Appendix B. : Genotoxicigty test results with rodent non-carcinogens.

ID	Chemical	CAS No	Ames	*in vitro* CA	*in vivo* MN	*in vivo* MN Ref.	TGR	TGR Ref.
NC1	Acetohexamide	968-81-0	–	+				
NC2	Acetonitrile [AKA ethyl nitrile]	75-05-8	–	E	+	[33]		
NC3	Acrolein	107-02-8	+	–	–	[50]		
NC4	Adipamide	628-94-4	–					
NC5	Agar	9002-18-0						
NC6	Aldicarb	116-06-3	–	–	E	[33]		
NC7	Aluminum potassium sulfate	10043-67-1	–	TC				
NC8	dl-Amphetamine sulfate	60-13-9	E	–	+ a	[30]		
NC9	Ampicillin trihydrate	7177-48-2	–	–	–	[31]~		
NC10	Anilazine	101-05-3	–	–				
NC11	p-Anisidine HCl	20265-97-8	+					
NC12	o-Anthranilic acid	118-92-3	–	–*	–	[89]		
NC13	l-Ascorbic acid	50-81-7	E	–	+	[34]		
NC14	Aspirin, phenacetin, and caffeine	8003-03-0	–					
NC15	Azinphosmethyl [AKA gusathion]	86-50-0	+					
NC16	Barium chloride dihydrate	10326-27-9	–	–				
NC17	Benzoate, sodium	532-32-1	–	+				
NC18	Benzoin	119-53-9	–	+	–	[34,145]		
NC19	1H-Benzotriazole	95-14-7	+	+				
NC20	Benzyl alcohol	100-51-6	–	–*	–	[81]		
NC21	Beryllium sulfate	13510-49-1	–		–	[146]		
NC22	Black PN [AKA Food Black 1]	2519-30-4						
NC23	Bromomethane	74-83-9	+	E	+	[36]		
NC24	n-Butyl chloride	109-69-3	–	–				
NC25	N-Butylurea	592-31-4	–	TC				
NC26	gamma-Butyrolactone	96-48-0	–		–	[147]		
NC27	Caffeine	58-08-2	–	+	E	[33]		
NC28	Caprolactam	105-60-2	–	E	–	[148]		
NC29	Carbromal	77-65-6	–	+				
NC30	2-Chloroacetophenone	532-27-4	–	E				
NC31	4-(Chloroacetyl)-acetanilide	140-49-8	+	+				
NC32	p-Chloroaniline	106-47-8	+	+	E	[33]		
NC33	o-Chlorobenzalmalonitrile [AKA malonitrile, o-chlorobenzylidene]	2698-41-1	E	+	–	[149]		
NC34	Chlorodifluoromethane [AKA fluorocarbon 22]	75-45-6	+					
NC35	(2-Chloroethyl)trimethylammonium chloride	999-81-5	–	–				
NC36	2-(Chloromethyl)pyridine HCl	6959-47-3	+	+	–	[50]		
NC37	3-Chloro-p-toluidine [AKA 4-methyl-5-chloro-1-aniline]	95-74-9	–	E				
NC38	Chlorpheniramine maleate	113-92-8	–	+	–	[50]		
NC39	Chlorpropamide	94-20-2	–	+	E	[33]		
NC40	C.I. acid orange 10	1936-15-8	–	+	–	[34]		
NC41	C.I. food red 3 [AKA Acid red 14]	3567-69-9	+	–	–	[34]		
NC42	C.I. pigment red 23 [AKA pigment red 23]	6471-49-4	+	–				
NC43	C.I. pigment yellow 12	6358-85-6	–	–				
NC44	Codeine	76-57-3	–		–	[150]		
NC45	Coumaphos	56-72-4	–	–	–	[151]		
NC46	Cyanamide, calcium	156-62-7	+	–	–	[152]		
NC47	Cyclohexanone	108-94-1	–	–				
NC48	Cyclohexylamine HCl	4998-76-9	–					
NC49	Deltamethrin	52918-63-5	–	–	+	[33]		
NC50	Diallyl phthalate	131-17-9	–	+	–	[33,34]		
NC51	4,4-Diamino-2,2-stilbenedisulfonic acid, disodium salt	7336-20-1	–					
NC52	2,6-Diaminotoluene 2HCl	15481-70-6	+	+	E	[33]	–	[16]~
NC53	2,5-Diaminotoluene sulfate	6369-59-1	+	+	–	[33]~		
NC54	Diazinon	333-41-5	–	+	+	[153]		
NC55	Dibenzo-p-dioxin	262-12-4	–					
NC56	1,2-Dichlorobenzene	95-50-1	–	–	–	[34,50]		
NC57	2,7-Dichlorodibenzo-p-dioxin	33857-26-0	–					
NC58	Dichlorodifluoromethane	75-71-8	–					
NC59	1,1-Dichloroethane	75-34-3	–	–	+	[154]		
NC60	2,4-Dichlorophenol	120-83-2	E	+	–	[155]		
NC61	N,N-Dicyclohexylthiourea	1212-29-9	–	–				
NC62	Dieldrin, photo-	13366-73-9	+	–				
NC63	Dimethoate	60-51-5	+	+	+	[33]		
NC64	Dimethoxane, commercial grade [AKA acetic acid ester with 2,6-dimethyl-m-dioxan-4-ol]	828-00-2	+	+				
NC65	2,4-Dimethoxyaniline HCl	54150-69-5	+	+	–	[50]		
NC66	6-Dimethylamino-4,4-diphenyl-3-heptanone HCl	1095-90-5						
NC67	Dimethylformamide	68-12-2	–	–	–	[156]		
NC68	Dimethyl terephthalate	120-61-6	–	–	–	[34]		

NC69	Dioxathion [AKA phosphorodithioic acid, S,S′-p-dioxane-2,3-diyl-O,O,O′,O′-tetraethyl-2,3-diyl-*O,O,O′,O′*-tetraethyl ester]	78-34-2	+	–				
NC70	Diphenhydramine HCl	147-24-0	–	+				
NC71	Diphenyl-p-phenylenediamine	74-31-7	+	+				
NC72	2,5-Dithiobiurea	142-46-1	–	–				
NC73	EDTA, trisodium salt trihydrate	150-38-9	–	–	– b	[55]		
NC74	Endrin	72-20-8	–	–				
NC75	Ephedrine sulphate	134-72-5	–	–				
NC76	Erythorbate, sodium	6381-77-7	–	–				
NC77	Erythromycin stearate	643-22-1	–	–				
NC78	Estazolam	29975-16-4						
NC79	p,p-Ethyl-DDD [AKA perthane]	72-56-0	+	–				
NC80	Ethyl tellurac	20941-65-5	–	+				
NC81	Etodolac	41340-25-4	–	–				
NC82	Eugenol	97-53-0	–	+	–	[34]	–	[16]
NC83	FD & C green no. 3 [AKA C.I. Food green 3]	2353-45-9	–	TC	E	[33]		
NC84	FD & C red no. 3 [AKA fluorescein, 2′, 4′, 5′, 7′-tetraiodo, disodium salt]	16423-68-0	–	+	E	[33]		
NC85	FD & C yellow no. 5 [AKA tartrazine]	1934-21-0	–	+				
NC86	FD & C yellow no. 6 [AKA Food yellow 3]	2783-94-0	–	–	–	[34]		
NC87	Fenaminosulf, formulated [AKA p-dimethylaminobenzenediazo sulphonic acid, sodium salt]	140-56-7	+	–				
NC88	Fenthion	55-38-9	E	+				
NC89	Fenvalerate [AKA cyano-3-phenoxyphenylmethyl-4-chloro-alpha-1-methylethylbenzene acetate]	51630-58-1	–	+	+	[33]		
NC90	Fluometuron [AKA urea, 1,1-dimethyl 3-(alpha, alpha, alpha-trifluoro-m-tolyl)-]	2164-17-2	–	–	–	[157]		
NC91	Fluoride, sodium	7681-49-4	–	+	E	+[33];–[50,57,58]		
NC92	Gemfibrozil	25812-30-0						
NC93	Guar gum	9000-30-0	–					
NC94	Gum arabic	9000-01-5	–					
NC95	HC blue no. 2 [AKA ethanol, 2,2′ ((4-(2-hydroxyethylamino)-3-nitrophenyl)imino)di-]	33229-34-4	+	–	–	[105]		
NC96	HC yellow 4	59820-43-8	+	–				
NC97	Hexachlorocyclopentadiene	77-47-4	–	+	–	[36]		
NC98	Hexachlorophene	70-30-4	–					
NC99	Hexamethylenetetramine	100-97-0	+					
NC100	4-Hexylresorcinol	136-77-6	–	–	–	[50]		
NC101	Hydrochlorothiazide	58-93-5	–	–				
NC102	8-Hydroxyquinoline [AKA 8-quinolinol]	148-24-3	+	+	–	[34]		
NC103	Iodoform [AKA methane, triiodo-]	75-47-8	+	–				
NC104	Isopropyl-N-(3-chlorophenyl)carbamate	101-21-3	–	E	–	[147]		
NC105	4,4′-isopropylidenediphenol	80-05-7	–	+	–	[158]		
NC106	Lead dimethyldithiocarbamate	19010-66-3	+	+				
NC107	Levobunolol HCl	27912-14-7						
NC108	Lithocholic acid	434-13-9	–	+				
NC109	Locust bean gum	9000-40-2	–					
NC110	Malaoxon	1634-78-2	–	E				
NC111	Malathion	121-75-5	–	+	+	[33]		
NC112	Maleic hydrazide	123-33-1	–	E	–	[159]		
NC113	Manganese (II) sulfate monohydrate	10034-96-5	+	+	+	[33]		
NC114	d-Mannitol	69-65-8	–	–	–	[34]		
NC115	Methotrexate	59-05-2	–	+	+	[33,122]		
NC116	Methoxychlor	72-43-5	–	–				
NC117	alpha-Methyldopa sesquihydrate	41372-08-1	–					
NC118	Methyl methacrylate	80-62-6	–	+	–	[160]		
NC119	Methyl parathion [AKA phosphorothioic acid, O, O-dimethyl o-(p-nitrophenyl)ester]	298-00-0	+	–	+	[33]		
NC120	Monochloroacetic acid	79-11-8	–	–	+	[161]		
NC121	N-(1-Naphthyl)ethylenediamine 2HCl [AKA PL-89779]	1465-25-4	+	+				
NC122	Nickel (II) sulfate hexahydrate	10101-97-0	–	+	–	[11]		
NC123	p-Nitroaniline	100-01-6	+	+				
NC124	4-Nitroanthranilic acid	619-17-0	+	–*				
NC125	3-Nitro-4-hydroxyphenylarsonic acid (AKA roxarsone)	121-19-7	–	–				
NC126	1-Nitronaphthalene	86-57-7	+	+			–	[16]~
NC127	4-Nitro-o-phenylenediamine	99-56-9	+	+	–	[162]		
NC128	3-Nitropropionic acid	504-88-1	+	E				
NC129	Omeprazole	73590-58-6	–		E	[33]		
NC130	gamma-Oryzanol	11042-64-1	–					
NC131	Oxamyl	23135-22-0	–					
NC132	Oxprenolol HCl	6452-73-9	–		E	[33]		
NC133	Oxytetracycline HCl	2058-46-0	–	–	+	[163]		

NC134	Parathion	56-38-2	–	–	–	[110]		
NC135	Penicillin VK	132-98-9	–	+	–	[164]		
NC136	Pentaerythritol tetranitrate with 80% d-lactose monohydrate	78-11-5	–	–				
NC137	Phenformin HCl	834-28-6	–	–				
NC138	Phenol	108-95-2	–	+	+ c	[43,59]		
NC139	p-Phenylenediamine 2HCl	624-18-0	+	+	–	[162]		
NC140	Phenylephrine HCl	61-76-7	–	–	–	[50]		
NC141	1-Phenyl-3-methyl-5-pyrazolone	89-25-8	–	–				
NC142	Phenyl-beta-naphthylamine [AKA N-phenyl-2-naphthylamine]	135-88-6	–	E				
NC143	N-Phenyl-p-phenylenediamine HCl [AKA C.I. Oxidation base 2A]	2198-59-6	–					
NC144	1-Phenyl-2-thiourea	103-85-5	–	–*				
NC145	Phthalamide	88-96-0	–	–				
NC146	Phthalic anhydride	85-44-9	–	+				
NC147	Picloram, technical grade	1918-02-1	–	TC				
NC148	Polysorbate 80	9005-65-6	–	TC				
NC149	Promethazine HCl	58-33-3	–	–				
NC150	Propylene [AKA propene]	115-07-1	+		–	[165]		
NC151	Propyl gallate	121-79-9	–	+	+	[33]	–	[40]
NC152	Resorcinol	108-46-3	–	–*	+	[50]		
NC153	Rhodamine 6 G [AKA basic red 1]	989-38-8	–	TC				
NC154	Rotenone	83-79-4	–	–	–	[166]		
NC155	Sodium chlorite	7758-19-2	+	+	+	[33]		
NC156	Sodium diethyldithiocarbamate trihydrate [AKA carbamic acid, diethyldithio, sodium salt]	148-18-5	–	–				
NC157	Sodium hypochlorite	7681-52-9	–	E	–	[81]		
NC158	Sorbic acid	110-44-1	–	TC	–	[167]		
NC159	Sotalol HCl	959-24-0						
NC160	Sulfisoxazole	127-69-5	–	–	–	[34]		
NC161	3-Sulfolene	77-79-2	–	–				
NC162	Tara gum	39300-88-4	–					
NC163	2,3,5,6-Tetrachloro-4-nitroanisole	2438-88-2	–	E				
NC164	Tetracycline HCl	64-75-5	–	+				
NC165	Tetraethylthiuram disulfide [AKA disulfide, bis(diethylthiocarbamoyl)]	97-77-8	–	+				
NC166	1-trans-delta-9-Tetrahydrocannabinol	1972-08-3	–		+	[33]		
NC167	Tetrakis(hydroxymethyl)phosphonium chloride	124-64-1	–	+	–	[50]		
NC168	Tetrakis(hydroxymethyl)phosphonium sulfate	55566-30-8	–	+	–	[168]		
NC169	Tetramethylthiuram disulfide	137-26-8	+		+	[33]		
NC170	4,4-Thiobis(6-tert-butyl-m-cresol) [AKA santonox-R]	96-69-5	–	–				
NC171	Tin (II) chloride	7772-99-8	–	+	–	[33,34]		
NC172	Tolazamide	1156-19-0	E	TC				
NC173	Tolbutamide	64-77-7	–	–	+	[33]		
NC174	1,1,1-Trichloroethane, technical grade	71-55-6	–	E	–	[50]		
NC175	Trichlorofluoromethane	75-69-4	–					
NC176	2,4,5-Trichlorophenoxyacetic acid	93-76-5	–		–	[33]~		
NC177	Tricresyl phosphate	1330-78-5	–	–				
NC178	Triphenyltin hydroxide	76-87-9	–	–	+	[169]		
NC179	Triprolidine HCl monohydrate	6138-79-0	–					
NC180	l-Tryptophan	73-22-3	–	–				
NC181	Turmeric oleoresin (79%-85% curcumin)	8024-37-1	–	E				
NC182	Urea	57-13-6	–	TC				
NC183	Vinyl toluene (65-71% m- and 32-35% p-) [AKA benzene,ethenylmethyl-]	25013-15-4	–	+	+	[33]		

For explanation of the *in vitro* results, *in vivo* MN and TGR results and other symbols see footnotes to Appendix A.

a, as free base (300-62-9).

b, as disodium salt.

c, maybe due to hypothermia.

References

[1] The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), Guidance on Genotoxicity Testing and Data Interpretation for Pharmaceuticals Intended for Human Use, Step 4 in November 2011. Available at http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Safety/S2_R1/Step4/S2R1_Step4.pdf (accessed 26.09.15).

[2] UK Committee on Mutagenicity (COM), Guidance on a strategy for genotoxicity testing of chemical substances, 1 December, 2011, Available at https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/315793/testing_chemicals_for_genotixicity.pdf (accessed 05.11.15).

[3] European Chemical Agency (ECHA), Guidance on Information Requirements and Chemical Safety Assessment, Chapter R.7a, Version 4.1, October, 2015, Available at http://echa.europa.eu/documents/10162/13632/information_requirements_r7a_en.pdf (accessed 05.11.15).

[4] HEuropean Food Safety Authority (EFSA), Scientific opinion on genotoxicity testing strategies applicable to food and feed safety assessment, 3 October 2012, EFSA Journal 9 (9) (2011) 2379, Available at http://www.efsa.europa.eu/sites/default/files/scientific_output/files/main_documents/2379.pdf (accessed 05.11.15).

[5] M. Hayashi1, M. Honma, M. Takahashi, A. Horibe, J. Tanaka1, M. Tsuchiya, T. Morita, Identification and evaluation of potentially genotoxic agricultural and food-related chemicals, Food Saf. 1 (2013) 32–42, Available at https://www.jstage.jst.go.jp/article/foodsafetyfscj/1/1/1_2013003/_pdf (accessed 05.11.15).

[6] EU, Directive 2003/15/EC of the European Parliament and of the Council of 27 February 2003 amending Council Directive 76/768/EEC on the approximation of the laws of the Member States relating to cosmetic products, Off. J. Eur. Union L66 (2003) 10.

[7] EU, Regulation (EC) No 1223/2009 of the European Parliament and of the Council of 30 November 2009 on cosmetic products, Off. J. Eur. Union L342 (2009) 151.

[8] R.W. Tennant, B.H. Margolin, M.D. Shelby, E. Zeiger, J.K. Haseman, J. Spalding, W. Caspary, M. Resnick, S. Stasiewicz, B. Anderson, R. Minor,

Prediction of chemical carcinogenicity in rodents from in vitro genetic toxicity assays, Science 236 (1987) 933–941.
[9] E. Zeiger, Identification of rodent carcinogens and noncarcinogensusing genetic toxicity tests: premisespromisesand performance, Regul. Toxicol. Pharmacol. 28 (1998) 85–95.
[10] D. Kirkland, M. Aardema, L. Henderson, L. Müller, Evaluation of the ability of a battery of three in vitro genotoxicity tests to discriminate rodent carcinogens and non-carcinogens I. Sensitivity, specificity and relative predictivity, Mutat. Res. 584 (2005) 1–256.
[11] T. Morita, N. Asano, T. Awogi, Y.F. Sasaki, S. Sato, H. Shimada, S. Sutou, T. Suzuki, A. Wakata, T. Sofuni, M. Hayashi, Evaluation of the rodent micronucleus assay in the screening of IARC carcinogens (Groups 1, 2A and 2B), The summary report of the sixth collaborative study by CSGMT/JEMS-MMS, Mutat. Res. 389 (1997) 3–122 (Erratum, Mutat. Res. 391 (1997) 259–267.).
[12] B.S. Kim, B.H. Margolin, Prediction of rodent carcinogenicity utilizing a battery of in vitro and in vivo genotoxicity tests, Environ. Mol. Mutagen. 34 (1999) 297–304.
[13] R. Benigni, C. Bossa, A. Worth, Structural analysis and predictive value of the rodent in vivo micronucleus assay results, Mutagenesis 25 (2010) 335–341.
[14] R. Benigni, C. Bossa, O. Tcheremenskaia, C.L. Battistelli, P. Crettaz, The new ISSMIC database on in vivo micronucleus and its role in assessing genotoxicity testing strategies, Mutagenesis 27 (2012) 87–92.
[15] I.B. Lambert, T.M. Singer, S.E. Boucher, G.R. Douglas, Detailed review of transgenic rodent mutation assays, Mutat. Res. 590 (2005) 1–280.
[16] Organisation for Economic Co-operation and Development (OECD), Detailed review paper on transgenic rodent mutation assays, Series on testing and assessment, Number 103 OECD Paris 2009, ENV/JM/MONO(2009)7. Available at http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono(2009)7&doclanguage=en (accessed 26.09.15).
[17] Carcinogenicity Genotoxicity eXperience (CGX) dataset, ver. 2, April, 2007, Available at https://eurl-ecvam.jrc.ec.europa.eu/databases/genotoxicity-carcinogenicity-db(accessed 26.09.15).
[18] A. Araki, N. Kamigaito, T. Sasaki, T. Matsushima, Mutagenicity of carbon tetrachloride and chloroform in Salmonella typhimurium TA98, TA100, TA1535, and TA1537, and Escherichia coli WP2uvrA/pKM101 and WP2/pKM101 using a gas exposure method, Environ. Mol. Mutagen. 43 (2004) 128–133.
[19] M. Ishidate Jr., M. Harnois, T. Sofuni, A comparative analysis of data on the clastogenicity of 951 chemical substances tested in mammalian cell cultures, Mutat. Res. 195 (1988) 151–213.
[20] D. Brusick, Analysis of genotoxicity and the carcinogenic mode of action for ortho-phenylphenol, Environ. Mol. Mutagen. 45 (2005) 460–481.
[21] H. Zhang, M.A. Cifone, H. Murli, G.L. Erexson, M.S. Mecchi, T.E. Lawlor, Application of simplified in vitro screening tests to detect genotoxicity of aristolochic acid, Food Chem. Toxicol. 42 (2004) 2021–2028.
[22] S. Chanda, G. Erexson, C. Riach, D. Innes, F. Stevenson, H. Murli, K. Bley, Genotoxicity studies with pure trans-capsaicin, Mutat. Res. 557 (2004) 85–97.
[23] R. Proudlock, C. Thompson, E. Longstaff, Examination of the potential genotoxicity of pure capsaicin in bacterial mutationchromosome aberration, and rodent micronucleus tests, Environ. Mol. Mutagen. 44 (2004) 441–447.
[24] R. Albanese, P.A. Watkins, The mutagenic activity of razoxane (ICRF159): an anticancer agent, Br. J. Cancer 52 (1985) 725–731.
[25] P.A. Lefevre, H. Tinwell, S.M. Galloway, R. Hill, J.M. Mackay, C.R. Elcombe, J. Foster, V. Randall, R.D. Callander, J. Ashby, Evaluation of the genetic toxicity of the peroxisome proliferator and carcinogen methyl clofenapate, including assays using Muta Mouse and Big Blue transgenic mice, Hum. Exp. Toxicol. 13 (1994) 764–775.
[26] P.R. Padma, A.J. Amonkar, S.V. Bhide, Mutagenic and cytogenetic studies of N-nitrosonornicotine and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone, Cancer Lett. 46 (1989) 173–180.
[27] D.J. Tweats, D.G. Gatehouse, Further debate of testing strategies, Mutagenesis 3 (1988) 95–102.
[28] Organisation for Economic Co-operation and Development (OECD) Guideline for the Testing of Chemicals, TG 473, in vitro Mammalian Chromosomal Aberration Test, Adopted 26 2014, Available at http://www.oecd-ilibrary.org/environment/oecd-guidelines-for-the-testing-of-chemicals-section-4-healt-effects_20745788 (accessed 18.01.16).
[29] T. Morita, A. Miyajima, A. Hatano, M. Honma, Effects of lowering the proposed top-concentration limit in an in vitro chromosomal aberration test on assay sensitivity and on the reduction of the number of false positives, Mutat. Res. 769 (2014) 34–49.
[30] K.H. Mavournin, D.H. Blakey, M.C. Cimino, M.F. Salamone, J.A. Heddle, The in vivo micronucleus assay in mammalian bone marrow and peripheral blood, a report of the U.S. Environmental Protection Agency Gene-Tox Program, Mutat. Res. 239 (1990) 29–80.
[31] D. Kirkland, P. Kasper, L. Müller, R. Corvi, G. Speit, Recommended lists of genotoxic and non-genotoxic chemicals for assessment of the performance of new or improved genotoxicity tests: a follow-up to an ECVAM workshop, Mutat. Res. 653 (2008) 99–108.
[32] D. Kirkland, G. Speit, Evaluation of the ability of a battery of three in vitro genotoxicity tests to discriminate rodent carcinogens and non-carcinogens III. Appropriate follow-up testing in vivo, Mutat. Res. 654 (2008) 114–132.
[33] D. Kirkland, L. Reeve, D. Gatehouse, V. Vanparys, A core in vitro genotoxicity battery comprising the Ames test plus the in vitro micronucleus test is sufficient to detect rodent carcinogens and in vivo genotoxins, Mutat. Res. 721 (2011) 27–73.
[34] M.D. Shelby, G.L. Erexson, G.J. Hook, R.R. Tice, Evaluation of a three-exposure mouse bone marrow micronucleus protocol: results with 49 chemicals, Environ. Mol. Mutagen. 21 (1993) 160–179.
[35] A. Wakata, Y. Miyamae, S. Sato, T. Suzuki, T. Morita, N. Asano, T. Awogi, K. Kondo, M. Hayashi, Evaluation of the rat micronucleus test with bone marrow and peripheral blood: summary of the 9th collaborative study by CSGMT/JEMSMMS. Collaborative Study Group for the Micronucleus Test. Environmental Mutagen Society of Japan, Mammalian Mutagenicity Study Group, Environ. Mol. Mutagen. 32 (1998) 84–100.
[36] K.L. Witt, A. Knapton, C.M. Wehr, G.J. Hook, J. Mirsalis, M.D. Shelby, J.T. MacGregor, Micronucleated erythrocyte frequency in peripheral blood of B6C3F1 mice from short-termprechronic, and chronic studies of the NTP carcinogenesis bioassay program, Environ. Mol. Mutagen. 36 (2000) 163–194.
[37] S. Hamada, S. Sutou, T. Morita, A. Wakata, S. Asanami, S. Hosoya, S. Ozawa, K. Kondo, M. Nakajima, H. Shimada, K. Osawa, Y. Kondo, N. Asano, S. Sato, H. Tamura, N. Yajima, R. Marshall, C. Moore, D.H. Blakey, L.M. Schechtman, J.L. Weaver, D.K. Torous, R. Proudlock, S. Itoh, C. Namiki, M. Hayashi, Evaluation of the rodent micronucleus assay by a 28-day treatment protocol: summary of the 13th collaborative study group for the micronucleus test (CSGMT)/Environmental Mutagen Society of Japan (JEMS)-Mammalian Mutagenicity Study Group (MMS), Environ. Mol. Mutagen. 37 (2001) 93–110.
[38] K. Masumori, Transgenic mouse gene mutation assay of sodium nitrite, Exp. No. B968 (079-452), 2011. Unpublished report for Ministry of Health Labour and Welfare, Japan (in Japanese).
[39] M. Nakajima, Transgenic mouse gene mutation assay of Food Red No.102, Exp. No. B518 (079-433), 2009, Unpublished report for Ministry of Health Labour and Welfare, Japan (in Japanese).
[40] M. Nakajima, Transgenic mouse gene mutation assay of propylgallate, Exp. No. B967 (079-451), 2011, Unpublished report for Ministry of Health Labour and Welfare, Japan (in Japanese).
[41] European Union Risk Assessment Report (EURAR), Acrylonitrile, 107-13-1, 2004, Available at http://echa.europa.eu/documents/10162/22bf49d3-e951-44b8-a45a-6973d3dc62f6 (accessed 26.09.15).
[42] S. Asanami, K. Shimono, S. Kaneda, Transient hypothermia induces micronuclei in mice, Mutat. Res. 413 (1998) 7–14.
[43] D.J. Tweats, D. Blakey, R.H. Heflich, A. Jacobs, S.D. Jacobsen, T. Morita, T. Nohmi, M.R. O'Donovan, Y.F. Sasaki, T. Sofuni, R. Tice, Report of the IWGT working group on strategies and interpretation of regulatory in vivo tests I. Increases in micronucleated bone marrow cells in rodents that do not indicate genotoxic hazards, Mutat. Res. 627 (2007) 78–91.
[44] A.O. Asita, M. Hayashi, Y. Kodama, A. Matsuoka, T. Suzuki, T. Sofuni, Micronucleated reticulocyte induction by ethylating agents in mice, Mutat. Res. 271 (1992) 29–37.
[45] A. Rothfuss, M. O'Donovan, M. De Boeck, D. Brault, A. Czich, L. Custer, S. Hamada, U. Plappert-Helbig, M. Hayashi, J. Howe, A.R. Kraynak, B.J. van der Leede, M. Nakajima, C. Priestley, V. Thybaud, K. Saigo, S. Sawant, J. Shi, R. Storer, M. Struwe, E. Vock, S. Galloway, Collaborative study on fifteen compounds in the rat-liver comet assay integrated into 2- and 4-week repeat-dose studies, Mutat. Res. 702 (2010) 40–69.
[46] S. Asanami, K. Shimono, Species-level differences between mice and rats in regards to micronucleus induction with the hypothermia-inducing drug haloperidol, Mutat. Res. 676 (2009) 102–105.
[47] J.R. Meier, P. Wernsing, J. Torsella, Feasibility of micronucleus methods for monitoring genetic damage in two feral species of small mammals, Environ. Mol. Mutagen. 33 (1999) 219–225.
[48] C.K. Grisolia, C.S. Takahashi, Assessment of interactions of the antimalarial drugs chloroquine and mefloquine with $NaNO_2$ and $HgCl_2$ in rodents, Mutat. Res. 305 (1994) 151–156.
[49] H. Suzuki, N. Ikeda, K. Kobayashi, Y. Terashima, Y. Shimada, T. Suzuki, T. Hagiwara, S. Hatakeyama, K. Nagaoka, J. Yoshida, Y. Saito, J. Tanaka, M. Hayashi, Evaluation of liver and peripheral blood micronucleus assays with 9 chemicals using young rats, a study by the collaborative study group for the micronucleus test (CSGMT)/Japanese environmental mutagen society (JEMS)-Mammalian mutagenicity study group (MMS), Mutat. Res. 583 (2005) 133–145.
[50] NTP Chemical Effects in Biological Systems (CEBS) database. Available at http://tools.niehs.nih.gov/cebs3/ui/ (accessed 26.09.15).
[51] M.Y. Kim, Y.C. Kim, M.H. Cho, Combined treatment with 4-(N-methyl-N-nitrosamino)-1- (3-pyridyl)-1-butanone and dibutyl phthalate enhances ozone-induced genotoxicity in B6C3F1 mice, Mutagenesis 17 (2002) 331–336.
[52] OECD Screening Information Data Set (SIDS), Sodium Nitrite, 7632-00-0, 2005, Available at http://webnet.oecd.org/HPV/UI/handler.axd?id=5d2b8a51-1de5-4fc9-b7f1-09b3f8725ef5 (accessed 26.09.15).
[53] T. Ono, T. Sekiya, Y. Takahashi, Y.F. Sasaki, T. Ohta, Species-specificity of pyrimethamine in the rodent bone marrow micronucleus test, Mutat. Res. 390 (1997) 167–170.
[54] The Carcinogenic Potency Database (CPDB), Available at http://toxnet.nlm.nih.gov/cpdb/pdfs/ChemicalTable.pdf (accessed 26.09.15).

[55] EURAR, Edetic Acid (EDTA), 60-00-4, 2004, Available at http://echa.europa.eu/documents/10162/65615721-ab6d-4f28-b48f-73cf9d8cc529 (accessed 26.09.15).
[56] A. Muralidhara, K. Narasimhamurthy, Assessment of in vivo mutagenic potency of ethylenediaminetetraacetic acid in albino mice, Food Chem. Toxicol. 29 (1991) 845–849.
[57] Y. Li, A.J. Dunipace, G.K. Stookey, Lack of genotoxic effects of fluoride in the mouse bone-marrow micronucleus test, J. Dent. Res. 66 (1987) 1687–1690.
[58] E. Zeiger, D.K. Gulati, P. Kaur, A.H. Mohamed, J. Revazova, T.G. Deaton, Cytogenetic studies of sodium fluoride in mice, Mutagenesis 9 (1994) 467–471.
[59] P.J. Spencer, B.B. Collapudi, J.M. Waechter Jr., Induction of micronuclei by phenol in the mouse bone marrow: I. Association with chemically induced hypothermia, Toxicol. Sci. 97 (2007) 120–127.
[60] D. Kirkland, E. Zeiger, F. Madia, N. Gooderham, P. Kasper, A. Lynch, T. Morita, G. Ouedraogo, J.M.P. Morte, S. Pfuhler, V. Rogiers, M. Schulz, V. Thybaud, J. van Benthem, P. Vanparys, A. Worth, R. Corvi, Can in vitro mammalian cell genotoxicity test results be used to complement positive results in the Ames test and help predict carcinogenic or in vivo genotoxic activity? I. Reports of individual databases presented at an EURL ECVAM Workshop, Mutat. Res. 775–776 (2014) 55–68.
[61] D. Kirkland, S. Pfuhler, D. Tweats, M. Aardema, R. Corvi, F. Darroudi, A. Elhajouji, H. Glatt, P. Hastwell, M. Hayashi, P. Kasper, S. Kirchner, A. Lynch, D. Marzin, D. Maurici, J.-R. Meunier, L. Müller, G. Nohynek, J. Parry, E. Parry, V. Thybaud, R. Tice, J. van Benthem, P. Vanparys, P. White, How to reduce false positive results when undertaking in vitro genotoxicity testing and thus avoid unnecessary follow-up animal tests: report of an ECVAM Workshop, Mutat. Res. 628 (2007) 31–55.
[62] T. Morita, M. Honma, K. Morikawa, Effect of reducing the top concentration used in the in vitro chromosomal aberration test in CHL cells on the evaluation of industrial chemical genotoxicity, Mutat. Res. 741 (2012) 32–56.
[63] P. Fowler, R. Smith, K. Smith, J. Young, L. Jeffrey, D. Kirkland, S. Pfuhler, P. Carmichael, Reduction of misleading (false) positive results in mammalian cell genotoxicity assays. II. Importance of accurate toxicity measurement, Mutat. Res. 747 (2012) 104–117.
[64] M.T. King, H. Beikirch, K. Eckhardt, E. Gocke, D. Wild, Mutagenicity studies with X-ray-contrast mediaanalgesics, antipyretics, antirheumatics and some other pharmaceutical drugs in bacterial, drosophila and mammalian test systems, Mutat. Res. 66 (1979) 33–43.
[65] OECD SIDS Initial Assessment Profile (SIAP), Acrylamide, 79-06-1, SIAM (SIDS Initial Assessment Meeting), 12, 27–29 June, 2001, Available at http://webnet.oecd.org/HPV/UI/handler.axd?id=c81f3f95-e5f8-4857-91af-4c76b9094edb (accessed 26.09.15).
[66] Acrylamide EURAR, 79-06-1, 2002, Available at http://echa.europa.eu/documents/10162/50218bf9-ba0f-4254-a0d9-d577a5504ca7 (accessed 26.09.15).
[67] Y. Ishii, K. Matsushita, K. Kuroda, Y. Yokoo, A. Kijima, S. Takasu, Y. Kodama, A. Nishikawa, T. Umemura, Acrylamide induces specific DNA adduct formation and gene mutations in a carcinogenic target site, the mouse lung, Mutagenesis 30 (2015) 117–235.
[68] M.V.U. Rani, O.S. Reddi, P.P. Reddy, Mutagenicity studies involving aldrinendosulfan, dimethoate, phosphamidon, carbaryl and ceresin, Bull. Environ. Contam. Toxicol. 25 (1980) 277–282.
[69] OECD SIAP, Allyl 2,3-epoxypropyl ether, 106-92-3, SIAM 25, 17–18 October, 2007, Available at http://webnet.oecd.org/HPV/UI/handler.axd?id=c4aeebe8-60de-411a-8f05-fca31da10165 (accessed 26.09.15).
[70] D.K. Gulati, J.P. Wojciechowski, P. Kaur, Comparison of single-, double- or triple-exposure protocols for the rodent bone marrow/peripheral blood micronucleus assay using 4-aminobiphenyl and treosulphan, Mutat. Res. 234 (1990) 135–139.
[71] T. Itoh, T. Suzuki, A. Nishikawa, F. Furukawa, M. Takahashi, W. Xue, T. Sofuni, M. Hayashi, In vivo genotoxicity of 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline in lacI transgenic (Big Blue) mice, Mutat. Res. 468 (2000) 19–25.
[72] N. Loprieno, G. Boncristiani, G. Loprieno, An experimental approach to identifying the genotoxic risk from cooked meat mutagens, Food Chem. Toxicol. 29 (1991) 377–386.
[73] E. Okada, Y. Fujiishi, K. Narumi, N. Yasutake, W. Ohyama, A four-day oral treatment regimen for simultaneous micronucleus analysis in the glandular stomachcolon, and bone marrow of rats, Mutat. Res. 758 (2013) 87–94.
[74] M.F. Salamone, J.A. Heddle, M. Katz, Mutagenic activity of 41 compounds in the in vivo micronucleus assay, Prog. Mutat. Res. 1 (1981) 686–697.
[75] E.M. Bomhard, High-dose clastogenic activity of aniline in the rat bone marrow and its relationship to the carcinogenicity in the spleen of rats, Arch. Toxicol. 77 (2003) 291–297.
[76] A. Kohara, T. Suzuki, M. Honma, T. Ohwada, M. Hayashi, Mutagenicity of aristolochic acid in the lambda/lacZ transgenic mouse (MutaMouse), Mutat. Res. 515 (2002) 63–72.
[77] T. Gebel, S. Kevekordes, K. Pav, R. Edenharder, H. Dunkelberg, In vivo genotoxicity of selected herbicides in the mouse bone-marrow micronucleus test, Arch. Toxicol. 71 (1997) 193–197.
[78] R. Barale, C. Scapoli, C. Meli, D. Casini, M. Minunni, A. Marrazzini, N. Loprieno, I. Barrai, Cytogenetic effects of benzimidazoles in mouse bone marrow, Mutat. Res. 300 (1993) 15–28.
[79] R. Ciranni, R. Barale, A. Marrazzini, Benzene and the genotoxicity of its metabolites, Mutat. Res. 208 (1988) 61–67.
[80] K.S. Subramanya, A.M. Motimaya, P.T. Curry, R.M. Kitchin, Assessment of genotoxicity of two anti-parkinsonian drugs (selegiline hydrochloride and bromocriptine mesylate) in vivo mouse bone marrow cells, Toxicol. Lett. 66 (1993) 221–230.
[81] M. Hayashi, M. Kishi, T. Sofuni, M. Ishidate Jr, Micronucleus tests in mice on 39 food additives and eight miscellaneous chemicals, Food Chem. Toxicol. 26 (1988) 487–500.
[82] A. Celik, B. Byunkakilli, B. Cimen, B. Tasdelen, M.I. Ozturk, D. Eke, Assessment of cadmium genotoxicity in peripheral blood and bone marrow tissues of male Wister rats, Toxicol. Mech. Methods 19 (2009) 135–140.
[83] L. Fabry, Relationship between the induction of micronuclei in marrow cells by chromium salts and their carcinogenic properties, C. R. Soc. Biol. 174 (1980) 889–892.
[84] L. Abramsson-Zetterberg, K. Svensson, Semicarbazide is not genotoxic in the flow cytometry-based micronucleus assay in vivo, Toxicol. Lett. 155 (2005) 211–217.
[85] A. Marrazzini, L. Chelotti, I. Barrai, N. Loprieno, R. Barale, In vivo genotoxic interactions among three phenolic benzene metabolites, Mutat. Res. 341 (1994) 29–46.
[86] J. Ashby, C. Loquet, M. Ishidate Jr., R.D. Callander, R. Albanese, Mutagenicity to bacteriacultured cells, and rodents of the human carcinogen chlornaphazine, Environ. Mol. Mutagen. 12 (1988) 365–374.
[87] R. Ebert, N. Fedtke, H. Certa, H.-J. Wiegand, J.-F. Regnier, R. Marshall, S.W. Dean, Genotoxicity studies with chloroethane, Mutat. Res. 322 (1994) 33–44.
[88] EURAR, Chloroform, 67-66-3, Human Health, 2008, Available at http://echa.europa.eu/documents/10162/166afc78-29a5-42df-bec8-20b978163b36 (accessed 26.09.15).
[89] A.F. McFee, P.P. Jauhar, K.W. Lowe, J.T. MacGregor, C.M. Wehr, Assays of three carcinogen/non-carcinogen chemical pairs for in vivo induction of chromosome aberrations, sister chromatid exchanges and micronuclei, Environ. Mol. Mutagen. 14 (1989) 207–220.
[90] OECD SIDS, 1-Chloro-4-nitrobenzene, 100-00-5, 2002, Available at http://webnet.oecd.org/HPV/UI/handler.axd?id=ad5cafea-2e80-4360-9aba-9056532403f0 (accessed 26.09.15).
[91] International Programme on Chemical Safety (IPCS), Environmental Health Criteria (EHC) 183, Chlorothalonil, 1996, Available at http://www.inchem.org/documents/ehc/ehc/ehc183.htm (accessed 26.09.15).
[92] B. Beije, Induction of unscheduled DNA synthesis in liver and micronucleus in bone marrow of rats exposed in vivo to the benzidine-derived azo dye, Direct Black 38, Mutat. Res. 187 (1987) 227–234.
[93] C. Westmoreland, D. Gatehouse, D and C red No. 9: genotoxic or nongenotoxic carcinogen, Mutat. Res. 281 (1992) 163–167.
[94] I.-D. Adler, U. Kliesch, P. van Hummelen, M. Kirsch-Volders, Mouse micronucleus tests with known and suspect spindle poisons: results from two laboratories, Mutagenesis 6 (1991) 47–53.
[95] OECD SIDS, 1,2-Dichloropropane, 78-, 2006, Available at http://webnet.oecd.org/HPV/UI/handler.axd?id=e6ce93c6-9dd5-41df-ade6-86ecabd0f533 (accessed 26.09.15).
[96] R. Cicchetti, M. Bari, G. Argentin, Induction of micronuclei in bone marrow by two pesticides and their differentiation with CREST staining: an in vivo study in mice, Mutat. Res. 439 (1999) 239–248.
[97] G.R. Douglas, A.P. Hugenholtz, D.H. Blakey, Genetic toxicology of phthalate esters: mutagenic and other genotoxic effects, Environ. Health Perspect. 65 (1986) 255–262.
[98] L. Henderson, T. Regan, Effects of diethylstilboestrol-dipropionate on SCEs, micronuclei, cytotoxicity, aneuploidy and cell proliferation in maternal and foetal mouse cells treated in vivo, Mutat. Res. 144 (1985) 27–31.
[99] H. Suzuki, T. Imamura, A. Koeda, K. Morimoto, Y. Wakasa, Y. Takei, R. Amemiya, H. Hatakeyama, H. Satoh, S. S. Sato, Genotoxicity studies of 2,6-dinitrotoluene (2,6-DNT), J. Toxicol. Sci. 36 (2011) 499–505.
[100] L. Müller, A. Korte, S. Madle, Mutagenicity testing of doxylamine succinate, an antinauseant drug, Toxicol. Lett. 49 (1989) 79–86.
[101] J. Ashby, K. Fletcher, C. Williams, J. Odum, H. Tinwell, Lack of activity of estradiol in rodent bone marrow micronucleus assays, Mutat. Res. 395 (1997) 83–88.
[102] B.J. Phillips, P. Jenkinson, Is ethanol genotoxic? a review of the published data, Mutagenesis 16 (2001) 91–101.
[103] R.L. Robison, W. Suter, R.H. Cox, Carcinogenicity and mutagenicity studies with fluvastatina new, entirely synthetic HMG-CoA reductase inhibitor, Fundam. Appl. Toxicol. 23 (1994) 9–20.
[104] M. Aranda, L.P. Perez-Alzola, M.R. Ellahuene, C. Sepulveda, Assessment of in vitro mutagenicity in Salmonella and in vivo genotoxicity in mice of the mycotoxin fuminosin B1, Mutagenesis 16 (2000) 469–471.
[105] J.W. Parton, J.E. Beyers, M.L. Garriott, Mutagenicity evaluation of HC Blue No. 1 and HC Blue No. 2. I. Effect on the induction of micronuclei in mouse bone marrow cells, Mutat. Res. 241 (1990) 139–144.
[106] EURAR, Hydrogen Peroxide, 7722-84-1, 2003, Available at http://echa.europa.eu/documents/10162/a6f76a0e-fe32-4121-9d9d-b06d9d5f6852 (accessed 26.09.15).
[107] M. Matsumoto, S. Masumori, M. Hirata-Koizumi, A. Ono, M. Honma, K. Yokoyama, A. Hirose, Evaluation of in vivo mutagenicity of hydroquinone in Muta™ mice, Mutat. Res. 775–776 (2014) 94–98.
[108] J.L. O'Donoghue, S.R. Haworth, R.D. Curren, P.E. Kirby, T. Lawlor, E.J. Moran, R.D. Phillips, D.L. Putnam, A.M. Rogers-Back, R.S. Slesinski, Mutagenicity

studies on ketone solvents: methyl ethyl ketone, methyl isobutyl ketone, and isophorone, Mutat. Res. 206 (1988) 149–161.
[109] J.C. Richardson, A preliminary assessment of the cytogenetic effects of METEPA on mouse bone marrow using the micronucleus test, Mutat. Res. 26 (1974) 391–394.
[110] S. Kevekordes, T. Gebel, K. Pav, R. Edenharder, H. Dunkelberg, Genotoxicity of selected pesticides in the mouse bone-marrow micronucleus test and in the sister-chromatid exchange test with human lymphocytes in vitro, Toxicol. Lett. 89 (1996) 35–42.
[111] T. Hashimoto, K. Takeuchi, S. Ohno, S. Komatsu, Mutagenicity tests of the antithyroid agent thiamazole, cytogenetic studies on male mice, J. Toxicol. Sci. 12 (1987) 23–32.
[112] M.A. Friedman, J. Staub, Induction of micronuclei in mouse and hamster bone marrow by chemical carcinogens, Mutat. Res. 43 (1977) 255–262.
[113] K.L. Witt, L. Hughes, L.T. Burka, A. McFee, J.B. Bishop, Mouse bone marrow micronucleus test results do not predict the germ cell mutagenicity of N-hydroxy-methylacrylamide in the mouse dominant lethal assay, Environ. Mol. Mutagen. 41 (2003) 111–120.
[114] S.V. Bhide, E.B. Bhalerao, A.V. Sarode, G.B. Maru, Mutagenicity and carcinogenicity of mono- and diacetyl hydrazine, Cancer Lett. 23 (1984) 235–240.
[115] A. Montaldi, T. Mariot, M. Zordan, M. Paleologo, A.G. Levis, Nitrilotriacetic acid (NTA) does not induce chromosomal damage in mammalian cells either in vitro or in vivo, Mutat. Res. 208 (1988) 95–100.
[116] EURAR Nitrobenzene, 98-95-3, Human Health, 2008, Available at http://echa.europa.eu/documents/10162/a4e6c593-b26c-4235-9549-3af825bdcab7 (accessed 26.09.15).
[117] International Agency for Research on Cancer (IARC), IARC Monographs on the Evaluation of Carcinogenic Risks to Human, Volume 57, Occupational Exposures of Hairdressers and Barbers and Personal Use of Hair Colourants; Some Hair Dyes, Cosmetic Colourants, Industrial Dyestuffs and Aromatic Amines, 1993, Available at http://monographs.iarc.fr/ENG/Monographs/vol57/index.php (accessed 26.09.15).
[118] H.E. Holden, D. Studwell, J.B. Majeska, Oxymetholone: I. Evaluation in a comprehensive battery of genetic toxicology and in vitro transformation assays, Toxicol. Pathol. 27 (1999) 501–506.
[119] J.A. Heddle, M. Hite, B. Kirkhart, K. Larsen, J.T. MacGregor, G.W. Newell, M.F. Salamone, The induction of micronuclei as a measure of genotoxicity. A report of the U.S. Environmental Protection Agency Gene-Tox Program, Mutat. Res. 123 (1983) 61–118.
[120] J.P. Seiler, The mutagenicity of mono- and di-functional aromatic glycidyl compounds, Mutat. Res. 135 (1984) 159–167.
[121] S. Balakrishnan, D.A. Eastmond, Micronuclei and cell proliferation as early biological markers of ortho-phenylphenol-induced changes in the bladder of male F344 rats, Food Chem. Toxicol. 44 (2006) 1340–1347.
[122] CSGMT (The Collaborative Study Group for the Micronucleus Test), Micronucleus test with mouse peripheral blood erythrocytes by acridine orange supravital staining: the summary report of the 5th collaborative study by CSGMT/JEMS.MMS, Mutat. Res. 278 (1992) 83–98.
[123] T. Suzuki, S. Itoh, M. Nakajima, N. Hachiya, T. Hara, Target organ and time-course in the mutagenicity of five carcinogens in Muta™ Mouse: a summary report of the second collaborative study of the transgenic mouse mutation assay by JEMS/MMS, Mutat. Res. 444 (1999) 259–268.
[124] C. Westmoreland, E. George, M. York, D. Gatehouse, In vivo genotoxicity studies with p-benzoquinone dioxime, Environ. Mol. Mutagen. 19 (1992) 71–76.
[125] A. Leonard, E.D. Leonard, Mutagenicity test with saccharin in the male mouse, J. Environ. Pathol. Toxicol. 2 (1979) 1047–1053.
[126] S. De Flora, M. Iltcheva, R.M. Balansky, Oral chromium(VI) does not affect the frequency of micronuclei in hematopoietic cells of adult mice and of transplacentally exposed fetuses, Mutat. Res. 610 (2006) 38–47.
[127] N. Chinnasamy, L.J. Fairbairn, J. Laher, M.A. Willington, J.A. Rafferty, Modulation of O^6-alkylating agent induced clastogenicity by enhanced DNA repair capacity of bone marrow cells, Mutat. Res. 416 (1998) 1–10.
[128] U. Liegibel, H. Tinwell, R.D. Callander, P. Schmezer, J. Ashby, Clastogenicity to the mouse bone marrow of the mouse germ cell genotoxin streptozotocin, Mutagenesis 7 (1992) 471–474.
[129] K.K. Vijayalaxmi, S.P. Rai, Studies on the genotoxicity of tamoxifen citrate in mouse bone marrow cells, Mutat. Res. 368 (1996) 109–114.
[130] D.M. Rodrigues Jr., A.R. dos Santos, A.A. Cavalcante, A.P. Alencar, P.V. Lopes-Costa, B.B. da Silva, Assessment of micronucleus frequency in the peripheral blood of female rats in persistent estrus treated with selective estrogen receptor modulators, Environ. Mol. Mutagen. 53 (2012) 51–54.
[131] P.P.W. van Buul, S. van Buul-Offers, Effect of hormone treatment on spontaneous and radiation-induced chromosomal breakage in normal and dwarf mice, Mutat. Res. 106 (1982) 237–246.
[132] J. Meyne, D.C. Allison, K. Bose, S.W. Jordan, P.F. Ridolpho, Hepatotoxic doses of dioxin do not damage mouse bone marrow chromosomes, Mutat. Res. 157 (1985) 63–69.
[133] IPCS, Concise International Chemical Assessment Document (CICAD), No. 49, Thiourea, 2003, Available at http://www.who.int/ipcs/publications/cicad/en/cicad49.pdf?ua=1 (accessed 26.09.15).
[134] H.K. Lindberg, A. Korpi, T. Santonen, K. Sakkinen, M. Jarvela, J. Tornaeus, N. Ahonen, H. Jarventaus, A.L. Pasanen, C. Rosenberg, H. Norppa, Micronucleihemoglobin adducts and respiratory tract irritation in mice after inhalation of toluene diisocyanate (TDI) and 4,4′-methylenediphenyl diisocyanate (MDI), Mutat. Res. 723 (2011) 1–10.
[135] K. Eckhardt, M. King, E. Gocke, D. Wild, Mutagenicity study of Remsen-Fahlberg saccharin and contaminants, Toxicol. Lett. 7 (1980) 51–60.
[136] J.L. Oud, P.W.J. Peters, A sequential screening of the cytogenetic damage induced by triaziquone, Mutat. Res. 54 (1978) 175–184.
[137] OECD SIDS, 1,1,2-Trichloroethane, 79-00-5, 2002, Available at http://webnet.oecd.org/HPV/UI/handler.axd?id=27dc50b4-f7fc-47e0-905c-10d61a673fc0 (accessed 26.09.15).
[138] E. Janik-Spiechowicz, K. Wyszynska, E. Dziubaltowska, Genotoxicity evaluation of trimethylbenzenes, Mutat. Res. 412 (1998) 299–305.
[139] W.R. Bruce, J.A. Heddle, The mutagenic activity of 61 agents as determined by the micronucleus, Salmonella, and sperm abnormality assays, Can. J. Genet. Cytol. 21 (1979) 319–334.
[140] S.G. Kim, Y.-J. Surh, J.A. Miller, Inhibitory effects of chlorophyllin on micronucleus formation induced by ethyl carbamate and its proximate and ultimate carcinogenic forms in mouse peripheral reticulocytes, Environ. Mol. Mutagen. 34 (1999) 57–60.
[141] C. Bevan, D.A. Keller, A.S. Panepinto, K.S. Bentley, Effect of 4-vinylcyclohexene on micronucleus formation in the bone marrow of rats and mice, Drug Chem. Toxicol. 24 (2001) 273–285.
[142] Z. Ouanes, S. Abid, I. Ayed, R. Anane, T. Mobio, E.E. Creppy, H. Bacha, Induction of micronuclei by Zearalenone in Vero monkey kidney cells and in bone marrow cells of mice: protective effect of Vitamin E, Mutat. Res. 538 (2003) 63–70.
[143] R. Crebelli, A. Zijno, L. Conti, B. Crochi, P. Leopardi, F. Marcon, L. Renzi, A. Carere, Further in vitro and in vivo mutagenicity assays with thiram and ziram fungicides: bacterial reversion assays and mouse micronucleus test, Teratog. Carcinog. Mutagen. 12 (1992) 97–112.
[144] The Joint FAO/WHO Meeting on Pesticide Residues (JMPR), Monographs of Toxicological Evaluations, Zineb (Pesticide Residues in Food 1993 Evaluations Part II Toxicology), 1993, Available at http://www.inchem.org/documents/jmpr/jmpmono/v93pr19.htm (accessed 26.09.15).
[145] M. De Boeck, B.J. Van der Leede, F. Van Goethem, A. De Smedt, M. Steemans, A. Lampo, P. Vanparys, Flow cytometric analysis of micronucleated reticulocytes: time- and dose-dependent response of known mutagens in miceusing multiple blood sampling, Environ. Mol. Mutagen. 46 (2005) 30–42.
[146] J. Ashby, M. Ishidate Jr., G.D. Stoner, M.A. Morgan, F. Ratpan, R.D. Callander, Studies on the genotoxicity of beryllium sulphate in vitro and in vivo, Mutat. Res. 240 (1990) 217–225.
[147] T. Tsuchimoto, B.E. Matter, Activity of coded compounds in the micronucleus test, Prog. Mutat. Res. 1 (1981) 705–711.
[148] M. Ishidate Jr., Y. Odagiri, Negative micronucleus tests on caprolactam and benzoin in ICR/JCL male mice, Mutat. Res. 224 (1989) 357–359.
[149] J. Grawe, M. Nusse, I.-D. Adler, Quantitative and qualitative studies of micronucleus induction in mouse erythrocytes using flow cytometry. I. Measurement of micronucleus induction in peripheral blood polychromatic erythrocytes by chemicals with known and suspected genotoxicity, Mutagenesis 12 (1997) 1–8.
[150] R. Mittal, P.A. Patil, S.S. Torgal, Screening of codeine, dextromethorphan and dextropropoxyphene for their genotoxicity in Swiss albino mice, Indian J. Med. Res. 129 (2009) 676–680.
[151] The European agency for the evaluation of medical products, Veterinary medicines and inspections, Committee for veterinary medicinal products, Coumafos, Summary Report (2), EMEA/MRL/769/00-FINAL, 2001, Available at http://www.ema.europa.eu/docs/en_GB/document_library/Maximum_Residue_Limits_-_Report/2009/11/WC500013015.pdf (accessed 28.09.15).
[152] A. Menargues, R. Obach, J.M. Valles, An evaluation of the mutagenic potential of cyanamide using the micronucleus test, Mutat. Res. 136 (1984) 127–129.
[153] A.T. Hariri, S.A. Moallem, M. Mahmoudi, H. Hosseinzadeh, The effect of crocin and safranalconstituents of saffron, against subacute effect of diazinon on hematological and genotoxicity indices in rats, Phytomedicine 18 (2011) 499–504.
[154] B.P. Patlolla, A.K. Patlolla, P.B. Tchounwou, Cytogenetic effects of 1,1-dichloroethane in mice bone marrow cells, Int. J. Environ. Res. Public Health 2 (2005) 101–106.
[155] OECD SIDS, 2,4-Dichlorophenol, 120-83-2, 2007, Available at http://webnet.oecd.org/HPV/UI/SIDS_Details.aspx?key=78392ccb-4242-4f2a-b59a-c4997c85cc58&idx=0 (accessed 26.09.15).
[156] J.L. Antoine, J. Arany, A. Leonard, J. Henrotte, G. Jenar-Dubuisson, G. Decat, Lack of mutagenic activity of dimethylformamide, Toxicology 26 (1983) 207–212.
[157] J.P. Seiler, Herbicidal phenylalkylureas as possible mutagens I. Mutagenicity tests with some urea herbicides, Mutat. Res. 58 (1978) 353–359.
[158] F. Pacchierotti, R. Ranaldi, U. Eichenlaub-Ritter, S. Attia, I.-D. Adler, Evaluation of aneugenic effects of bisphenol A in somatic and germ cells of the mouse, Mutat. Res. 651 (2008) 64–70.
[159] R.C. Chaubey, B.R. Kavi, P.S. Chauhan, K. Sundaram, The effect of hycanthone and maleic hydrazide on the frequency of micronuclei in the bone-marrow erythrocytes of mice, Mutat. Res. 57 (1978) 187–191.
[160] IPCS, CICAD No. 4, Methyl Methacrylate, 1998, Available at http://www.who.int/ipcs/publications/cicad/en/cicad04.pdf (accessed 26.09.15).
[161] M.F. Siddiqui, R. Ahmad, W. Ahmad, A. Hasnain, Micronuclei induction and chromosomal aberrations in rattus norvegicus by chloroacetic acid and chlorobenzene, Ecotoxicol. Environ. Saf. 65 (2006) 159–164.

[162] L. Soler-Niedziela, X. Shi, J. Nath, T. Ong, Studies on three structurally related phenylenediamine with the mouse micronucleus assay system, Mutat. Res. 259 (1991) 43–48.
[163] D. Blitek, K. Pieńkowska, H. Gajcy, J. Koziorowska, Mutagenicity of oxytetracycline, Mutat. Res. 117 (1983) 193–199.
[164] G. Stemp, S. Pascoe, D. Gatehouse, In vitro and in vivo cytogenetic studies of three beta-lactam antibiotics (penicillin VK, ampicillin and carbenicillin), Mutagenesis 4 (1989) 439–445.
[165] L.H. Pottenger, L.A. Malley, M.S. Bogdanffy, E.M. Donner, P.B. Upton, Y. Li, V.E. Walker, J.R. Harkema, M.I. Banton, J.A. Swenberg, Evaluation of effects from repeated inhalation exposure of F344 rats to high concentrations of propylene, Toxicol. Sci. 97 (2007) 336–347.
[166] S.M. Amer, E.I. Aboul-ela, Cytogenetic effects of pesticides III. Induction of micronuclei in mouse bone marrow by the insecticides cypermethrin and rotenone, Mutat. Res. 155 (1985) 135–142.
[167] R. Jung, C. Cojocel, W. Muller, D. Bottger, E. Luck, Evaluation of the genotoxic potential of sorbic acid and potassium sorbate, Food Chem. Toxicol. 30 (1992) 1–7.
[168] T.H. Connor, J. Meyne, M.S. Legator, The mutagenic evaluation of tetrakis (hydroxymethyl) phosphonium sulfate using a combined testing protocol approach, J. Environ. Pathol. Toxicol. 4 (1980) 145–158.
[169] J.S. Chao, L.Y. Wei, M.C. Huang, S.C. Liang, H.H. Chen, Genotoxic effects of triphenyltin acetate and triphenyltin hydroxide on mammalian cells in vitro and in vivo, Mutat. Res. 444 (1999) 167–174.

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シリーズ：日本毒性学会との連携

基礎から臨床へのトランスレーショナルトキシコロジー （第4回）

LD50値による毒性評価手法の変遷

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はじめに

化学物質による中毒とは，生体内に入った化学物質やその代謝物によって病態や機能障害が生じることとされる。急性中毒による帰結のうちもっとも重篤なものが「致死・死亡」といえる。そのため，化学物質の急性致死毒性を定量化したLD50値（半数致死量）は，さまざまな種類の化学物質の致死毒性の強さの定量的比較を可能とし，医療処置や化学物質管理に利用されてきた。しかしながら，実験動物を用いるLD50値の厳密な測定は，その科学的有用性（半数致死用量の意義，すなわち数値の実験間差や用いたげっ歯類の種差・系統差を考慮したヒトへの外挿性）ならびに動物福祉（多数の動物をその致死を指標として使用）の観点から疑問が呈された。それを受け，時代背景ならびに科学的進展を踏まえ，LD50値の概念および測定方法は従来とは異なったものとなった。

本稿ではLD50値による毒性評価手法の変遷を概説し，“LD50値”に対する認識について基礎と臨床の相互理解を図りたい。

I　LD50値と毒性試験ガイドライン

急性毒性試験に関する最初のOECD試験ガイドライン（TG）401（急性経口毒性）は1981年に採択された。その目的は，化学物質を経口経路により短期間曝露したときに生じる可能性のある健康障害の情報を得ることにあり，症状と死亡を観察する。実際には，死亡用量の特定に重みが置かれ，投与した動物の半数に死亡を引き起こすと期待される統計学的に得られた被験物質の1回投与量，すなわちLD50値を求めることとされた。統計学的評価を可能とするために，試験は通常，1群雌雄各5匹の動物を用い，6群（5用量段階および溶媒対照）を設けて実施し，プロビット法などを用いてLD50値を算出した。そのため1回の試験で60匹の動物を使用した。

一方，医薬品については，1984年2月15日付薬審第118号別添「医薬品のための毒性試験法ガイドライン」にて「急性毒性試験」としてOECD TG401と類似の内容が示されていた。しかしながら，1989年9月11日付薬審1第24号にて，急性毒性試験は「単回投与毒性試験」に改称され，その目的は，被験物質を哺乳動物に1回投与したときの毒性を質的量的に明らかにすることであった[1]。そこでは，使用するげっ歯類は1種でよく，また，LD50値ではなく概略の致死量を求めればよいとされた。そして，概略の致死量とは，「いくつかの異なる用量で観察された死亡の発現率から推定されるものであり，従来行われてきたような多数の動物を用いる計算法は必要としない，との意味である」とされた。しかしながら，使用動物数は，げっ歯類では1群5匹以上であった。

さらに，ICH（日米欧三極医薬品承認審査ハーモナイゼーション国際会議）での議論を受け，1993年

本稿は，第37回日本中毒学会「合同シンポジウム：臨床中毒学と毒性学―そのコラボレーション」における講演をもとに執筆した。

Table 1　急性経口毒性試験に関する新旧OECD毒性試験ガイドラインの比較

項目	OECD TG401	OECD TG423
方法	従来法	毒性等級法
採択年	1981	2001
動物種(げっ歯類)	ラット優先	ラット優先
1群の動物数と性	雌雄各5匹	雌3匹
用量段階	3用量以上	初回の規定投与量から2～4ステップ
一般的使用動物数	40～60匹	5～20匹
目的	LD 50値の決定	死亡が予想される特定の用量範囲の決定

8月10日には薬新薬第88号によりこの単回投与毒性試験ガイドラインが改正された。すなわち，動物数に関する規定が削除されるとともに，LD50値を求める必要のないことをより明確にするため，概略の致死量について「いくつかの異なる用量で観察された動物の生死及び毒性の徴候から判断されるおおよその最小致死量を意味するもの」と説明された[2]。この医薬品安全性評価におけるLD50値の概念変更の影響は，一般化学物質にも波及し，OECDでは，科学的進展や評価法の変化を踏まえ，2001年12月17日に急性経口毒性試験に関する新しいガイドラインTG420(固定用量法)やTG423(毒性等級法)が採択され，これに伴いTG401は2002年12月20日に廃止された。このため，欧州では2003年以降，動物愛護の観点からTG401による試験結果は受け入れ不可とされた。

さらに，2006年3月23日にはTG425(上げ下げ法)が新たな急性経口毒性試験法として採択された。TG401に代わるこれらの試験法では，より少ない動物数で評価が可能となっている。例えばTG423急性経口毒性(毒性等級法)では，その目的は，死亡が予想される特定の用量範囲の決定にあり，まず，被験物質を既定投与用量(5，50，300，2,000 mg/kg)から1用量を選択し，雌3匹(通常ラット)に投与する。次に，その結果に基づき段階的に試験を実施し，2匹以上死亡の場合は用量を下げ，それ以外は用量を上げて，同様の処置を行い，同一投与量による2回の試験での死亡が計6匹中1匹以下になる用量を求めLD50値の範囲を推定する。本法では，平均5～20匹の動物で評価可能とされている(Table 1)。雌動物が選択された理由は，従来のLD50値を検証した結果，雄より若干感度が高いことが示されたこと，ならびに雄の過剰供給の抑制につながるとされたことによる[3]。なお，TG401をはじめとするOECD毒性試験ガイドラインの翻訳は，国立医薬品食品衛生研究所のホームページ(http://www.nihs.go.jp/hse/chem-info/oecdindex.html)から参照できる。

Ⅱ　LD50値とGHS区分ならびに毒物・劇物との関係

GHS(Globally Harmonized System of Classification and Labeling of Chemicals，化学品の分類および表示に関する世界調和システム)とは，2003年7月に採択された国連勧告で，化学物質の危険有害性情報の国際的に調和された分類・表示方法を規定している[4]。その目的は健康の維持，環境の保護，貿易の促進にあり，GHSの利用対象者は消費者，労働者，輸送担当者および緊急時対応者(医療関係者を含む)である。

GHSでは，欧州，米国および日本で異なっていた経口急性毒性における“Toxic(劇物)”表示の上限値(それぞれLD50値で200 mg/kg，500 mg/kgおよび300 mg/kg)を300 mg/kgに統一し，有害性分類を用量範囲による区分として設定した。すなわち，経口では区分1を5 mg/kg以下，区分2を5～50 mg/kg，区分3を50～300 mg/kg，区分4を300 mg/kg超と定め，例えば吸入(気体の場合)では，区分1を100 ppm以下，区分2を100～500 ppm，区分3を500～2,500 ppm，区分4を2,500～20,000 ppm

Table 2　GHS急性毒性区分と急性毒性推定値(ATE)ならびに毒物・劇物との関係

曝露経路	急性毒性値(LD/LC50値)あるいはATE					
	GHS	区分1	区分2	区分3	区分4	区分5
	毒劇法	毒物	毒物	劇物	普通物	普通物
経口(mg/kg)		≦5	≦50	≦300	≦2,000	≦5,000
経皮(mg/kg)		≦50	≦200	≦1,000	≦2,000	-
吸入：気体(ppm/4 hr)		≦100	≦500	≦2,500	≦20,000	-
吸入：蒸気(mg/L/4 hr)		≦0.5	≦2.0	≦1,000	≦20	-
吸入：粉塵・ミスト(mg/L/4 hr)		≦0.05	≦0.5	≦1.0	≦5	-

と定めた。その結果，経口，経皮および吸入急性毒性ともに，GHS区分1あるいは区分2に該当するものがわが国における毒物に相当し，GHS区分3に該当するものがわが国における劇物に相当することとなった(Table 2)。

なお，GHSでは，区分の規定指標に急性毒性値あるいは急性毒性推定値(Acute Toxicity Estimate value，ATE値)を用いており，これは既存のLD50値ならびにOECD毒性試験ガイドラインなどに準拠した毒性範囲試験で得た急性毒性範囲値に基づいている。このATE値は，医薬品における概略の致死量(おおよその最小致死量)ともほぼ同義であり，したがって，現在の急性毒性試験は，このGHS区分を求める試験デザインとなっている。GHSによって化学物質固有の有害性を世界共通基準で分類することが可能となり，公衆衛生への寄与が期待されている。

Ⅲ　LD50値と毒薬・劇薬の関係

毒薬および劇薬はともに医薬品であることから，その扱い(指定基準，管理・表示方法等)は毒物および劇物とは異なる。

1998年3月12日の中央薬事審議会常任部会にて了承された毒薬・劇薬の指定基準では，概略の致死量(薬審1第24号に準拠)が，①経口投与の場合，毒薬が30 mg/kg以下，劇薬が300 mg/kg以下の値を示すもの，②皮下投与の場合，毒薬が20 mg/kg以下，劇薬が200 mg/kg以下の値を示すもの，③静脈内(腹腔内)投与の場合，毒薬が10 mg/kg以下，劇薬が100 mg/kg以下の値を示すもの，とされている[5]。経口における基準毒性値が毒物(50 mg/kg以下)と毒薬(30 mg/kg以下)とで異なるほか，経口経路以外の評価曝露経路が毒物・劇物での経皮，吸入に対し，毒薬・劇薬では皮下，静脈内(腹腔内)となっている。注意すべきは，毒性値の概念は毒物・劇物，毒薬・劇薬ともにLD50値ではなく概略の致死量となっていることである。

なお，医薬品は，基本的には医師の指導・管理のもと，利用者(患者)が意図して摂取するものであり，また，各国で独自の規制が取られていることから，GHS対象の例外となっている。

ま と め

化学物質や医薬品の急性致死毒性の評価ならびに分類は，基本的にはLD50値に基づいている。しかしながら，動物愛護の問題を受け，急性毒性試験はLD50値を求める試験から概略の致死量(あるいはLD50値の範囲)を求める試験へとデザインが変更され，もはやLD50値は求められなくなった。すなわち，急性致死毒性による評価自体は変わっていないが，LD50値の概念が特定の値から範囲値へ変化したといえる。

今後は，「物質AとBの急性経口毒性はそれぞれGHS区分3およびGHS区分4だから，物質Aに対する懸念が高く，適切に管理する必要がある」というように利用されていくであろう。GHS区分を利用して，化学物質を適正に管理・使用することが，事故を軽減し，国民の健康につながるものと考える。

【文　献】

1)　厚生省薬務局：医薬品の製造(輸入)承認申請に必要な毒性試験のガイドラインについて，薬務局審査第一・審査第二・生物製剤課長連名通知，薬審一第二四号，平成元年9月11日．
https://www.pmda.go.jp/files/000205438.pdf