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研究課題名：インシリコ予測技術の高度化・実用化に基づく化学物質のヒト健康リスクの評価ストラテジーの開発
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分担研究報告書
代謝予測モデルの改良による MoA に基づいた *in vivo* 遺伝毒性予測性の向上に関する研究

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研究要旨

In silico による *in vivo* 遺伝毒性予測技術の高度化・実用化を実現するためには、*in vitro* 陰性で *in vivo* 陽性の物質を抽出し、その要因を *in vitro/in vivo* における代謝の比較解析から検討する必要がある。その前提として利用するデータの妥当性・適切性が極めて重要となるため、既存の各種データベースから *in vitro* 染色体異常試験（CA）陰性で *in vivo* 小核試験（MN）陽性と報告されている 21 物質および Ames 試験（Ames）陰性でげっ歯類トランスジェニック突然変異試験（TGR）陽性と報告されている 13 物質を抽出し、当該試験結果の妥当性を評価した。その結果、前者では 21 物質中 11 物質が、後者では 13 物質中 3 物質が当初の評価通り陰性/陽性で妥当と判断された。また、一部の Ames 陰性・TGR 陽性物質については、その代謝様式の違い、ならびに想定される警告構造について調査し、暫定的な結果を得た。

A. 研究目的

In vitro（肝 S9 画分）と *in vivo*（生物個体）間の代謝の違いは、異なる遺伝毒性結果を引き起こす可能性がある。特に、*in vitro* 遺伝毒性試験では陰性だが、*in vivo* 試験で陽性となる物質の存在は、遺伝毒性試験戦略ならびに評価において極めて重要な意味を持つ。*In vitro* と *in vivo* における代謝の違いは、概念的には、1) *in vivo* における第 2 相代謝反応の存在、2) 臓器（肝臓 S9 画分）に対する生物全体の代謝レベルの相違、3) *in vivo* における受容体介在性の基質特異的酵素の存在、が挙げられる。すなわち、*in vitro* の代謝系では *in vivo* の代謝系を完全には再現できないと仮定すべきである。しかしながら、この「代謝の違い」を考慮した *in silico* 遺伝毒性予測モデルが構築できれば、特に *in vivo* における予測性の向上につながることが期待できる。最終的には、*in vitro/in vivo* の代謝の相違を反映した代謝予測シミュレータを開発し、Mode of Action（MoA）に基づく *in vivo* 遺伝毒性の予測性の向上を目指

す。そのために、*in vitro* 陰性で *in vivo* 陽性の物質を抽出し、*in vivo* 特異的陽性の要因を *in vitro/in vivo* における代謝の比較解析等から検討する。

B. 研究方法

In vitro 陰性で *in vivo* 陽性の物質を抽出し、その要因を *in vitro/in vivo* における代謝の比較解析等から検討し、*in silico* 予測技術の高度化・実用化を実現するためには、利用する実データの妥当性・適切性が極めて重要となる。*In vitro* あるいは *in vivo* を問わず、質の高い遺伝毒性試験データに基づき、陽性・陰性を判断する必要がある。それらの正しい結果を利用することにより、MoA に基づく *in vivo* 遺伝毒性の *in silico* 予測性の向上が可能となる。そのため、既存の各種データベースから *in vitro* 染色体異常試験（CA）陰性で *in vivo* 小核試験（MN）陽性と報告されている 21 物質を抽出し、原著論文等の精査により当該試験結果の妥当性を評価した。また、同様に Ames 試験（Ames）陰性でげっ歯類トランスジェニック突然変異試験（TGR）陽性と報告されている 13 物質を抽出し、当該試験結果の妥当性を評価した。それら遺伝毒性試験データの精査に基づき、今後の研究に活用すべきデータか否かを検証した。また、一部の Ames 陰性・TGR 陽性物質については、暫定的にその代謝様式の違い、ならびに想定される警告構造について調査した。

なお、当初の研究計画では初年度に *in vitro* CA 陰性・*in vivo* MN 陽性物質の抽出とそのデータの妥当性評価ならびに *in vitro/in vivo* 比較解析を、次年度に同様に Ames 陰性・TGR 陽性物質についての検討を実施するものであった。しかしながら、作業効率の観点から、初年度に *in vitro* CA 陰性・*in vivo* MN 陽性物質および Ames 陰性・TGR 陽性物質のデータの妥当性評価ならびに一部物質についての *in vitro/in vivo* 比較解析を実施し、次年度以降、*in vitro/in vivo* 比較解析を重点的に実施することとした。

（倫理面への配慮）本研究は動物を用いた研究を行わないため対象外である。

C. 研究結果

C.1. *In vitro* CA 陰性・*in vivo* MN 陽性物質の検証

抽出した 21 物質について評価した試験結果の妥当性の要約を表 1 に、評価の詳細を Appendix 1 に示す。21 物質中 11 物質が、当初の評価通り陰性・陽性（-ve/+ve）で妥当と判断された。1 物質は、*in vitro* CA 陰性と明確には断定できず、残り 9 物質はいずれか、あるいは両方の結果の評価が異なった。今後の研究に活用すべきデータとしては、前者の 12 物質（すなわち、Thioacetamide、1,1,2,2-Tetrachloroethane、CI Solvent yellow 14、C.I. Direct black 38 、 Urethane 、 Chlordiazepoxide、Procarbazine hydrochloride、Diazepam 、 Atrazine 、 Amphetamine 、 Dimethylvinyl chloride および Salicylazosulfapyridine）を用いることが妥当と考えられた。また、データベースおよび *in silico* 評価における問題点として、*in vitro* CA における数的異常（異数性/倍数性）を含むか否かが挙げられた。必要に応じ、これに該当する 1 物質（Thiabendazole）も使用データに含めることが妥当と考えられた。

C.2. Ames 陰性・TGR 陽性物質の検証

抽出した 13 物質について評価した試験結果の妥当性の要約を表 2 に、評価の詳細を Appendix 2 および Appendix 3 に示す。13

物質中 3 物質が、当初の評価通り-ve/+ve で妥当と判断された。2 物質は Ames 陰性の妥当性が確定できず、また、別の 2 物質は TGR 陽性の妥当性が確認できなかった。残り 6 物質はいずれか、あるいは両方の結果の評価が異なるかデータが認められなかった。今後の本研究に活用すべきデータとしては、前者の 3 物質（Cyproterone acetate、Tamoxifen、Oxazepam）および必要に応じ妥当性に疑問の残る 4 物質（Dicyclanil、Leucomalachite green、Hexachlorobutadiene、Procarbazine HCl）を用いることが妥当と考えられた。

C.3. 一部の Ames 陰性・TGR 陽性物質の要因解析と TGR 特異的警告構造の抽出

In vitro/in vivo における代謝の相違の例として、対象物質数の少なさから *in vitro* Ames と *in vivo* TGR を対象とした。Ames 陽性および TGR 陰性は、物質が生体内において代謝解毒される、すなわち、*in vivo* における第 2 相代謝反応の存在によると考えられる。一方、Ames 陰性および TGR 陽性は、物質が生体内において代謝活性化される、すなわち、生体活性化第 2 相硫酸抱合反応の存在、あるいは、*in vivo* における追加的第 1 相代謝反応の存在によると考えられる。本研究では、Ames 陰性および TGR 陽性を対象とし、以下の物質について検討した。

C.3.1. Tamoxifen と Cyproterone acetate

Tamoxifen は α-水酸化された後に硫酸抱合され、脱硫酸すると求電子性の活性中間体となり DNA 付加体を形成すると考えられている。すなわち、第 1 相アリル水酸化を受けた後、第 2 相硫酸抱合されることにより代謝活性化される。これは、*in vivo* 個体においてのみ生じ、*in vitro* の S9 系では、特別な補酵素を添加しない限り生じない。類似の例として Cyproterone acetate が挙げられた。これらの知見から、アリルフラグメントは TGR で陽性を示す特異的警告構造として考えられた。4223 物質の Ames データベースには本警告構造を有するものが 161 物質認められ、その大部分（153 物質）は、Ames 陰性であった。このことは、第 2 相硫酸抱合が *in vitro* の S9 系では生じないことを示唆している。

C.3.2. Oxazepam

Oxazepam の複数の代謝経路の内、環収縮代謝反応による酸化的脱炭酸と脱水素環化で生ずる第 1 相代謝産物が DNA 反応性代謝物と考えられているが、本物質は *in vitro* では認められていない。本知見から、ベンゾジアゼピンフラグメントは TGR で陽性を示す特異的警告構造として考えられた。4223 物質の Ames データベースには本警告構造を有するものは 5 物質しか認められなかったが、いずれも Ames 陰性であった。このことは、*in vivo* で追加的第 1 相代謝反応が生じていることを示唆している。

D. 考察

既存の各種データベース等に基づく試験結果は必ずしも正確ではないことが図らずも明らかとなった。最終的に *in vitro* CA 陰性・*in vivo* MN 陽性あるいは Ames 陰性・TGR 陽性と評価されたのは、それぞれ 21 物質中 11 物質あるいは 13 物質中 3 物質と、半分以下であった。その要因は、単純な記載間違い、原著論文の読み込み不足、間違った二次資料からの引用などが想定されるが、明らかではない。また、類似の試験結果が存在する場合には、試験の信頼性・妥当性など証拠の重みづけ（WOE）による専門家判断に基づき、結果が異なってくることもある。Ames 陰性・TGR 陽性において結果が正し

いものと判断された 3 物質について、暫定的に *in vitro* と *in vivo* における代謝様式の相違を検討し、また、警告構造を抽出したが、これらについてはより詳細に検討する必要があろう。

E. 結論

既存の各種データベースから抽出した *in vitro* CA 陰性・*in vivo* MN 陽性の 21 物質中 11 物質、ならびに Ames 陰性・TGR 陽性の 13 物質中 3 物質が当該結果通りで妥当と判断され、以降の代謝様式あるいは警告構造の検討に利用すべきと考えられた。また、一部の Ames 陰性・TGR 陽性物質について、代謝様式の違いおよび想定される警告構造の暫定的な結果を得た。これらについてはより詳細に検討する必要がある。

F. 研究発表

誌上発表

1. Morita T, Shigeta Y, Kawamura T, Fujita Y, Honda H, Honma M: *In silico* prediction of chromosome damage: Comparison of three QSAR models. *Mutagenesis*, **34**, 91-100, 2019.
2. Fujita Y, Honda H, Matsumura S, Yamane M, Morita T, Matsuda T, Morita O: A decision tree–based integrated testing strategy for the tailor-made carcinogenicity evaluation of test substances using genotoxicity test results and chemical spaces, *Mutagenesis*, **34**, 101-109, 2019.
3. Tennant RE, Guesné SJ, Canipa S, Cayley A, Drewe WC, Honma M, Masumura K, Morita T, Stalford SA, Williams RV : Extrapolation of *in vitro* structural alerts for mutagenicity to the *in vivo* endpoint, *Mutagenesis*, **34,** 111-121, 2019.

学会発表

1. 森田　健；遺伝毒性評価のための *in vivo* 試験実施戦略、日本毒性学会シンポジウム：動き始めた遺伝毒性評価の新たな潮流、第 45 回日本毒性学会学術年会（2018.7、大阪）
2. Morita T, Shigeta Y, Kawamura T, Fujita Y, Honda H, Honma M; Current Situation of *in silico* Prediction of Chromosome Aberration, Environmental Mutagenesis & Genomics Society, 49th Annual Meeting (2018.9, サンアントニオ、米国)
3. Fujita Y, Honda H, Yamane M, Morita T, Matsuda T and Morita O; Integrated testing strategy for carcinogenicity evaluation of chemicals using genotoxicity tests and chemical properties, 20th International Congress on *In Vitro* Toxicology (2018.10, ベルリン、ドイツ)

G. 知的財産権の出願・登録状況

なし

表 1.　*In vitro* CA 陰性・*in vivo* MN 陽性と報告されている 21 物質のデータ評価

#	物質名	CAS	評価結果 CA/MN[1)]	備考
1	Thioacetamide	62-55-5	-ve?/+ve	CA陰性は不明確知見
2	1,1,2,2-Tetrachloroethane	79-34-5	-ve/+ve	問題なし
3	4,4'-methylenebis(2-chlorobenzenamine)	101-14-4	+ve/-ve	倍数体誘発によりCA陽性；WOEによりMN陰性
4	1,4-dichlorobenzene	106-46-7	-ve/-ve	WOEによりMN陰性
5	Thiabendazole	148-79-8	+ve/+ve	異数体誘発によりCA陽性
6	CI Solvent yellow 14	842-07-9	-ve/+ve	問題なし
7	C.I. Direct black 38	1937-37-7	-ve/+ve	問題なし
8	Urethane	51-79-6	-ve/+ve	10 mM以上でCA陽性
9	Carbon tetrachloride	56-23-5	-ve/-ve	MN陽性知見の信頼性疑問
10	Chlordiazepoxide	58-25-3	-ve/+ve	問題なし
11	Acetaldehyde	75-07-0	+ve/+ve	MN陰性知見は減数分裂時
12	Thiram	137-26-8	+ve/+ve	情報取集不足によるCA陰性
13	Procarbazine hydrochloride	366-70-1	-ve/+ve	問題なし
14	Diazepam	439-14-5	-ve/+ve	In vitro MN陽性（異数性）
15	Atrazine	1912-24-9	-ve/+ve	問題なし
16	Chloracetophone	74940-61-6	-ve/-ve	ハムスターによるMN陽性知見は疑問；ラットでは陰性
17	Amphetamine	300-62-9	-ve/+ve	問題なし
18	Dimethylvinyl chloride	513-37-1	-ve/+ve	問題なし
19	Propylene glycol mon-t-butyl ether	57018-52-7	-ve/-ve	マウス3か月吸入による雌のMN陽性は疑問；雄は陰性
20	Cypermethrin	52315-07-8	-ve/-ve	GLP試験結果に基づくWOEにより陰性
21	Salicylazosulfapyridine	599-79-1	-ve/+ve	In vitro MN陽性（異数性）

1)　In vitro CA/in vivo MN

:評価結果が変更したもの

表 2.　Ames 陰性・TGR 陽性（-ve/+ve）と報告されている 13 物質のデータ評価

#	物質名	CAS	評価結果 Ames/TGR	備考
1	Cyproterone acetate	427-51-0	-ve/+ve	問題なし
2	Dicyclanil	112636-83-6	-ve/+ve?	TGRの陽性知見は行政目的では疑問的。
3	Adozelesin	110314-48-2	No data/+ve	Ames知見は確認できず。
4	Leucomalachite green	129-73-7	-ve/+ve?	TGRの陽性知見は行政目的では疑問的。
5	Solasodine	126-17-0	No data/-ve	Ames知見は確認できず。本物質のTGR知見は陰性。
6	Rachelmycin	69866-21-3	+ve/+ve	Ames陰性は不適切な情報収集による
7	Tamoxifen	10540-29-1	-ve/+ve	問題なし
8	alpha-Hydroxytamoxifen	97151-02-5	No data/+ve	Ames知見は確認できず。
9	Oxazepam	604-75-1	-ve/+ve	問題なし
10	Benzene	71-43-2	-ve/-ve	12週吸入曝露によるTGR陽性知見は信頼性に乏しい。経口投与では陰性。
11	Hexachlorobutadiene	87-68-3	-ve?/+ve	Amesは33 ug/plateまでの試験。最高用量の適切性疑問。
12	Procarbazine HCl	366-70-1	-ve?/+ve	Amesは333 ug/plateまでの試験。最高用量の適切性疑問。
13	Uracil	66-22-8	-ve/-ve	TGR陽性は細胞増殖亢進による二次的なもの

：評価結果が変更したもの

Appendix 1. Evaluation of *in vivo* bone marrow (BM) or peripheral blood (PB) micronucleus (MN) data on certain chemicals which showed negative in *in vitro* chromosomal aberration (CA) test but positive in *in vivo* MN test.

#	Name (CAS)	In vitro CA	In vivo MN	Evaluation of in vitro CA and in vivo MN data
1	Thioacetamide (62-55-5)	-ve → OK, but nonclear -ve	+ve → OK	Mirkova reported positive in mouse BM MN test (Mutat Res., 352, 23-30, 1996). Thioacetamide showed reproducible positive responses in C57BL/6 male and female mice treated by oral gavage up to 1500 mg/kg. In BALB/c male and female mice, weak response was shown (no clear dose-response, no reproducible) in mortality dose range; I suppose it was negative in BALB/c mice. Morita et al summarized as positive in in vivo MN to thioacetamide (Mutat Res., 786-788, 14-44, 2015), in which thioacetamide is metabolized in vivo to acetamide. Acetamide was negative both in Ames test and mouse BM MN test (Mirkova, 1996; Morita et al., 1997). On the other hand, thioacetamide was negative in repeated dose rat BM MN and liver MN tests for 14- or 28-days treatment up to 20 mg/kg/day (Hamada et al., Mutata. Res., 780-781, 2-17, 2015). Data from in vitro CA test of thioacetamide was assigned TC (technically compromised, original call by the authors was negative) in CGX database (Kirkland et al., Mutat. Res., 584, 1-256, 2005; Morita et al., Mutat Res., 802, 1-29, 2016). Overall evaluation is non-clear negative in in vitro CA test and positive in in vivo MN test by single high dose oral exposure.
2	1,1,2,2-Tetrachloroethane (79-34-5)	-ve → OK	+ve → OK	Morita et al., 1997 does not mention in vivo MN test result on 1,1,2,2-tetrachloroethane. It will be Morita et al., 2016. The compound showed positive response in mouse peripheral blood (normochromatic erhthrocytes) MN test by feeding for 14 weeks up to 9100 ppm (Morita et al., 2016; NTP TOX 49, https://ntp.niehs.nih.gov/ntp/htdocs/st_rpts/tox049.pdf). The response was weak both male and females, but statistically significant (pair wise and tread tests). Overall evaluation is negative in in vitro CA and positive in in vivo MN test using mouse normochromatic erythrocytes by feeding for 14 weeks.
3	4,4'-methylenebis(2-chlorobenzenamine) (101-14-4) Synonym: 4,4'-methylene-bis-[2-chloroaniline] (MOCA)	-ve → +ve in numerical aberration (polyploidy)	+ve → -ve	Heddle et al (Mutation Res., 123, 61-118, 1983) summarized MN data on this compound; -u in Tsuchinmoto & Matter, 1981, + in Salamone et al., 1981. Mavournin et al (Mutat Res., 239, 29-80, 1990) also summarized the positive data. However, no details were given on the positive finding, thus no conclusion can be given. Morita et al (1997) reported negative in CD-1 mouse BM MN test by ip injection in 3 independent assays and positive in MS mouse PB MN test by ip injection in one assay. In conclusion, the data was "inconclusive" in Morita et al (1997). Wakata et al (Environ Mol Mutagen., 32, 84-100, 1998) reported also negative in rat BM and PB MN test by ip dosing. Based on these two reports, Morita et al (2016) assigned negative in in vivo MN on this compound. With respect to in vitro CA/MN, this compound induced polyploidy in CA (http://dra4.nihs.go.jp/mhlw_data/home/file/file101-14-4.html) and MN (Matsushima et al., Mutagenesis, 14, 569-580, 1999). Therefore, Morita et al (2016) assigned positive in in vitro CA on this compound. Overall evaluation is positive in in vitro CA as polyploidy and negative in in vivo MN test in usual mouse strains and rat treated by ip injection.
4	1,4-dichlorobenzene (106-46-7)	-ve → OK	+ve → -ve	Mohtashamipur et al (Mutagenesis, 2, 111-113, 1987) reported as positive in in vivo mouse BM MN test by ip injection. However, this data is doubtful; one control group for 8 test compounds, MN frequencies in the lowest dose in all 8 compounds were higher than the control, benzene was positive by ip injection (usually, benzene was positive by po route, but negative by ip route). Morita et al (1997) and Witt et al (Environ Mol Mutagen., 36, 163-194, 2000) reported negative in in vivo mouse PB MN by ip or po route, thus Morita et al (2016) assigned negative in in vivo MN. Overall evaluation is negative in in vitro CA and negative in in vivo MN test. Please also see EU risk assessment report (https://ec.europa.eu/jrc/en/publication/eur-scientific-and-technical-research-reports/european-union-risk-assessment-report-14-dichlorobenzene-cas-no-106-46-7-einecs-no-203-400-5) and EPA toxicological review (https://ofmpub.epa.gov/eims/eimscomm.getfile?p_download_id=457549).
5	Thiabendazole (148-79-8)	-ve → +ve (aneugenicity)	+ve → OK	Mudry de Pargament et al (Mutat Res., 188, 1-6, 1987) reported that thiazendazole (TBZ) was positive in mouse BM MN test by ip dosing up to 200 mg/kg. JECFA reported positive in in vitro CA (aneuploidy) and negative in in vivo CA (http://www.inchem.org/documents/jecfa/jecmono/v39je02.htm). Food Safety Committee Japan (FSCJ, 2014; Japanese) reported positive in in vitro CA (aneugenicity) and positive in vivo BM MN by ip injection. The mechanism of aneugenicity by TBZ is tubulin polymerization inhibition. Overall evaluation is positive in in vitro CA (aneugenicity) and positive in in vivo MN by ip injection.

6	1-Phenylazo-2-naphthol (CI Solvent yellow 14) (842-07-9)	-ve → OK	+ve → OK	Elliott et al (Mutagenesis, 12, 2555-258, 1987) reported positive in rat BM MN (clear effect) and in mouse BM MN (weak effect) by oral gavage at 5000 mg/kg. Wakata et al (1998) reported solvent yellow 14 was positive in rat BM (clear) and PB (weak) MN test by oral route up to 2000 mg/kg. Hamada et al (2015) also reported positive in rat repeated dose BM MN test (14 days), but negative in rat repeated dose liver MN test up to 600 mg/kg/day. Overall evaluation is positive in in vivo rat BM MN by po single or 14-days treatment. Pleas also see EFSA opinion (http://www.efsa.europa.eu/sites/default/files/scientific_output/files/main_documents/263.pdf).
7	C.I. Direct black 38 (1937-37-7)	-ve → OK	+ve → OK	Beije (Mutat Res., 187, 227-234, 1987) reported positive (weak effect) in rat BM MN test by oral gavage up to 1000 mg/kg. Overall evaluation is positive in in vivo rat BM MN by po treatment.
8	Urethane (51-79-6)	MNT -ve → OK. (CA -ve, but +ve at more than 10 mM)	+ve → OK	Odagiri et al (Mutat Res., 170, 79-83, 1986) reported positive in in vivo mouse BM MN by inhalation up to 150 min at 13 mg/L. Urethane also showed positive in in vivo mouse PB MN by oral gavage up to 1000 mg/kg (CSGMT, Mutat. Res., 278, 83-98, 1992; Morita et al., 2016). With respect to in vitro CA, urethane showed positive response at equal or more than 90 mM, resulting in negative call based on the recent test guidelines (limit to 10 mM or 2 mg/mL) (Morita et al., Mutat Res., 769, 34-49, 2014; Morita et al., 2016).
9	Carbon tetrachloride (56-23-5)	-ve → OK	+ve → -ve	Ye et al (2004, Chinese) reported positive in mouse BM MN test (for abstract, http://en.cnki.com.cn/Article_en/CJFDTotal-HJYJ200406008.htm). The mice were treated by ip injection up to 25 mg/kg. I suppose that this finding is quite doubtful. Morita et al reported clear negative in mouse BM/PB MN test by ip or po treatment up to 2000 mg/kg in four independent assays (Morita et al., 389, 3-122, 1997). In vitro CA data by Sofuni (1998) is negative. Morita et al summarized to in vitro CA-negative and in vivo MN-positive (Morita et al., Mutat Res., 802, 1-29, 2016). Overall evaluation is negative in in vitro CA and negative in several reliable in vivo MN tests.
10	Chlordiazepoxide (58-25-3) [Chlordiazepoxide HCL (438-41-5)]	-ve → OK	+ve → OK	Kirkland et al summarized genotoxicity data on this chemical as in vitro CA-negative and in vivo MN-positive (Kirkland et al., Mutat Res., 721, 27-73, 2011). It cited Snyder (2009) as one of two references, which is based on the PDR (Snyder, Enviro. Mol. Mutagen., 50, 435-450, 2009). In the other data, Susheela reported positive in in vivo mouse BM MN test (Susheela and Rao, Toxicol Lett., 18, 45-48, 1983). The mice treated with chlordiazepoxide HCL by ip or po route up to 201 mg/kg or 562 mg/kg, respectively, resulting in both posityive. In vitro CA data by Sofuni (1998) is negative. Overall evaluation is negative in in vitro CA and positive in in vivo MN tests.
11	Acetaldehyde (75-07-0)	-ve → +ve	+ve → OK	The paper by Lahdetie (1988) is not good reference for this project; it showed negative in meiotic MN. Morita et al (1997) reported positive in mouse BM MN by ip dosing up to 400 mg/kg in two independent assays. In vitro CA data by Sofuni (1998) is positive with and without S9. CGX database summarize in vitro CA-positive and in vivo MN-positive ((Kirkland et al., Mutat. Res., 584, 1-256, 2005; Morita et al., 2016). Overall evaluation is positive in in vitro CA and positive in in vivo MN tests.
12	Tetramethylthiuram disulfide (Thiram) (137-26-8)	-ve → +ve	+ve → OK	In vitro CA data by Sofuni (1998) is positive with S9 mix and equivocal without S9. Kirkland et al (2011) summarized to in vitro CA-positive and in vivo MN-positive. IARC mono V53 (https://www.gezondheidsraad.nl/sites/default/files/0015090osh.pdf) and Netherland (https://monographs.iarc.fr/ENG/Monographs/vol53/mono53-16.pdf) also presented positive results in both tests. Overall evaluation is positive in both in vitro CA and in vivo MN tests.
13	Procarbazine hydrochloride (366-70-1)	-ve → OK	+ve → OK	Morita et al (2016) summarized to in vitro CA-negative and in vivo MN-positive, which cited CSGMT data presenting clear positive (CSGMT, Muatat Res., 278, 83-98, 1992). The data from CSGMT will be more reliable than Bruce et al (1979). In vitro CA data by Sofuni (1998) is negative. Overall evaluation is negative in in vitro CA and positive in in vivo MN tests.
14	Diazepam (439-14-5)	-ve → OK, but in vitro MN +ve (aneugenicity)	+ve → OK	Morita et al (2016) summarized to in vitro CA-negative using Kirkland et al (2005) and in vivo MN-positive. Garza et al (Arch Med Res., 29, 285-289, 1998) also showed positive in mouse BM MN test. In vitro CA data by Sofuni (1998) is negative, but short-term treatment (e.g., 6 hrs) was not employed. So, this negative is technically compromised (TC) negative. On the other hand, Snyder (2009) reported positive in in vitro CA based on the data from PDR. Schuler et al also showed positive in in vitro MN test (Mutat Res., 702, 219-229, 2010). Diazepam is considered aneugen. Overall evaluation is negative in in vitro CA (but positive in in vitro MN due to aneugenicity) and positive in in vivo MN tests.
15	Atrazine (1912-24-9)	-ve → OK	+ve → OK	CGX database showed in vitro CA-negative and in vivo MN-positive (Kirkland et al, 2005; Morita et al., 2016). In vitro CA data by Sofuni (1998) is negative. Overall evaluation is negative in in vitro CA and positive in in vivo MN tests.

16	2-chloroacetophenone (532-27-4) [Maybe Chloracetophone (74940-61-6)]	-ve → OK	+ve → -ve (in rats)	2-Chloroacetophenone (CAS 532-27-4) will not be correct chemical. Because NTP TR379 reported weak positive in vitro CA test without S9. The chemical to be evaluated will be chloracetophone (74940-61-6; Kirkland et al., 2011; but, the name of 'chloroacetophone' is described in this paper.). Kirkland et al (2011) summarized to Ames-positive (TA100, with and without S9, less than x2, up to 6000 ug/p), in vitro MN-negative with TC, in vitro CA-negative with TC, in vivo MN-negative in rats, but -positive in hamsters, in vivo CA-negative in rats and hamsters. All data were from Kappas et al (Mutat Res., 240, 203-208, 1990). In Kappas paper, other negative in rat BM MN test was described. From WOE approach, the positive finding in hamster BM MN is questionable. Overall evaluation is negative in in vitro CA and negative in in vivo rat MN tests.
17	Amphetamine; alpha-methylphenethylamine (300-62-9)	-ve → OK	+ve → OK	Morita et al (2016) summarized to in vitro CA-negative and in vivo MN-positive. Tariq et al reported the in vivo MN-positive originally (Mutat Res., 190, 153-157, 1987). NTP TR387 reported in vitro CA-negative. US FDA (https://www.accessdata.fda.gov/drugsatfda_docs/label/2017/208147s003lbl.pdf) also mentioned in vitro CA-negative and in vivo MN-positive for d, l-Amphetamine (1:1 enantiomer ratio). However, amphetamine, in the enantiomer ratio present in DYANAVEL XR (d- to l-ratio of 3.2 to 1), was not clastogenic in the mouse bone marrow micronucleus test in vivo. Overall evaluation is negative in in vitro CA and positive in in vivo MN tests.
18	Dimethylvinyl chloride (513-37-1)	-ve → OK	+ve → OK	Morita et al (2016) summarized to in vitro CA-negative and in vivo MN-positive, based on the US NTP data. Overall evaluation is also negative in in vitro CA and positive in in vivo MN tests.
19	Propylene glycol mon-t-butyl ether (57018-52-7)	-ve → OK	+ve → -ve	NTP TR515 reported Ames-positive (TA97 without S9, up to 10000 ug/p, x2.1-2.4 at 10000 ug/p), in vitro CA-negative, in vivo mouse PB (normochromatic erythrocytes) MN-negative in males, but -positive (statistically significant) in females by inhalation for 3 months at 1200 ppm (max. conc.). The MN frequency were 1.05/1000 in cont vs 1.10/1000 at 1200 ppm in male, and 0.70/1000 in cont vs 1.25/1000 at 1200 ppm in female. The effect was very small, and biological significance is questionable. Overall evaluation is negative in in vitro CA and negative in in vivo MN tests.
20	Cypermethrin (52315-07-8)	-ve → OK	+ve → -ve	Pluijmen et al (1984) reported negative in V79 HGPRT and OUA mutation test, not in vitro CA test (Mutat Res., 137, 7-15, 1984). PIM 163 cited Amer et al as positive in mouse BM CA by ip route (180 mg/kg of cis:trans=1:1), not BM MN (not examined) (Amer et al., J Appl. Toxicol. 13, 341-345, 1993). Amer et al also reported positive in mouse BM MN by oral route (feeing for 14 days up to 900 ppm), but negative by ip route up to 180 mg/kg of cis:trans=1:1 (Amer et al, Mutat Res., 155, 135-142, 1985). Kirkland et al (2011) summarized to Ames-negative, in vitro MN-positive, in vitro CA-positive, in vivo MN-positive and in vivo CA-positive. Regulatory genotoxicity data are generally negative in EC (https://circabc.europa.eu/sd/a/2a9e841c-ed61-4922-93fc-f8b2d61742a2/Cypermethrin%20(assessment%20report%20as%20finalised%20on%2012.07.13).pdf, which says "In vivo, cypermethrin cis:trans/40:60 did not produce micronuclei in the immature erythrocytes of the mouse bone marrow micronucleus assay (single oral dose), and was, therefore considered negative for mutagenicity. The open literature provides inconsistent evidence of genotoxicity in vitro as well as in vivo. ..... The global weight-of-evidence suggests that cypermethrin cis:trans/40:60 should not be considered a genotoxicant.") and Japanese pesticide evaluation (negative in Ames, in vitro CA, hamster in vivo BM CA by po route up to 40 mg/kg, in vivo rat liver UDS by po route up to 200 mg/kg for cypermethrin; negative in Ames, MLA, in vitro CA, in vivo rat BM CA by po route up to 8 mg/kg, in vivo mouse MN by po route up to 10 mg/kg for alpha-cypermethrin; negative in Ames (small response (x2) at 10000 ug/p in TA100), CHO HGPRT mutation, in vitro CA, in vivo rat BM CA by po route up to 125 mg/kg for zeta-cypermethrin), but mixed results (evaluated by regulatory and published data) in Canada (http://publications.gc.ca/site/archivee-archived.html?url=http://publications.gc.ca/collections/collection_2016/sc-hc/H113-27-2016-18-eng.pdf). Cypermethrin has several formula/preparations, and regulatory data and published data are inconsistent, which makes evaluation difficult. Evaluation based on the regulatory data is negative in both in vitro CA and in vivo MN tests. On the other hand, evaluation based on the published data is positive in both in vitro CA

21	Salicylazosulfapyridine; sulfasalazine; (599-79-1)	-ve → OK, but in vitro MN +ve (aneugenicity)	+ve → OK	Kirkland et al (2011) summarized to Ames-negative with TC, in vitro MN-positive, in vitro CA-negative with TC, in vivo MN-positive and in vivo CA-negative. Bishop et al (1990) reported in vitro CA-negative and in vivo MN-positive (Mutagenesis, 5, 549-554, 1990). Bishop et al also reported in vivo MN-positive by kinetochore positive (Mutat Res., 283, 53-57, 1992). This, aneugenic effect of this chemical, will be due to negative in in vitro and in vivo CA-negative, and in vitro and in vivo MN-positive. Iatropoulos et al also summarized genotoxicity data (in vitro CA-negative, in vitro MN-positive, in vivo MN-positive) and reported that "SASP and its major metabolites are not genotoxic. Folate deficiency associated with SASP administration is probably responsible for aneuploidy in lymphocytes and erythrocytes" (Iatropoulos et al., Exp. Toxic Pathol, 49, 15-28, 1997). Overall evaluation is negative in in vitro CA (but positive in in vitro MN due to aneugenicity and positive in in vivo MN tests.

Appendix 2. Evaluation of 9 substances having *in vitro* Ames negative and *in vivo* TGR positive data

#	Name (CAS)	In vitro CA	In vivo MN	Evaluation of Ames and TGR data
1	Cyproterone acetate (427-51-0)	-ve → OK	+ve → OK	Negative in the Ames test by Lang & Reimann (Environ Mol Mutagen, 21, 272-304, 1993) has been confirmed. There was no Ames data on E. coli or TA102. However, it will be OK that this chemical is considered as Ames-negative (Kasper, Pharmacol. Toxicol, 88, 223-231, 2001). Positive in TGR test by Krebs et al (Carcinogenesis, 19, 241-245, 1998) has been also confirmed, in which the chemical was administered by single oral dose of 20 to 100 mg/kg to female Big Blue F344 rat for investigation of Lac I mutation in the liver. The mutant frequencies were less than 4 times above the control at the highest dose of 200, 100 or 100 mg/kg in the expression periods of 6, 11 or 22 days, respectively. However, the effect was reproducible (Krebs et al, 1998; Topinka et al, Pharmacol. Toxicol. 85, S1, 22, 1999, Abstract; Kasper, 2001). Overall evaluation is negative in Ames and positive in TGR.
2	Dicyclanil (112636-83-6)	-ve → OK	+ve → Questionable +ve	The references of Umemura et al (Mutat Res., 633, 46-54, 2007) and Moto et al (J Toxicol Sci, 28, 173-179, 2003) are not suitable for Ames-negative, because no Ames data are presented. The JECFA/WHO Toxicological evaluation of certain veterinary drug residues in food, WHO Food Additives Series, No. 45, 2000 will be useful (http://www.inchem.org/documents/jecfa/jecmono/v45je04.htm). Ames test results are negative by in house reports. Umemura et al showed positive in TGR assay in female mice, but not male, in which dicyclanil was administered by feed (0.15%) for 13 weeks to male and female B6C3F1 gpt delta mice for 6-TG mutation in the liver. The mutant frequency in the treatment group was about 5 times above the control group. I suppose the data has several limitations as follows: 1) only single dose employed (difficult for evaluation of dose-response relationship, 2) 13 weeks treatment (longer than the guideline length of 4 weeks), 3) no positive control employed (difficult for evaluation of data quality), 4) increase of liver weight observed (mitogenic effect), and 5) no clear differences identified in oxidative stress as 8-OHdG level between male and female (difficult for clear explanation of the difference between male and female). Therefore, the positive TGR data is questionable for regulatory use. Thus, overall evaluation is negative in Ames and questionable positive in TGR.
3	Adozelesin (110314-48-2)	-ve → No data	+ve → OK	The reference on Ames-negative (Harbach et al, Cancer Res., 48, 32-36, 1988) does not present any Ames data on adozelesin. Based on the TGR review paper by Lambert et al (Mutat Res., 590, 1-280, 2005), there is no Ames data. Positive in TGR test by Monroe and Mitchell (Cancer Res., 53, 5690-5696, 1993) has been confirmed, in which adozelesin was administered by single iv injection of 0.036 mg/kg to male Big Blue mice for investigation of Lac I mutation in the liver (also in review papers by Lambert et al, 2005, and OECD, 2009: SERIES ON TESTING AND ASSESSMENT, Number 103, DETAILED REVIEW PAPER ON TRANSGENIC RODENT MUTATION ASSAYS, 2009 (http://www.oecd.org/mwg-internal/de5fs23hu73ds/progress?id=vDjt3QSQicizf02YTLEpG6LU2h8yH1IoaOCHSJz2j6w,&dl). The mutant frequencies in the treatment group were about 3 times higher than the control group at 3 or 15 days after the treatment. The effect was expression time dependent (negative at 18 hrs after the treatment). Overall evaluation is no data in Ames and positive in TGR.
4	Leucomalachite green (129-73-7)	-ve → OK	+ve → Questi	Negative in the Ames test by Fessard et al (J Appl. Toxicol. 19, 421-430, 1999) has been confirmed, in which 4 strains of S, thyphimurium (TA97a, TA98, TA100, TA102) were employed and tested up to 2 mg/plate (precipitation at >0.5 mg/plate). Positive in TGR test by Mittelstaedt et al (Mutat Res., 561, 127-138, 2004) has been confirmed, in which the chemical was administered by feeding of 204 or 408 ppm to female Big Blue mice for 4 or 16 weeks. No increase in lymphocyte Hprt mutant frequencies were observed. On the other hand, weak increase in liver cII mutant frequencies (less than 2 times of the control) were observed at 408 ppm for 16 weeks treatment (no data presented by 204 ppm- or 4 weeks-treatment). The weak effect will be due to 2 of 6 high responder mice. In female Big Blue rats treated by feeding at 543 ppm for 16 weeks, no increase in liver cII mutant frequencies were observed. I suppose the data has several limitations as follows: 1) only single dose data presented (difficult for evaluation of dose-response relationship, 2) 16 weeks treatment (longer than the guideline length of 4 weeks), and 3) no positive control employed (difficult for evaluation of data quality). Therefore, the positive TGR data is questionable for regulatory use. Thus, overall evaluation is negative in Ames and questionable positive in TGR.

5	Solasodine (126-17-0)	-ve → No data	+ve → -ve	The reference of Friedman et al (Fd Chem Toxicol, 41, 61-71, 2003) is not for Ames test data. Based on the review document by Kirkland et al (Mutat Res., 721, 27-73, 2011), no Ames data is existed. Positive in TGR test by Crawford and Myhr (Fd Chem Toxicol, 33, 191-194, 1995) has been confirmed, in which solasodine was administered by ip injection at 300 mg/kg (MTD) for 3 days to impregnated female MutaMouse. The reference mentions that no increase in lacZ mutant frequencies in the dam livers was obtained by solasodine, but three or four times above spontaneous background were shown by alpha-solanine, solanidine or alpha-chaconine. Therefore, solasodine is not positive in TGR assay. Thus, overall evaluation is no data in Ames and negative in TGR. Kirkland et al (2011) summarizes that solanidine and alpha-solanine are negative in the Ames test, but positive in TGR assay. Solasodine is also shown as TGR-positive, but it is a mistake. Instead of solasodine, selection of solanidine or alpha-solanine might be better. However, quality of both positive data in the TGR assay is not suitable for regulatory use.
6	Rachelmycin (69866-21-3) Synonym: CC-1065	-ve → OK	+ve → OK	The reference cited as Ames-negative is ChemID which is the acute toxicity database for toxicology. Genotoxicity data is not included in the database. Synonym of rachelmycin is CC-1065 based on ChemID; the Ames positive data in TA100 is shown in Harbach et al, 1988 (see #3 Adozelesin). Thus, the reference (Harbach et al, 1988) cited as TGR-positive is not suitable, it is for Ames-negative. A reference cited as TGR-positive should be Monroe and Mitchell, 1993 (see #3 Adozelesin). It has been confirmed, in which CC-1065 was administered by single iv injection of 0.050 mg/kg to male Big Blue mice for investigation of Lac I mutation in the liver (also in review paper by Lambert et al, 2005, and OECD, 2009). The mutant frequencies in the treatment group were about 3 times above the control group at 3 days after the treatment. CC-1065 binds to double-strand DNA irreversibly, and CC-1065 is mutagenic bacteria and mammalian cells (Harbach et al, 1988). From the mode of action of CC-1065, the positive finding in TGR assay is reasonable. Overall evaluation is both positive in Ames and TGR.
7	Tamoxifen (10540-29-1)	-ve → OK	+ve → OK	The reference of Glatt et al (Carcinogenesis, 19, 1709-1713, 1998) cited as Ames-negative is not suitable reference; it contains modified Ames data on alpha-hydroxytamoxifen, but not tamoxifen. PDR genotoxicity database by Snyder (Mutata Res., 488, 151-169, 2001; Environ Mol Mutagen, 50, 435-450, 2009) shows negative in Ames test. TGR database shows also Ames-negative (Lambert et al, 2005; OECD, 200). The reference of White (Carcinogenesis, 20, 1153-1160, 1999) cites as TGR-positive is not good reference. Davies et al (Cancer Res, 57, 1288-1293, 1997, http://cancerres.aacrjournals.org/content/canres/57/7/1288.full.pdf) or Kawamura et al (Toxicology, 312, 56-62, 2013) are better references. Review paper by Nohmi et al (Genes Environ, 39:11, 2017, https://genesenvironment.biomedcentral.com/track/pdf/10.1186/s41021-016-0072-6) is also good reference for this purpose. Tamoxifen showed TGR-positive in female Big Blue or gpt delta rats liver treated by ip injection, gavage or diet. Overall evaluation is negative in Ames and positive in TGR.
8	alpha-Hydroxytamoxifen (97151-02-5)	-ve → No data	+ve → OK	The reference of Glatt et al (1998) cited as Ames-negative is not suitable reference; it shows negative in TA1538 and positive in TA 1538-rHSTa (expressing rat hydroxysteroid sulfotransferase a). These data do not provide any positive/negative conclusion in the Ames test. Kirkland et al (2011) concluded as +M (positive by modified assay including sulfotransferases) to the result. TGR database papers say no data for Ames test (Lambert et al, 2005; OECD, 2009). The reference of Boocoke et al (Carcinogenesis, 20, 153-160, 1999) cites as TGR-positive is not suitable reference; there is no TGR data. White et al (Carcinogenesis, 22, 553-557, 2001, https://academic.oup.com/carcin/article/22/4/553/2529895), Chen et al (Carcinogenesis, 23, 1751-1757, 2002, https://academic.oup.com/carcin/article/23/10/1751/2896663), and Costa et al (Cancer Lett, 176, 37-53, 2002, https://ac.els-cdn.com/S0304383501007418/1-s2.0-S0304383501007418-main.pdf?_tid=d5f1636f-3d5b-4747-8525-4910fff1516ca&acdnat=1533015238_68dd1f533d8a355c64935430dc174d3f) are better references. Review paper by Nohmi et al (2017) is also good reference for this purpose. Alpha-hydroxytamoxifen showed TGR-positive in female Big Blue rat liver treated by ip injection or gavage. Overall evaluation is no data in Ames and positive in TGR.

9	Oxazepam (604-75-1)	-ve → OK	+ve → OK	The reference of Griffin and Burka (Drug Metab. Dispos. 23, 232-239, 1995) cited as Ames-negative is not suitable reference; there is no Ames data. PDR genotoxicity database by Snyder (2009) shows negative in Ames test. Definitive Ames data is presented by NTP TR443, 2003 (https://ntp.niehs.nih.gov/ntp/htdocs/lt_rpts/tr443.pdf), in which oxazepam was negative in TA102, TA100, TA1535, TA97, or TA98 up to 3333 ug/plate. Positive in TGR test by Shane et al (Carcinogenesis, 20, 1315-1321, 1999) has been confirmed, in which oxazepam was administered by feeding of 2500 ppm to male Big Blue mice for 180 days. About 2 times increase of mutant frequency (Lac I) in the liver was observed. Additional work by Singh et al (Biochem Pharamacol, 62, 685-692, 2001, https://www.sciencedirect.com/science/article/pii/S0006295201007225?via%3Dihub) showed similar positive result, in which oxazepam was administered by feeding of 2500 ppm to male Big Blue mice for 180 days. About 2 times increase of mutant frequency (cII) in the liver was observed. Overall evaluation is negative in Ames and positive in TGR.

Appendix 3.　発がん性物質で Ames 陰性で TGR 陽性 4 物質の再評価結果

			Ames			TGR		
#	Chemical	CAS No.	Ames Result	Ref Ames		TGR Result	Ref TGR	TGR Comments
1	benzene	71-43-2	N	Zeiger E. and Haworth S., Test with a preincubation modification of the Salmonella/microsome assay, In Evaluation of Short-Term Tests for C arcinogens, Ashby, J., de Serres, F. J., Draper, M., Ishidate, M., Jr., Margolin, B. H., Matter, B. E. … F. J., Draper, M., Ishidate, M., Jr., Margolin, B. H., Matter. B., and Shelby. M. D. (Eds), Elsevier/North Holland,		P→N	Mullin, A.H. et al. (1995) Inhalation of benzene leads to an increase in the mutant frequencies of a lacI transgene in lung and spleen tissues of mice. Mutat. Res., 327(1-2): 121-129. Provost, G.S. et al. (1996) Mutagenic response to benzene and tris(2,3-dibromopropyl)-phosphate in the lambda lacI transgenic mouse mutation assay: a standardized approach to in vivo mutation analysis. Environ. Mol. Mutagen., 28(4): 342-347.	Mulinの論文は陽性判断。BigBlueマウス、lacI。300ppm(1用量）で吸入暴露。1日6時間、週5日、12週間暴露した。肺臓で1.7倍、脾臓で1.5倍のMFの増加。肝臓では変異の増加無し Provostの論文は陰性。BugBlueマウス、LacI,経口投与。2000，400，750mg/kg5日間投与後14日後にとさつ。肺（X1.4）、骨髄、脾臓（X1.6)でわずかな増加。ただし統計学的には陰性 前者は大学、後者はCROのデータ。試験デザイン、信頼性を考慮し、陰性判断
2	Hexachlorobutadiene	87-68-3	N	Haworth S, Lawlor T, Mortelmans K, Speck W, Zeiger E. Salmonella mutagenicity test results for 250 chemicals. Environ. Mutagen. 5 (Suppl.1), 3-142, 1983. TA1535, TA100, TA1537, TA98で、33ug/plateまでしか試験をしていな		P	Unpublished data	
3	Procarbazine HCl	366-70-1	N	Dunkel VC, Zeiger E, Brusick D, McCoy E, McGregor D, Mortelmans K, Rosenkranz HS, Simmon VF , Reproducibility of microbial mutagenicity assays: I. Tests with Salmonella typhimurium and Escherichia coli using a standardized protocol. Environ. Mutagen. 6 (suppl. 2),1-251.1984 全試験菌株で行っているが、333ug/plateまでしか試験を実施していない（Dunkelの論文に関しては最高用量が333ug/plateまで）	ECVAMのAmes陽性DBではEquvocal。陽性の報告は以下の文献。vivo特異的な代謝が重要と考えられる D.G. Gatehouse, D.J. Paes, A Demonstration Of The In Vitro Bacterial Mutagenicity Of Procarbazine, Using The Microtitre Fluctuation Test And Large Concentrations Of S9 Fraction, Carcinogenesis, 4 (1983) 347-352. S. Parodi, S. De Flora, M. Cavanna, A. Pino, L. Robbiano, C. Bennicelli, G. Brambilla, DNA-damaging activity in vivo and	P	Suzuki, T. et al. (1999b) Procarbazine genotoxicity in the MutaMouse; strong clastogenicity and organspecific induction of lacZ mutations. Mutat. Res., 444(2): 269-281. Pletsa, V. et al. (1997) DNA damage and mutagenesis induced by procarbazine in lambda lacZ transgenic mice: evidence that bone marrow mutations do not arise primarily through miscoding by O6-methylguanine. Carcinogenesis, 18(11): 2191-2196. Myhr, B.C. (1991) Validation studies with Muta Mouse: a transgenic mouse model for detecting mutations in vivo. Environ. Mol. Mutagen., 18(4): 308-315.	4つの論文とも陽性反応。肝、腎、肺、骨髄 陽性判定
4	Uracil	66-22-8	N			P→N	Takahashi, S. et al. (2000) Mutation induction by mechanical irritation caused by uracil-induced urolithiasis in Big Blue rats. Mutat. Res., 447(2): 275-280.	BigBlueラット、3％ウラシル（1用量）50週間（2－50週まで経時的観察。膀胱で10週目からMFの増加を観察。ただし変異体の絶対数が少なすぎる（2，5，3個）、著者はMFの増加は細胞増殖亢進によるものと考察している 変異原性とは無関係と判断する陰性判断