる領域において、適切に用量間隔を設けて詳細な用量反応曲線（直線）を描くことにより、低用量での反応性を実験を行わずに予測するわけである。この際、概ね用量反応性は直線性を示すので、直線回帰を行い傾きが重要となるが、中には小核試験におけるメタンスルホン酸の場合のように、指数関数的な用量反応性を示すものもあり、注意が必要である。今後は、実際の動物実験データから、このモデルを用いたアプローチの妥当性を評価していきたい。

2. *In vivo* 遺伝毒性試験の発がん性との定量的相関関係モデルの一般化

我々はこれまでに、発がん性とトランスジェニックマウス変異原性試験の定量的相関について、図 4 に示したモデルにて比較し、両者が良い相関関係にあることを示してきた。このモデルにおいては、発がん性の強さを定量的に表現するために、発がん性の強さの指標として、毎日動物に投与して半分の個体に癌が発生する用量である TD_{50} 値（Gold et al., 1991）を用いた。一方、トランスジェニックマウス試験での活性の強さは、総投与量（mg/kg）あたりの変異頻度の上昇率（fold-increase）で表し、両者をグラフ上にプロットしたところ、図 4 に示すように非常に良い相関性が得られた。この結果から、トランスジェニックマウスを用いた試験は、定量的な発がん性の予測のために有用であることが示された。

この相関には旧来から使用されていた、Muta™Mouse および BigBlue®マウスに関するデータを用いたが、多くは我々のラボにて取得されたデータである。変異原性に関するデータは、発がん標的部位におけるデータを用いたことも、相関が良かった原因といえる。

ここで、このモデルの一般化にあたり、重要であったのは変異原性の強さの尺度として、fold-increase/mg/kg という指標を用いた点にある。本邦に置いては、この後 *gpt* delta という新たなトランスジェニックマウスモデルが開発され、データも蓄積してきていることから、このモデルにおいても発がん性との定量的相関性を検証する必要性が出てきた。*gpt* delta マウスモデルの特徴として、前二者と比べて、コントロールの自然突然変異頻度が低いことが挙げられる。表 1 に既に報告されている *gpt* delta マウスおよびラットの各種臓器での自然突然変異頻度を示すが、

Muta™Mouse および BigBlue®マウスにおける各標的遺伝子の変異頻度が 10^{-5} オーダーであるのに比べ、*gpt* delta マウスにおいては、*gpt* および Spi⁻変異ともに 10^{-6} オーダーと概ね一桁近く低い頻度を示している。このことより、*gpt* delta マウスにおける変異原活性の強さを他のモデルと比較する際には、変異頻度の実測値を用いることは難しく、変異頻度の上昇率（fold-increase）の値を指標として用いざるを得ない。幸い、発がん性との相関を調べたモデルにおいては前述のように fold-increase/mg/kg の値を用いていたので、同一物質においてデータが得られているアリストロキア酸を用いて Muta™Mouse と *gpt* delta マウスでのデータの比較を行った。

その結果、変異頻度の値は両者においてかなり差があったものの、fold-increase を用いて現した活性値は図4に示したようにほぼ同じ位置にプロットされた。

次に、異なる遺伝毒性試験系においても同様の相関関係が得られるかどうかを検討するため、トランスジェニックマウス変異原性試験データが得られている化合物のうちで、小核試験のデータがあるものに関して、その活性の強さを、単回投与量あたりの Fold-increase 値として、TD_{50} 値との相関を図5にプロットした。小核試験に関して、陽性結果が得られている化合物に関しては、TD_{50} 値との間に良い相関関係が見られた。また、多くの化合物に関しては、小核試験において陰性結果が得られており、これらの化合物に関しては、相関関係から外れた。

D．考　察

Fold-increase/mg/kg の値を定量的評価の指標として発がん性との相関を調べることにより、*in vivo* 遺伝毒性試験全体に適応可能であることがわかった。個々の試験法と発がん性の相関関係については、今後より詳しい解析を行う必要があるが、今回小核試験に関して一部の化合物にて比較を行ったところ、陽性結果の得られている化合物に関しては、発がん性との間に比較的良い相関関係があることが明らかとなった。一方で、陰性結果を示した化合物も多く、これらは当然相関からは外れることになるが、これら陰性結果を示した化合物に関しては、その標的臓器が肝臓など骨髄以外の臓器である場合が多く、トランスジェニックマウスの場合にも、標的臓器でのデータを使ってよい相関が得られたことから、小核試験においても標的臓器でのデータを使う必要性があると考えられる。基本的には、小核試験では骨髄系の細胞が使われるが、最近ではラット肝臓を用いた小核試験法も確立されつつあり、今後より適応は広がると期待される。その他、コメットアッセイや *Pig-a* 試験など、他の試験形についてもこの評価方法を適応し、発がん性と各試験との相関性に関して検討を深めたい。

低用量での用量反応性およびリスク評価法に関しては、実際に低用量域での実験的なアプローチの限界から、むしろ高用量での用量相関をきちんと評価し、その外挿による低用量領域での反応性の予測を行う手法を提案した。これには、用量反応の連続性（直線性ではない）が前提となるが、現実的にはこのようなアプローチを取らざるを得ない。また、低用量において逆の作用すなわち、ホルミシス効果が存在する場合には、リスク評価に大きな影響を与えるが、やはりそれを実験的に証明することは非常に難しいことから、より N 数を増やせる *in vitro* での実験から、その存在を予測する必要があると考えられる。ただし、ホルミシス効果が存在する場合には、リスクを過大評価することはあっても、過小評価することは無いため、fail safe の概念からすれば、無視しても構わないと考えられる。今後、高用量の用量反応の詳細な検討から低用量での作用を的確に予測可能かどうかについて、検証を行いたい。

E．結　論

In vivo 遺伝毒性試験の定量的評価のための指標として、Foldincrease/total dose を用いることにより、発がん性の強度との間に異なる種間および異なる試験系を統一して、一般化された相関関

係を描くことが可能となった。

F. 健康危機情報

なし

G. 研究発表

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H. 知的財産権の出願・登録状況

なし

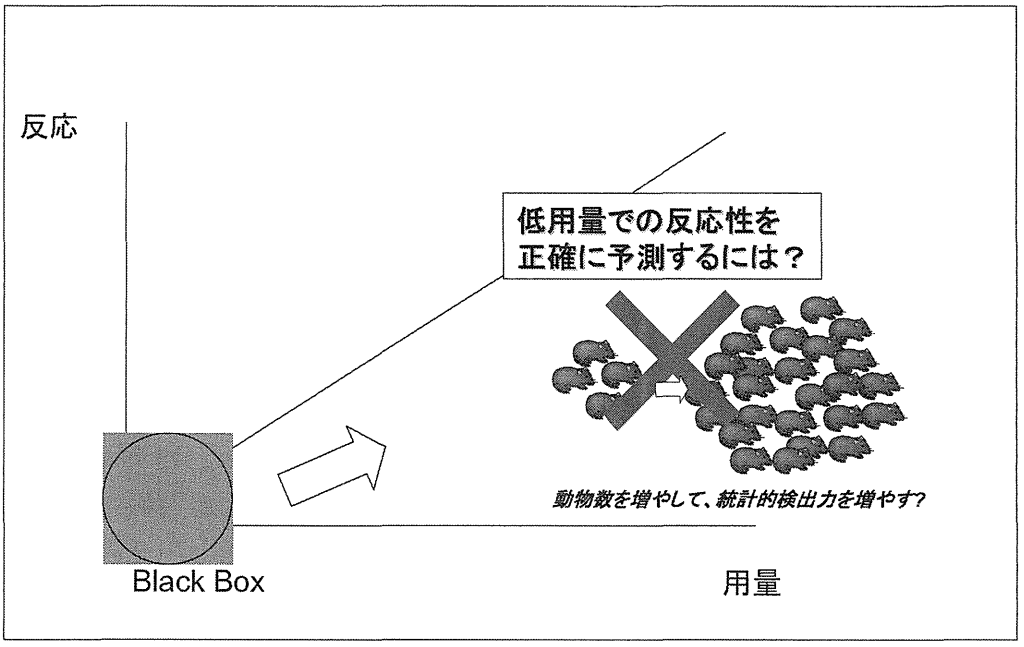

図１　動物実験において低用量評価をする上で何が重要か

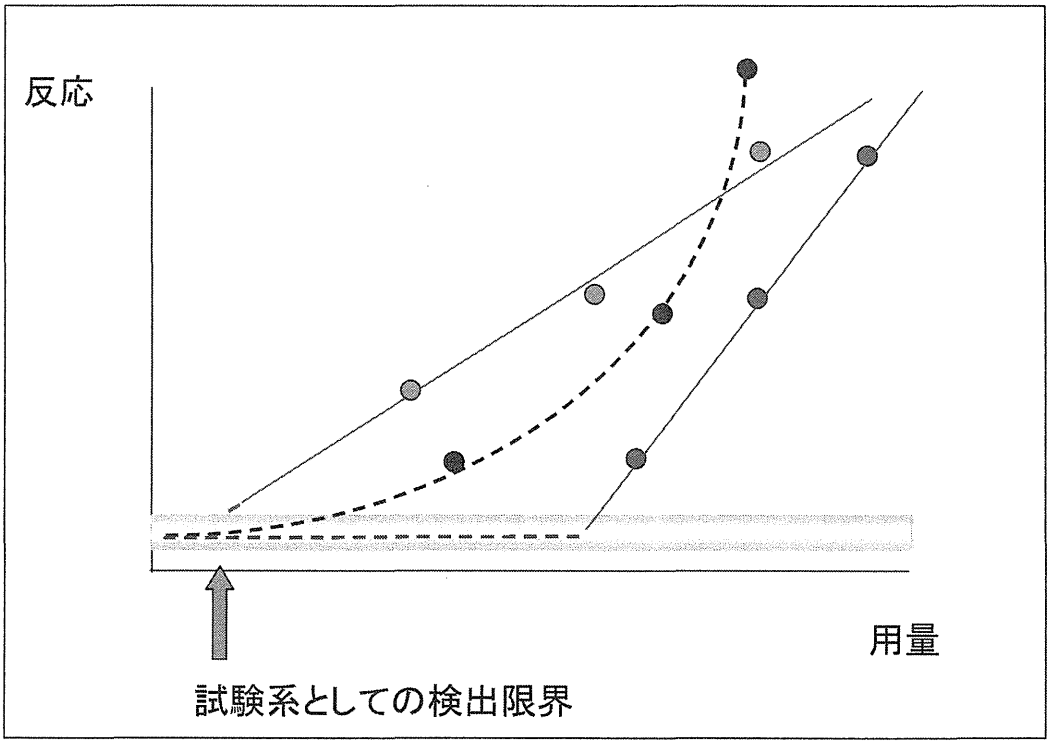

図２　低用量での反応性を予測するためには

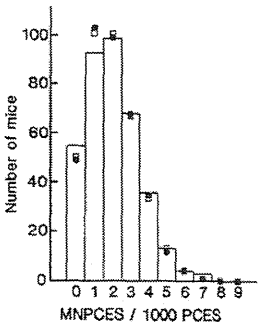

FIGURE 1. The distribution of frequency of micronucleated polychromatic erythrocytes (PCEs) based on 1000 PCEs observation in the negative historical control (bars), the fitted binomial distribution B(0.002, 1000) (□), and binomial random numbers (●) (2).

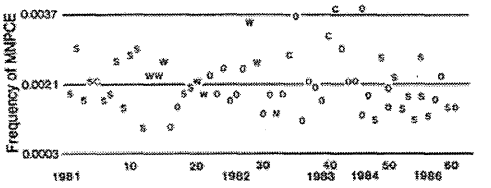

FIGURE 2. A control chart of the historical data of the proportion of micronucleated polychromatic erythrocytes in the negative control groups using six mice per group during the period from 1981 to 1986 (2). C, 0.5% carboxymethyl cellulose sodium salt aqueous solution; D, dimethyl sulfoxide; N, no treatment; O, olive oil; S, physiological saline; W, distilled water.

Environ Health Perspect. 1994 102(Suppl 1): 49–52.
Statistical analysis of data in mutagenicity assays: rodent micronucleus assay.
M Hayashi, S Hashimoto, Y Sakamoto, C Hamada, T Sofuni, and I Yoshimura

ヒストリカルコントロール値の範囲により、生物学的有意性を評価

ヒストリカルコントロールの平均値は約2であり、処理群の平均値が4を超えたら経験的に陽性と判断

図３　小核試験におけるコントロール値の範囲とばらつき

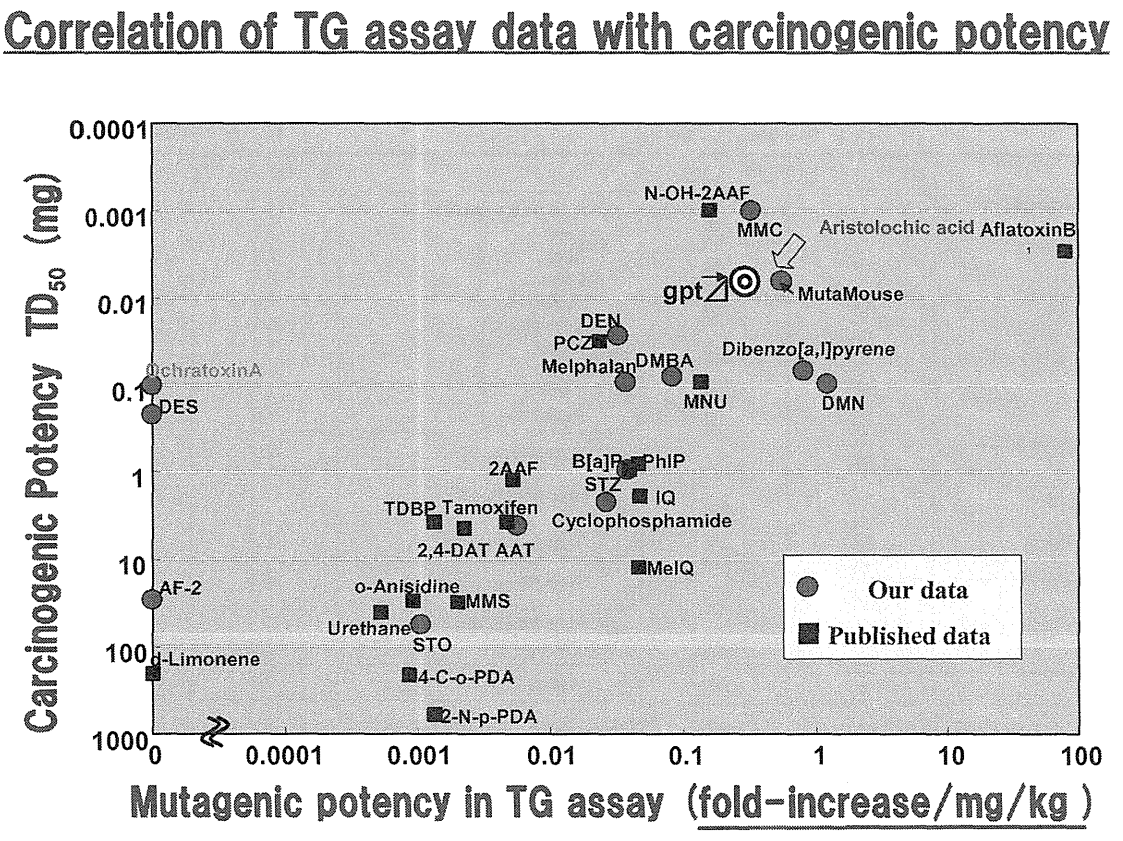

図４　発がん性データとトランスジェニックマウス変異原性試験データの定量的相関

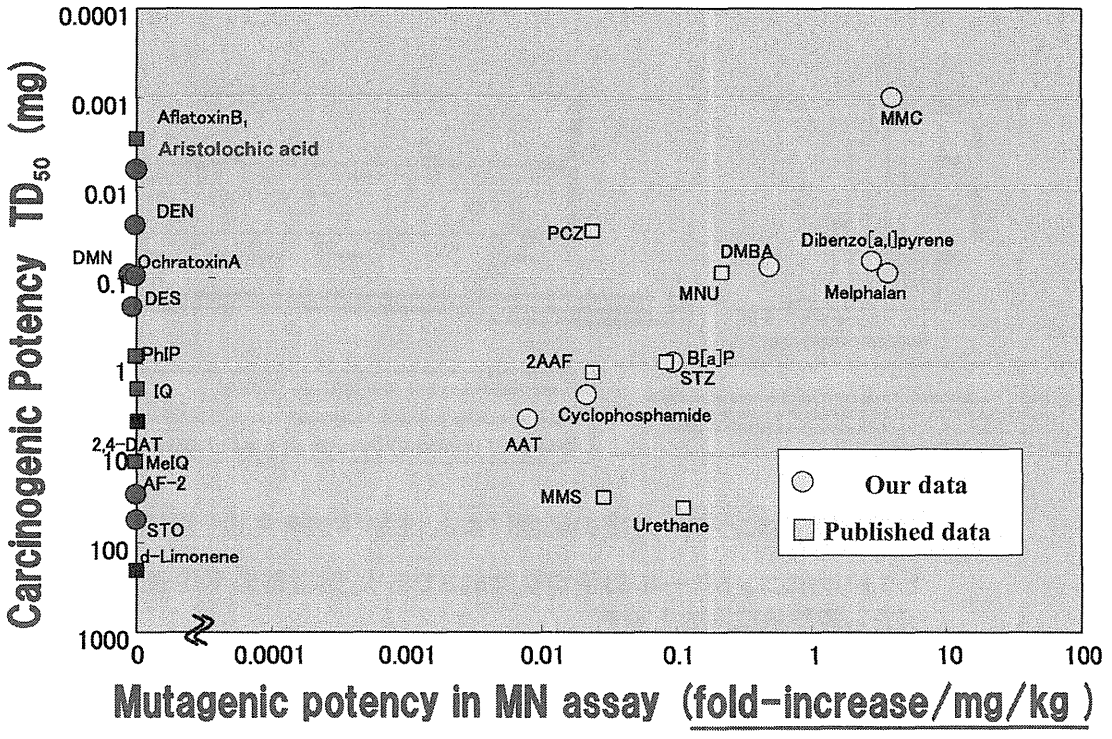

図５　発がん性データとマウス小核試験データの定量的相関

表１　*gpt* delta マウス、ラットの各種臓器の自然突然変異頻度

Table 1.　Spontaneous gpt MF

	gpt MF (×10⁻⁶)	No. of an
gpt delta Mouse (C57BL/6J)		
Liver	5.8	45
Lung	3.4	15
Colon	7.4	11
Epidermis	11.5	6
Bone marrow	2.9	5
Kidney	5.7	5
Stomach mucosa	5.0	5
Spleen	3.3	3
Testis	3.0	3
Dermis	12.1	2
Brain	5.0	2
Average	6.0	
gpt delta Rat (SD)		
Liver	4.6	21
Mammary gland	4.4	5
Kidney	4.0	5
Average	4.5	
gpt delta Rat (F344)		
Liver	4.4	10
Average	4.4	

*Instead of mutant frequency, mutation frequency is cited. U: Unpublisl

Table 2.　Spontaneous Spi⁻ MFs

	Spi⁻ MF (×10⁻⁶)	No. of animal
gpt delta Mouse (C57BL/6J)		
Liver	2.0	35
Lung	2.8	16
Colon	2.4	6
Epidermis	1.5	6
Bone marrow	5.4	5
Kidney	5.1	9
Spleen	3.2	17
Testis	2.2	4
Brain	1.8	12
Average	2.7	
gpt delta Rat (SD)		
Liver	4.4	5
Kidney	1.3	5
Average	2.9	
gpt delta Rat (F344)		
Liver	2.8	3
Average	2.8	

*Instead of mutant frequency, mutation frequency is cited. U: Unpublished data.

Spontaneous and Induced gpt and Spi? Mutant Frequencies in gpt delta Transgenic Rodents

Kenichi Masumura et al より改変

Genes and Environment　Vol. 31 (2009) 105-118 .

別添 5

III.　研究成果の刊行に関する一覧表

(別紙４)

III. 研究成果の刊行に関する一覧表

書　籍

著者氏名	論文タイトル名	書籍全体の編集者名	書　籍　名	出版社名	出版地	出版年	ページ
該当なし							

雑　誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
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Validation of the (Q)SAR combination approach for mutagenicity prediction of flavor chemicals

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ABSTRACT

Most exposure levels of flavor in food are considered to be extremely low. If at all, genotoxic properties should be taken into account in safety evaluations. We have recently established a (quantitative) structure–activity relationship, (Q)SAR, combination system, which is composed of three individual models of mutagenicity prediction for industrial chemicals. A decision on mutagenicity is defined as the combination of predictive results from the three models. To validate the utility of our (Q)SAR system for flavor evaluation, we assessed 367 flavor chemicals that had been evaluated mainly by JECFA and for which Ames test results were available. When two or more models gave a positive evaluation, the sensitivity was low (19.4%). In contrast, when one or more models gave a positive evaluation, the sensitivity increased to 47.2%. The contribution of this increased sensitivity was mainly due to the result of the prediction by Derek for Windows, which is a knowledge-based model. Structural analysis of false negatives indicated some common sub-structures. The approach of improving sub-structural alerts could effectively contribute to increasing the predictability of the mutagenicity of flavors, because many flavors possess categorically similar functional sub-structures or are composed of a series of derivatives.

1. Introduction

Many flavor chemicals in current food use have been evaluated under the Threshold of Toxicological Concern concept at the FAO/WHO Joint Expert Committee on Food Additives (JECFA). Most exposure levels of flavor in food are considered to be extremely low. In such cases, genotoxic properties should be taken account in safety evaluations in addition to the empirical threshold. Recently, (quantitative) structure–activity relationship ((Q)SAR) systems have been used to quickly assess the human hazards of chemicals for regulatory purposes (Cronin et al., 2003).

We had developed (Q)SAR models for assessment of chemical genotoxicity, which was optimized for application to industrial chemicals using three commercially available (Q)SAR systems, Derek for Windows and MultiCase, which are used widely by regulatory agencies, and ADMEWorks, which we customized. The results of previous evaluations of our (Q)SAR models using industrial chemical sets independent of the chemicals used for the model development indicated that the sensitivity, specificity and concordance rates were increased when we combined the three (Q)SAR systems to make a definitive decision on mutagenicity. Accordingly, we concluded that the (Q)SAR evaluation could be optimized by combining the evaluations from different systems (Hayashi et al., 2005).

Currently, about 3000 synthetic flavors are distributed commercially in Japan. About 900 of these originate from Japan and have not yet been assessed for their effect on human health. The Japan Flavor and Fragrance Materials Association (JFFMA) has been revaluating these flavor compounds, based on the safety assessment processes of the JECFA; however, for a number of these compounds there is insufficient information on their genotoxicity to be able to follow the JECFA process. It is not realistic for all of the flavor chemicals already used widely in Japan to be examined for genotoxicity because they are so numerous. Therefore, if we could make a reliable prediction of their genotoxicity (the results of the Ames

Abbreviations: JECFA, FAO/WHO Joint Expert Committee on Food Additives; (Q)SAR, (quantitative) structure–activity relationship; JFFMA, Japan Flavor and Fragrance Materials Association; FAS, WHO Food Additives Series; JFSC, Japan Food Safety Commission.

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Table 1
Performances of each (Q)SAR model.

	Ames results	(Q)SAR		
		+	–	Total
Derek for Windows	Positive	10	14	24
	Equivocal	4	8	12
	Negative	22	309	331
	Total	36	331	367
MultiCASE	Positive	6	18	24
	Equivocal	3	9	12
	Negative	19	312	331
	Total	28	339	367
ADMEWorks	Positive	4	20	24
	Equivocal	1	11	12
	Negative	28	303	331
	Total	33	334	367

test or chromosomal aberration test) based on their chemical structures *in silico*, it would be useful in the assessment of flavor chemicals originally used in Japan.

The purpose of our study was to develop an *in silico* system in order to define the priorities for conducting genotoxicity studies of many existing flavors unevaluated and/or flavors newly synthesized; furthermore, in future, to enable exemption from actual genotoxicity studies for evaluating specific chemical groups. In the present study, we applied our previously developed (Q)SAR combination system for predicting the Ames test results of flavors, which we selected from the series of JECFA reports. The prediction performance was not so high, because our system had been customized for industrial chemicals, but the results of this study indicated that our system is capable of improving the predictability of Ames test results for flavors.

2. Materials and methods

2.1. Set of chemicals for validation

The WHO Food Additives Series (FAS) from 1965 to 2008 and evaluation reports published by the Japan Food Safety Commission (JFSC) were used to select a set of flavor chemicals with information from the Ames test.

There were 367 flavor items with information on their activity in the Ames assay. We considered optical and geometrical isomers to be the same compound because sometimes isomers are not distinguished in Ames tests. The 2D structures of chemicals prepared by JFFMA were used for *in silico* evaluation. Moreover, because the results of the (Q)SAR models were not considered by the differences in strains, and with or without S9 mix in Ames tests on a training set, we did not consider their differences in the validation set of 367 compounds.

In the current study, flavors were defined as positive if at least one positive result had been reported. In order to confirm the positive results, we reviewed the corresponding reports in detail, and justified the positive results according to the following criteria. In the case of results obtained by standard methods, a positive result was assigned when a revertant count that exceeded twice the background revertant count was obtained. However, for results by typical methods that were slightly greater than twice or, in the case of positive results obtained by atypical methods, experts reviewed the data of a report, did not consider the report to have clear positive data and judged the report equivocal. If a flavor has reports only with Ames-equivocal results other than Ames-negative results, we considered that flavor to be equivocal. As a result, the judgment consists of "positive," "equivocal," and "negative." Among these 367 flavors, 24 were positive, 12 were equivocal, and 331 were negative compounds in the Ames assay. Overall, 367 flavoring compounds evaluated, and their Ames test and (Q)SAR results are shown in Appendix A. To move closer to our current purpose, we put weight on the findings of Ames-positive alerts, and then considered Ames-equivocal flavors to be positive.

2.2. (Q)SAR programs and in silico definition of positive and negative responses

In silico evaluation of potential mutagenicity was carried out using three commercially available (Q)SAR programs. Derek for Windows (version 10.0.2; Lhasa Ltd., UK) is a specialized or toxic chemical sub-structure rules-based system (Greene et al., 1999). When the system gave an evaluation as "certain", "probable", "plausible" or "equivocal", we considered this as "positive", and when the system gave "doubted", "improbable", "impossible" or "no alert", we considered this as "negative." MultiCase (version 1.90; Multicase Co. Ltd., Japan) is a hybrid system of 2D chemical descriptors based (Q)SAR and known toxic sub-structure identification (Rosenkranz et al., 1999). When the system showed "active", "borderline" or "probably inactive", we considered this as positive, and only when the system showed "inactive" did we consider this as negative. ADMEWorks (version 4.0; Fujitsu Kyushu Systems Ltd., Japan) is a system based mainly on 2D (sometimes 3D) descriptors, such as topological, topographical, physicochemical, and sub-structural parameters. When the system showed "positive," we considered this as "positive", and when the system showed "negative" we considered this as "negative". We selected these systems for the combined prediction system because of their different modes of analysis. In this study, *in silico* prediction of the mutagenicity of 367 flavor chemicals was performed using prediction models developed in our previous study (Hayashi et al., 2005), and compared with the reported experimental results.

2.3. Definitions in (Q)SAR models

We calculated sensitivity, specificity, concordance, applicability, false positive, and false negative as follows:

$$\text{Sensitivity}\ (\%) = N_{AS+}/N_{A+} \times 100,\ \text{Specificity}\ (\%) = N_{AS-}/N_{A-} \times 100,$$

$$\text{Concordance}\ (\%) = (N_{AS+} + N_{AS-})/N_{eval} \times 100,\ \text{Applicability}\ (\%) = N_{eval}/N_{all} \times 100,$$

$$\text{False positive}\ (\%) = (N_{A-} - N_{AS-})/N_{S+} \times 100,\ \text{False negative}\ (\%) = (N_{A+} - N_{AS+})/N_{S-} \times 100,$$

where N_{A+} is the number of chemicals that are positive in an *in vitro* assay (Ames test); N_{A-} is the number of chemicals negative in an *in vitro* assay (Ames test); N_{AS+} is the number of chemicals positive by both the Ames test and (Q)SAR evaluation; N_{AS-} is the number of chemicals negative in both the Ames test and (Q)SAR evaluation; N_{all} is the total number of chemicals analyzed; N_{eval} is the number of chemicals evaluated; N_{S+} is the number of chemicals positive in (Q)SAR evaluation; and N_{S-} is the number of chemicals negative in (Q)SAR evaluation.

3. Results

The predictions were performed by the single (Q)SAR model, the performances of each (Q)SAR model are shown in Table 1 and the results of their evaluations are summarized in Table 2. The predictions were performed also by combined evaluation of the three (Q)SAR models in three different ways: combination-1, -2 and -3. In combination-1, *in silico* mutagenicity evaluated using (Q)SAR systems was considered to be positive (or negative) only when all three models gave unanimous evaluations. In combination-2, *in silico* mutagenicity was considered to be positive (or negative) when two or more models gave the same evaluations. In combination-3, *in silico* mutagenicity was considered to be positive when one or more models gave a positive evaluation and to be negative when all three models gave negative evaluations. Performances of each combination of three (Q)SAR modes are shown in Table 3 and results of their evaluations are summarized in Table 4.

Table 2
Results of evaluation of each (Q)SAR model.

	Sensitivity (%)	Specificity (%)	Concordance (%)	Applicability (%)	False positive (%)	False negative (%)
Derek for Windows	38.9	93.4	88.0	100.0	61.1	6.6
MultiCase	25.0	94.3	87.5	100.0	67.9	8.0
ADMEWorks	13.9	91.5	83.9	100.0	84.8	9.3

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.

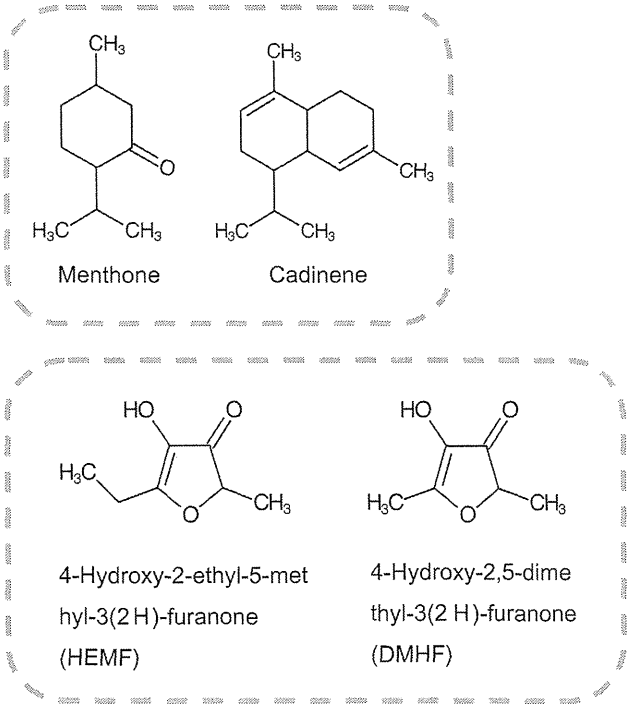

Fig. 1. Structures of false negative chemicals with a common sub-structure.

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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