Table 1 (*Continued*)

CGX ID	C/NC	Chemical name	CAS no.	MW	CA (original call)	Equiv. to 10 mM (mg/mL)	LEC (mg/mL)	LEC (mM)	Ref.	1997-OECD[a]	r-OECD[b]	ICH[c]
										CA	CA	CA
101	C	*Ethyl methanesulphonate*	*62-50-0*	*124.2*	+	*1.24*	*3.00E-06*	*0.000024*	*17*	+	+	+
102	C	*N-Ethyl-N'-nitro-N-nitrosoguanidine*	*63885-23-4*	*161.1*	+	*1.61*	*0.0025*	*0.016*	*17*	+	+	+
103	C	*1-Ethyl-1-nitrosourea*	*759-73-9*	*117.1*	+	*1.17*	*0.0117*	*0.1*	*17*	+	+	+
104	C	*5-Fluorouracil*	*51-21-8*	*130.1*	+	*1.30*	*0.001*	*0.008*	*17*	+	+	+
105	C	*Formaldehyde*	*50-00-0*	*30.0*	+	*0.30*	*0.006*	*0.2*	*17*	+	+	+
106	C	Fumonisin B1	116355-83-0	721.8	+	7.22	0.001	0.0014	30	+	+	+
107	C	*Furan*	*110-00-9*	*68.1*	+	*0.68*	*0.16*	*2.35*	*22*	+	+	- (+)
108	C	*Furfural*	*98-01-1*	*96.1*	+	*0.96*	*0.2*	*2.08*	*22*	+	+	- (+)
109	C	Furosemide	54-31-9	330.7	+	3.31	2	6	17	+	+	-
110	C	Furylfuramide (AF-2)	3688-53-7	248.2	+	2.48	0.005	0.02	17	+	+	+
111	C	*Glycidol*	*556-52-5*	*74.1*	+	*0.74*	*0.03*	*0.4*	*17*	+	+	+
112	C	Griseofulvin	126-07-8	352.8	+	3.53	0.04	0.11	17	+	+	+
113	C	Haloperidol	52-86-8	375.9	+	3.76	0.01	0.026	31	+	+	+
114	C	HC Blue 1 (impure and purified)	2784-94-3	255.3	+	2.55	0.96	3.76	20	+	+	-
115	C	Heptachlor	76-44-8	373.3	+	3.73	0.025	0.07	22	+	+	+
116	C	*Hexanamide*	*628-02-4*	*115.2*	+	*1.15*	*4*	*34.73*	*22*	-	-	-
117	C	*Hydrazine sulphate*	*10034-93-2*	*130.1*	+	*1.30*	*0.158*	*1.2*	*17*	+	+	- (+)
118	C	*Hydrazobenzene*	*122-66-7*	*184.2*	+	*1.84*	*0.0014*	*0.01*	*22*	+	+	+
119	C	*Hydrogen peroxide*	*7722-84-1*	34.0	+	0.34	0.00034	0.01	*17*	+	+	+
120	C	N-Hydroxy-2-acetylaminofluorene	53-95-2	239.3	+	2.39	0.001	0.0042	17	+	+	+
121	C	*Isobutyl nitrite*	*542-56-3*	*103.1*	+	*1.03*	*0.051*	*0.49*	*22*	+	+	+
122	C	*Isoniazid*	*54-85-3*	*137.1*	+	*1.37*	*0.44*	*3.2*	*17*	+	+	- (+)
123	C	*Isophorone*	*78-59-1*	*138.2*	+	*1.38*	*1.25*	*9.044*	*28*	+	+	- (+)
124	C	Lasiocarpine	303-34-4	411.5	+	4.12	0.206	0.5	17	+	+	+
125	C	Lead acetate	301-04-2	325.3	+	3.25	0.0033	0.01	17	+	+	+
126	C	Manganese ethylenebisthiocarbamate	12427-38-2	265.3	+	2.65	0.015	0.057	17	+	+	+
127	C	Melphalan	148-82-3	305.2	+	3.05	0.0001	0.0033	17	+	+	+
128	C	*2-Mercaptobenzothiazole*	*149-30-4*	*167.2*	+	*1.67*	*0.374*	*2.23*	*22*	+	+	- (+)
129	C	Methapyrilene hydrochloride	135-23-9	297.8	+	2.98	0.747	2.51	22	+	+	-
130	C	*Methimazole*	*60-56-0*	*114.2*	+	*1.14*	*0.37*	*3.2*	*17*	+	+	- (+)
131	C	*4-Methoxyphenol*	*150-76-5*	*124.1*	+	*1.24*	*0.031*	*0.25*	*32*	+	+	+
132	C	8-Methoxypsoralen	298-81-7	216.2	+	2.16	0.1	0.46	22	+	+	+
133	C	*Methylazoxymethanol acetate*	*592-62-1*	*132.1*	+	*1.32*	*0.00013*	*0.001*	*17*	+	+	+
134	C	*alpha-Methylbenzyl alcohol*	*98-85-1*	*122.2*	+	*1.22*	*1*	*8.19*	*22*	+	+	- (+)
135	C	3-Methylcholanthrene	56-49-5	268.3	+	2.68	0.002	0.0075	17	+	+	+
136	C	3'-Methyl-4-dimethylaminoazobenzene	55-80-1	239.3	+	2.39	0.05	0.21	17	+	+	+
137	C	4,4'-Methylenedianiline 2HCl	13552-44-8	271.2	+	2.71	0.8	2.95	22	+	+	-
138	C	*Methyl methanesulphonate*	*66-27-3*	*110.1*	+	*1.10*	*3.00E-06*	*0.000027*	*17*	+	+	+
139	C	2-Methyl-1-nitroanthraquinone	129-15-7	267.2	+	2.67	0.005	0.02	22	+	+	+
140	C	*N-Methyl-N'-nitro-N-nitrosoguanidine*	*70-25-7*	*147.1*	+	*1.47*	*3.00E-06*	*0.00002*	*17*	+	+	+
141	C	*Methylnitrosocyanamide*	*33868-17-6*	*85.1*	+	*0.85*	*0.00085*	*0.01*	*17*	+	+	+
142	C	*N-Methylolacrylamide*	*924-42-5*	*101.1*	+	*1.01*	*0.25*	*2.47*	*22*	+	+	- (+)
143	C	Methylphenidate HCl	298-59-9	267.0	+	2.67	1	3.71	18	+	+	-
144	C	*Metronidazole*	*443-48-1*	*171.2*	+	*1.71*	*0.0001*	*0.0006*	*33*	+	+	+
145	C	Mitomycin C	50-07-7	334.3	+	3.34	0.00017	0.00005	17	+	+	+
146	C	Monocrotaline	315-22-0	325.4	+	3.25	0.065	0.2	17	+	+	+
147	C	Nafenopin	3771-19-5	310.4	+	3.10	0.0093	0.03	29	+	+	+
148	C	*Naphthalene*	*91-20-3*	*128.2*	+	1.28	*0.03*	*0.23*	*22*	+	+	+
149	C	*1,5-Naphthalenediamine*	*2243-62-1*	*158.2*	+	1.58	*0.001*	*0.01*	*22*	+	+	+
150	C	*2-Naphthylamine*	*91-59-8*	*143.2*	+	1.43	0.00333	*0.023*	*17*	+	+	+
151	C	*Nitrite sodium*	*7632-00-0*	*69.0*	+	0.69	*4*	*58.0*	*17*	-	-	-
152	C	*o-Nitroanisole*	*91-23-6*	*153.1*	+	1.53	*1.06*	*6.92*	*18*	+	+	- (+)

153	*C*	*Nitrobenzene*	*98-95-3*	*123.1*	+	1.23	*6.15*	*50*	*34*	-	-	-
154	*C*	*6-Nitrobenzimidazole*	*94-52-0*	*163.1*	+	1.63	*0.5*	*3.06*	*22*	+	+	- (+)
155	*C*	*p-Nitrobenzoic acid*	*62-23-7*	*167.1*	+	1.67	*0.875*	*5.24*	*22*	+	+	- (+)
156	*C*	*5-Nitro-2-furaldehyde semicarbazone*	*59-87-0*	*198.1*	+	1.98	*0.023*	*0.12*	*22*	+	+	+
157	C	1-[(5-nitrofurfurylidene)amino]hydantoin	67-20-9	238.2	+	2.38	0.747	3.14	22	+	+	-
158	*C*	*Nitrogen mustard*	*51-75-2*	*156.1*	+	*1.56*	*0.00002*	*0.0001*	*17*	+	+	+
159	*C*	*2-Nitro-p-phenylenediamine*	*5307-14-2*	*153.1*	+	*1.53*	*0.3*	*1.96*	*22*	+	+	- (+)
160	C	1-Nitropyrene	5522-43-0	247.2	+	2.47	0.1	0.404	35	+	+	+
161	*C*	*4-Nitroquinoline-N-oxide*	*56-57-5*	*190.2*	+	*1.90*	*0.00002*	*0.00011*	*17*	+	+	+
162	*C*	*p-Nitrosodiphenylamine*	*156-10-5*	*198.2*	+	*1.98*	*0.00025*	*0.0013*	*22*	+	+	+
163	*C*	*N-Nitrosodiethylamine (diethylnitrosamine)*	*55-18-5*	*102.1*	+	*1.02*	*3*	*29*	*17*	-	-	-
164	*C*	*N-Nitrosodimethylamine (dimethylnitrosamine)*	*62-75-9*	*74.1*	+	*0.74*	*0.5*	*6.7*	*17*	+	+	- (+)
165	*C*	*N-Nitroso-N-methylurea*	*684-93-5*	*103.1*	+	*1.03*	*0.01*	*0.1*	*17*	+	+	+
166	*C*	*5-Nitro-o-toluidine*	*99-55-8*	*152.2*	+	*1.52*	*0.5*	*3.29*	*22*	+	+	- (+)
167	C	4,4'-Oxydianiline	101-80-4	200.2	+	2.00	0.1	0.50	22	+	+	+
168	C	*N*-Oxydiethylene thiocarbamyl-*N*-oxydiethylene sulphenamide	13752-51-7	248.4	+	2.48	0.005	0.02	17	+	+	+
169	C	Pentachloroethane	76-01-7	202.3	+	2.02	0.008	0.395	28	+	+	+
170	C	Pentachloronitrobenzene	82-68-8	295.3	+	2.95	0.0024	0.01	22	+	+	+
171	C	Petasitenine	60102-37-6	381.4	+	3.81	1.91	5	17	+	+	-
172	*C*	*Phenacetin*	*62-44-2*	*179.2*	+	*1.79*	*0.4*	*2.2*	*17*	+	+	- (+)
173	C	Phenazopyridine HCl	136-40-3	249.7	+	2.50	0.105	0.42	22	+	+	+
174	C	Phenobarbital	50-06-6	232.2	+	2.32	0.1	0.43	17	+	+	+
175	C	Phenolphthalein	28-37-6	318.3	+	3.18	0.05	0.16	22	+	+	+
176	C	Phenoxybenzamine HCl	63-92-3	340.3	+	3.40	0.03	0.09	22	+	+	+
177	C	Phenylbutazone	50-33-9	308.4	+	3.08	1.6	5.19	18	+	+	-
178	*C*	*o-Phenylphenol*	*90-43-7*	*170.2*	+	*1.70*	*0.1*	*0.59*	*25*	+	+	+
179	*C*	*Propane sultone*	*1120-71-4*	*122.1*	+	*1.22*	*0.012*	*0.1*	*17*	+	+	+
180	*C*	*beta-Propiolactone*	*57-57-8*	*72.1*	+	*0.72*	*0.03*	*0.42*	*17*	+	+	+
181	*C*	*1,2-Propylene oxide*	*75-56-9*	*58.1*	+	*0.58*	*0.5*	*8.61*	*22*	+	+	- (+)
182	*C*	*N-Propyl-N'-nitro-N-nitrosoguanidine*	*13010-07-6*	*175.2*	+	*1.75*	*0.01*	*0.057*	*17*	+	+	+
183	C	Pyrimethamine	58-14-0	248.7	+	2.49	0.05	0.201	36	+	+	+
184	C	Quercetin	117-39-5	302.2	+	3.02	0.006	0.02	17	+	+	+
185	*C*	*p-Quinone dioxime*	*105-11-3*	*138.1*	+	*1.38*	*0.01*	*0.07*	*22*	+	+	+
186	C	Retinol acetate	127-47-9	328.5	+	3.29	0.0656	0.2	23	+	+	+
187	C	Saccharin, sodium	128-44-9	205.2	+	2.05	8	39	17	-	-	-
188	*C*	*Safrole*	*94-59-7*	*162.2*	+	*1.62*	*0.0833*	*0.5*	*17*	+	+	+
189	*C*	*Selenium sulphide*	*7446-34-6*	*111.0*	+	*1.11*	*0.0005*	*0.0045*	*20*	+	+	+
190	C	Sodium dichromate	10588-01-9	262.0	+	2.62	0.0001	0.0019	17	+	+	+
191	*C*	*Styrene*	*100-42-5*	*104.2*	+	*1.04*	*0.25*	*2.4*	*17*	+	+	- (+)
192	*C*	*Styrene oxide*	*96-09-3*	*120.2*	+	*1.20*	*0.00375*	*0.031*	*17*	+	+	+
193	*C*	*1,1,1,2-Tetrachloroethane*	*630-20-6*	*167.8*	+	*1.68*	*0.1*	*0.596*	*28*	+	+	+
194	C	12-O-tetradecanoylphorbol 13-acetate	16561-29-8	616.8	+	6.17	6.20E-06	0.00001	17	+	+	+
195	*C*	*Tertanitromethane*	*509-14-8*	*196.0*	+	*1.96*	*0.02*	*0.10*	*22*	+	+	+
196	C	4,4'-Thiodianiline	139-65-1	216.3	+	2.16	0.1	0.46	22	+	+	+
197	*C*	*Thio-tepa*	*52-24-4*	*189.2*	+	*1.89*	*0.00094*	*0.0049*	*17*	+	+	+
198	*C*	*o-Toluidine*	*95-53-4*	*107.2*	+	*1.07*	*0.012*	*0.13*	*17*	+	+	+
199	C	Trenimon	68-76-8	231.3	+	2.31	1.00E-08	4.3E-08	17	+	+	+
200	C	Triamterene	396-01-0	253.3	+	2.53	0.00375	0.015	17	+	+	+
201	C	Tribromomethane	75-25-2	252.7	+	2.53	0.116	0.46	17	+	+	+
202	*C*	*1,1,2-Trichloroethane*	*79-00-5*	*133.4*	+	*1.33*	*0.377*	*2.83*	*22*	+	+	- (+)
203	C	*N*-(Trichloromethylthio)phthalimide	133-07-3	296.6	+	3.00	0.005	0.017	37	+	+	+
204	*C*	*1,2,3-Trichloropropane*	*96-18-4*	*147.4*	+	*1.47*	*0.0595*	*0.40*	*22*	+	+	+

Table 1 (*Continued*)

CGX ID	C/NC	Chemical name	CAS no.	MW	CA (original call)	Equiv. to 10 mM (mg/mL)	LEC (mg/mL)	LEC (mM)	Ref.	1997-OECD[a]	r-OECD[b]	ICH[c]
										CA	CA	CA
205	C	*2,4,5-Trimethylaniline*	*137-17-7*	*135.2*	+	*1.35*	*0.415*	*3.07*	*20*	+	+	- (+)
206	C	*Trimethylphosphate*	*512-56-1*	*140.1*	+	*1.40*	*3*	*21.42*	*22*	-	-	-
207	C	Tris(2,3-dibromopropyl)phosphate	126-72-7	697.9	+	6.98	0.125	0.18	17	+	+	+
208	C	*Urethane*	*51-79-6*	*89.1*	+	*0.89*	*8*	*90*	*17*	-	-	-
209	C	Zearalenone	17924-92-4	318.4	+	3.18	0.015	0.05	22	+	+	+
210	C	Zinc dimethyldithioearbamate (Ziram)	137-30-4	305.8	+	3.06	0.000025	0.00008	22	+	+	+
211	NC	Acetohexamide	968-81-0	324.4	+	3.24	2	6	17	+	+	-
212	*NC*	*o-Anthranilic acid*	*118-92-3*	*137.1*	+	*1.37*	*4*	*29.2*	*22*	-	-	-
213	*NC*	*Benzoate, sodium*	*532-32-1*	*144.1*	+	*1.44*	*0.29*	*2*	*17*	+	+	- (+)
214	NC	Benzoin	119-53-9	212.2	+	2.12	0.02	0.1	17	+	+	+
215	*NC*	*1H-Benzotriazole*	*95-14-7*	*119.1*	+	*1.19*	*1.257*	*10.55*	*22*	-	-	-
216	*NC*	*Benzyl alcohol*	*100-51-6*	*108.1*	+	*1.08*	*4*	*36.99*	*22*	-	-	-
217	*NC*	*Caffeine*	*58-08-2*	*194.2*	+	*1.94*	*0.08*	*0.4*	*17*	+	+	+
218	NC	Carbromal	77-65-6	237.1	+	2.37	1	4.22	22	+	+	-
219	NC	4-(Chloroacetyl)-acetanilide	140-49-8	211.6	+	2.12	0.0025	0.01	22	+	+	+
220	*NC*	*p-Chloroaniline*	*106-47-8*	*127.6*	+	*1.28*	*0.5*	*3.92*	*22*	+	+	- (+)
221	*NC*	*o-Chlorobenzalmalonitrile*	*2698-41-1*	*188.6*	+	*1.89*	*0.006*	*0.03*	*22*	+	+	+
222	*NC*	*2-(Chloromethyl)pyridine HCl*	*6959-47-3*	*164.0*	+	*1.64*	*0.0302*	*0.18*	*22*	+	+	+
223	NC	Chlorpheniramine maleate	113-92-8	390.9	+	3.91	0.5	1.28	22	+	+	-
224	NC	Chlorpropamide	94-20-2	276.7	+	2.77	1	3.6	17	+	+	-
225	NC	C.I. acid orange 10	1936-15-8	452.4	+	4.52	1.25	2.76	22	+	+	-
226	NC	Diallyl phthalate	131-17-9	246.3	+	2.46	0.2	0.81	22	+	+	+
227	NC	2,5-Diaminotoluen sulphate	6369-59-1	220.3	+	2.20	0.04	0.18	22	+	+	+
228	*NC*	*2,6-Diaminotoluene 2HCl*	*15481-70-6*	*195.1*	+	*1.95*	*1*	*5.13*	*22*	+	+	- (+)
229	NC	Diazinon	333-41-5	304.4	+	3.04	0.1	0.32	17	+	+	+
230	*NC*	*2,4-Dichlorophenol*	*120-83-2*	*163.0*	+	*1.63*	*0.0978*	*0.6*	*38*	+	+	+
231	NC	Dimethoate	60-51-5	229.2	+	2.29	0.5	2.2	17	+	+	-
232	*NC*	*Dimethoxane, commercial grade*	*828-00-2*	*174.2*	+	*1.74*	*0.0126*	*0.07*	*22*	+	+	+
233	*NC*	*2,4-Dimethoxyaniline HCl*	*54150-69-5*	*189.6*	+	*1.90*	*0.5*	*2.64*	*22*	+	+	- (+)
234	NC	Diphenhydramine HCl	147-24-0	291.8	+	2.92	0.1	0.34	22	+	+	+
235	NC	Diphenyl-*p*-phenylenediamine	74-31-7	260.3	+	2.60	0.001	0.0038	39	+	+	+
236	NC	Ethyl tellurac	20941-65-5	720.7	+	7.21	0.000032	0.00004	22	+	+	+
237	*NC*	*Eugenol*	*97-53-0*	*164.2*	+	*1.64*	*0.125*	*0.76*	*18*	+	+	+
238	NC	FD & C red no. 3 (MW as anhydrous)	16423-68-0	879.9	+	8.80	0.6	0.68	17	+	+	-
239	NC	FD & C yellow no. 5 [AKA tartrazine]	1934-21-0	534.4	+	5.34	2	3.7	17	+	+	-
240	NC	Fenthion	55-38-9	278.3	+	2.78	0.0015	0.005	40	+	+	+
241	NC	Fenvalerate	51630-58-1	419.9	+	4.20	0.01	0.024	41	+	+	+
242	*NC*	*Fluoride sodium*	*7681-49-4*	*42.0*	+	*0.42*	*0.02*	*0.48*	*17*	+	+	+
243	NC	Hexachlorocyclopentadiene	77-47-4	272.8	+	2.73	0.0075	0.03	22	+	+	+
244	*NC*	*8-Hydroxyquinoline*	*148-24-3*	*145.2*	+	*1.45*	*0.0058*	*0.04*	*42*	+	+	+
245	NC	4,4'-Isopropylidenediphenol	80-05-7	228.3	+	2.28	0.0912	0.4	43	+	+	+
246	NC	Lead dimethyldithiocarbamate	19010-66-3	447.6	+	4.48	0.000025	0.000056	18	+	+	+
247	NC	Lithocholic acid	434-13-9	376.6	+	3.77	0.56	1.5	17	+	+	-
248	NC	Malathion	121-75-5	330.4	+	3.30	<0.303	<0.92	18	+	+	+
249	*NC*	*Manganese(II) sulfate monohydrate*	*10034-96-5*	*169.0*	+	*1.69*	*0.18*	*1.065*	*18*	+	+	- (+)
250	NC	Methotrexate	59-05-2	454.4	+	4.54	0.001	0.0022	17	+	+	+
251	*NC*	*Methyl methacrylate*	*80-62-6*	*100.1*	+	*1.00*	*1.6*	*15.98*	*44*	-	-	-
252	NC	N-(1-Naphthyl)ethylenediamine 2HCl	1465-25-4	259.2	+	2.59	0.2	0.77	45	+	+	+
253	*NC*	*p-Nitroaniline*	*100-01-6*	*138.1*	+	*1.38*	*1.6*	*11.58*	*18*	-	-	-
254	*NC*	*4-Nitroanthranilic acid*	*619-17-0*	*182.1*	+	*1.82*	*2.2*	*12.08*	*22*	-	-	-
255	*NC*	*1-Nitronaphthalene*	*86-57-7*	*173.2*	+	*1.73*	*0.016*	*0.09*	*45*	+	+	+

256	NC	Penicillin VK	132-98-9	388.5	+	3.89	1.25	3.2	46	+	+	-	
257	NC	*Phenol*	*108-95-2*	*94.1*	+	*0.94*	*2*	*21.25*	22	-	-	-	
258	NC	*p-Phenylenediamine 2HCl*	*624-18-0*	*181.1*	+	*1.81*	*0.016*	*0.09*	45	+	+	+	
259	NC	*1-Phenyl-2-thiourea*	*103-85-5*	*152.2*	+	*1.52*	*3*	*19.71*	22	-	-	-	
260	NC	*Phthalic anhydride*	*85-44-9*	*148.1*	+	*1.48*	*1.48*	*10*	38	+	+	- (+)	
261	NC	*Resorcinol*	*108-46-3*	*110.1*	+	*1.10*	*4*	*36.33*	22	-	-	-	
262	NC	*Sodium chlorite*	*7758-19-2*	*90.4*	+	*0.90*	*0.02*	*0.22*	17	+	+	+	
263	NC	Tetracycline HCl	64-75-5	480.9	+	4.81	0.01	0.02	17	+	+	+	
264	NC	Tetraethylthiuram disulfide	97-77-8	296.5	+	2.97	5.00E-06	0.00002	22	+	+	+	
265	NC	*Tetrakis(hydoxymethyl)phosphonium chloride*	*124-64-1*	*190.6*	+	*1.91*	*0.03*	*0.16*	22	+	+	+	
266	NC	Tetrakis(hydoxymethyl)phosphonium sulphate	55566-30-8	251.2	+	2.51	0.005	0.02	44	+	+	+	
267	NC	*Tin(II) chloride*	*7772-99-8*	*189.6*	+	*1.90*	*0.025*	*0.13*	22	+	+	+	

C, Carcinogen; NC, Non-carcinogen; MW, Molecular weight; CA, Chromosomal aberration test; LEC, Lowest effective concentration; Equivalent to 10 mM means the equal concentration of weight per volume (mg/mL) to 10 mM.
+, Positive; –, Negative.
(+) shows positive after the appication of the r-OECD TGfor the chemicals MW less than 200 (n = 46).
Italics means chemicals MW less than 200 (n = 142).
Highilight to the negative result by the re-evaluation.
[a] Current OECD test guideline adopted in 1997 (10 mM or 5 mg/mL whichever is lower).
[b] Draft revised OECD test guideline (10 mM or 2 mg/mL whichever is lower).
[c] ICH S2(R1) guideline (1 mM or 0.5 mg/mL whichever is lower).

approach, because there were no carcinogenicity data for nearly all the 124-CA positives from the JEC database. This approach consisted of the identification of effects from extreme culture conditions (*e.g.*, low pH, precipitation, cytotoxicity) and a review of the literature (*e.g.*, *in vivo* genotoxicity and carcinogenicity for the chemical, and for closely related chemicals). The level of concern for 'different' chemicals – *to be used in human health-risk assessment* – was defined and based on previously described analyses [9]. The general criteria were as follows: (1) negligible concern, negative result(s) in the *in vivo* genotoxicity or carcinogenicity test, clear evidence(s) of non-relevance (*e.g.*, extreme culture condition) for CA-induction and/or mode of action of the non-DNA target; (2) minimal concern, some evidence(s) of non-relevance of CA-induction or of an increasing level of negligible concern or negative result(s) in the *in vivo* genotoxicity tests with some limitations; (3) some concern, positive result(s) in the Ames test with negative result(s) or no data in the *in vivo* genotoxicity test, positive result(s) in the *in vivo* genotoxicity or carcinogenicity test in related chemicals or no supporting evidence(s) for reducing the level of concern; and (4) real concern, positive result(s) in the Ames or *in vivo* genotoxicity tests, or when mentioned in the list of IARC carcinogens in Group 2B or higher.

2.6. Distribution of the MWs of the chemicals

The distribution of the MWs of the 267 CA-positives from the CGX database and 124 CA-positives from the JEC database was investigated.

3. Results

3.1. Sensitivity and specificity analyses

Results from the re-evaluation of 267 CA-positive chemicals (210 carcinogens and 57 non-carcinogens) from the CGX database are shown in Table 1. The results of the sensitivity and specificity analyses on the 435 chemicals, including the 168 CA-negatives from the CGX database are shown in Table 3. In addition, 267 CA-positives in the original call of the CGX database included 19 positive chemicals (10 carcinogens, *i.e.*, CGX IDs 5, 65, 95, 116, 151, 153, 164, 187, 206, 208; and nine non-carcinogens, *i.e.*, CGX IDs 212, 215, 216, 251, 253, 254, 256, 259, 260) at more than 10 mM. The IARC Group-2A agents (probable carcinogens), acrylamide (CGX ID5), *N*-nitrosodiethylamine (CGX ID163) and urethane (CGX ID208) were also included in these 10 carcinogens. The number of CA-positive chemicals was reduced to 248, 248 or 176 from the 267 chemicals in the original call when the 1997-OECD, r-OECD or ICH TG was applied, respectively. Because these chemicals were considered negative, the number of CA-negative chemicals increased to 187, 187 or 259 from 168 in the original call by the application of the 1997-OECD, r-OECD or ICH TG, respectively. The sensitivity and specificity against carcinogenicity based on the re-evaluation for the 435 chemicals from the CGX database are shown in Table 3. The sensitivity was reduced to 63.1%, 63.1% or 45.4% from 66.2% based on the original call, and the specificity had increased to 59.3%, 59.3% or 72.9% from 51.7% based on the original call, by the application of the 1997-OECD, r-OECD or ICH TG, respectively. The application of the r-OECD TG did not affect the sensitivity and specificity of the application of the 1997-OECD TG. However, the application of the ICH TG reduced sensitivity and increased specificity by approximately 15%.

3.2. Analysis of the alteration of the number of CA-positives

The results of the re-evaluation of 124 CA-positives from the JEC database are shown in Table 2. Because the 124 CA-positives by the original call in the JEC database included six positive chemicals (*i.e.*, JEC IDs 2, 11, 87, 99, 106, 111) at more than 10 mM, 118 chemicals were considered positive under the 1997-OECD TG. Alterations in the number of positive chemicals are presented in Table 4. Application of r-OECD TG showed a small reduction in the number of CA-positives (113 out of 124 chemicals by 1997-OECD TG), but ICH TG reduced this number to approximately half (60 out of 124 chemicals). Moreover, the number of CA-positive chemicals decreased remarkably upon application of the ICH TG.

Table 2
Re-evaluation of chromosomal aberration test results on the 124 CA-positive chemicals from the JEC database, based on the different top-concentration limits in several test guidelines.

JEC ID	Chemical name	CAS No.	MW	CA (original call)	Equiv. to 10 mM (mg/mL)	LEC (mg/mL)	LEC (mM)	Ref.	1997-OECD[a]	r-OECD[b]	ICH[c]
									CA	CA	CA
1	Acenaphthene	83-32-9	154.2	+	1.54	0.2	1.3	47	+	+	- (+)
2	o-Acetoacetotoluidine	93-68-5	191.2	+	1.91	2.5	13.1	47	-	-	-
3	3-Aminobenzenesulfonic acid	121-47-1	173.2	+	1.73	0.4	2.3	47	+	+	- (+)
4	2-Amino-5-chloro-4-methylbenzenesulfonic acid	88-53-9	221.5	+	2.22	2.0	9.0	47	+	+	-
5	N-(Aminoethyl)ethanolamine	111-41-1	104.2	+	1.04	1.0	9.6	47	+	+	- (+)
6	2-Amino-5-methylbenzenesulfonic acid	88-44-8	187.2	+	1.87	1.0	5.1	47	+	+	- (+)
7	2-Amino-1-naphthalenesulfonic acid	81-16-3	223.3	+	2.23	1.1	4.9	47	+	+	-
8	3-Aminophenol	591-27-5	109.1	+	1.09	0.03	0.3	47	+	+	+
9	4-Aminophenol	123-30-8	109.1	+	1.09	0.003	0.03	47	+	+	+
10	Azodicarbonamide	123-77-3	116.1	+	1.16	0.9	7.8	47	+	+	- (+)
11	Benzyltrimethylammonium chloride	56-93-9	185.7	+	1.86	1.9	10.2	47	-	-	-
12	4,4'-Biphenyldiol	92-88-6	186.2	+	1.86	0.03	0.2	47	+	+	+
13	1,3-Bis(aminomethyl)cyclohexane (mixtures of cis-, trans-)	2579-20-6	142.3	+	1.42	0.4	2.8	47	+	+	- (+)
14	1,2-Bis(2-chloroethoxy)ethane	112-26-5	187.1	+	1.87	0.06	0.3	47	+	+	+
15	Bis(1-methylethyl)naphthalene	38640-62-9	212.3	+	2.12	0.14	0.7	47	+	+	+
16	1,3-Bis(2-methylphenyl)guanidine	97-39-2	239.3	+	2.39	0.6	2.5	47	+	+	-
17	1-Bromo-3-chloropropane	109-70-6	157.4	+	1.57	0.3	1.6	47	+	+	- (+)
18	N-tert-Butyl-2-benzothiazolesulfenamide	95-31-8	238.4	+	2.38	0.2	0.8	47	+	+	+
19	tert-Butyl-methacrylate	585-07-9	142.2	+	1.42	0.4	2.8	47	+	+	- (+)
20	o-sec-Butylphenol	89-72-5	150.2	+	1.50	0.02	0.1	47	+	+	+
21	6-tert-Butyl-m-cresol	88-60-8	164.3	+	1.64	0.01	0.06	47	+	+	+
22	2-tert-Butylphenol	88-18-6	150.2	+	1.50	0.01	0.07	47	+	+	+
23	p-tert-Butylphenol	98-54-4	150.2	+	1.50	0.03	0.2	47	+	+	+
24	Cadmium nitrate tetrahydrate	10022-68-1	308.5	+	3.09	0.01	0.02	47	+	+	+
25	1-Chloro-2-(chloromethyl)benzene	611-19-8	161.0	+	1.61	0.1	0.6	47	+	+	+
26	4-Chloro-o-cresol	1570-64-5	142.6	+	1.43	0.1	0.7	47	+	+	+
27	Chloropentabromocyclohexane	87-84-3	513.1	+	5.13	0.03	0.06	47	+	+	+
28	2-Chlorophenol	95-57-8	128.6	+	1.29	0.3	2.3	47	+	+	- (+)
29	4-Chlorophenol	106-48-9	128.6	+	1.29	0.05	0.4	47	+	+	+
30	Chromic acid disodium salt dihydrate	7789-12-0	297.8	+	2.98	0.001	0.003	47	+	+	+
31	C.I. Fluorescent brightner 271	41267-43-0	1347.1	+	13.47	5.0	3.7	47	+	-	-
32	2,4-Diamino-6-phenyl-s-triazine	91-76-9	187.2	+	1.87	0.08	0.4	47	+	+	+
33	1,4-Dibromobenzene	106-37-6	235.9	+	2.36	0.6	2.5	47	+	+	-
34	1,3-Dibromopropane	109-64-8	201.9	+	2.02	0.06	0.3	47	+	+	+
35	Dibutyl adipate	105-99-7	258.4	+	2.58	0.7	2.5	47	+	+	-
36	2-(Di-n-butylamino)ethanol	102-81-8	173.3	+	1.73	0.3	1.7	47	+	+	- (+)
37	2,6-Di-tert-butyl-4-ethylphenol	4130-42-1	234.4	+	2.34	0.045	0.19	47	+	+	+
38	2,4-Di-tert-butylphenol	96-76-4	206.3	+	2.06	0.01	0.05	47	+	+	+
39	o-Dichlorobenzene	95-50-1	147.0	+	1.47	0.2	1.4	47	+	+	- (+)
40	3,4-Dichloro-1-butene	760-23-6	125.0	+	1.25	0.01	0.08	47	+	+	+
41	1,2-Dichloro-3-nitrobenzene	3209-22-1	192.0	+	1.92	0.1	0.6	47	+	+	+
42	1,4-Dichloro-2-nitrobenzene	89-61-2	192.0	+	1.92	0.15	0.8	47	+	+	+
43	α,4-Dichlorotoluene	104-83-6	161.0	+	1.61	0.0125	0.08	47	+	+	+
44	1,2-Dicyanobenzene	91-15-6	128.1	+	1.28	0.3	2.3	47	+	+	- (+)
45	Dicyclohexylamine	101-83-7	181.3	+	1.81	0.6	3.3	47	+	+	- (+)
46	N,N-Dicyclohexyl-2-benzothiazolesulfenamide	4979-32-2	346.6	+	3.47	0.2	0.6	47	+	+	+
47	2-(Diethylamino)ethyl methacrylate	105-16-8	185.3	+	1.85	0.6	3.2	47	+	+	- (+)
48	O,O'-Diethyl dithiophosphate	298-06-6	186.2	+	1.86	0.12	0.6	47	+	+	+
49	Diethyl fumarate	623-91-6	172.2	+	1.72	0.01	0.06	47	+	+	+
50	2-(Dimethylamino)ethyl acrylate	2439-35-2	143.2	+	1.43	0.05	0.3	47	+	+	+
51	2-(Dimethylamino)ethyl methacrylate	2867-47-2	157.2	+	1.57	0.6	3.8	47	+	+	- (+)
52	2,3-Dimethylaniline (2,3-Xylidine)	87-59-2	121.2	+	1.21	0.6	5.0	47	+	+	- (+)
53	2,6-Dimethylaniline (2,6-Xylidine)	87-62-7	121.2	+	1.21	0.3	2.5	47	+	+	- (+)

54	3,5-Dimethylaniline (3,5-Xylidine)	108-69-0	121.2	+	1.21	0.9	7.4	47	+	+	- (+)
55	N,N-Dimethylbenzylamine	103-83-3	135.2	+	1.35	0.4	3	47	+	+	- (+)
56	N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylenediamine	793-24-8	268.4	+	2.68	0.005	0.02	47	+	+	+
57	2,4-Dinitrophenol	51-28-5	184.1	+	1.84	1.2	6.5	47	+	+	- (+)
58	Diphenyl cresyl phosphate	26444-49-5	340.3	+	3.40	0.04	0.1	47	+	+	+
59	Disperse Red 206	26630-87-5	580.1	+	5.80	2.5	4.3	47	+	-	-
60	Disperse Yellow 42	5124-25-4	369.4	+	3.69	0.08	0.2	47	+	+	+
61	2,3-Epoxypropyl methacrylate	106-91-2	142.2	+	1.42	0.02	0.1	47	+	+	+
62	Ethenyltrimethoxysilane	2768-02-7	148.2	+	1.48	0.8	5.4	47	+	+	- (+)
63	4-Ethoxybenzeneamine (p-Phenetidin)	156-43-4	137.2	+	1.37	0.05	0.4	47	+	+	+
64	N-Ethylaniline	103-69-5	121.2	+	1.21	1.1	9.1	47	+	+	- (+)
65	2-Ethylanthraquinone	84-51-5	236.3	+	2.36	0.16	0.6	47	+	+	+
66	2-Ethylbutyric acid	88-09-5	116.2	+	1.16	0.4	3.4	47	+	+	- (+)
67	3-Ethylphenol	620-17-7	122.2	+	1.22	0.05	0.4	47	+	+	+
68	4-Ethylphenol	123-07-9	122.2	+	1.22	0.04	0.3	47	+	+	+
69	Ferrous sulfate heptahydrate	7782-63-0	278.0	+	2.78	0.5	1.8	47	+	+	-
70	Glycerol triacetate	102-76-1	218.2	+	2.18	2.2	10.0	47	+	-	-
71	Hydrazine monohydrate	7803-57-8	50.1	+	0.50	0.06	1.2	47	+	+	- (+)
72	2-Hydroxybenzaldehyde	90-02-8	122.1	+	1.22	0.1	0.8	47	+	+	+
73	4-Hydroxy-benzenesulfonic acid, tin (2+) tetrahydride	70974-33-3	465.1	+	4.65	0.528	1.1	47	+	+	-
74	4-Hydroxybenzoic acid	99-96-7	138.1	+	1.38	0.7	5.1	47	+	+	- (+)
75	2-Hydroxyethyl methacrylate	868-77-9	130.2	+	1.30	0.7	5.4	47	+	+	- (+)
76	3-Hydroxy-2-naphthalenecarboxylic acid	92-70-6	188.2	+	1.88	0.75	4.0	49	+	+	- (+)
77	2-Hydroxypropanenitrile	78-97-7	71.1	+	0.71	0.7	10.0	47	+	+	- (+)
78	2-Mercaptobenzimidazole	583-39-1	150.2	+	1.50	0.8	5.3	47	+	+	- (+)
79	Methacrylic acid, monoester with propane-1,2-diol	27813-02-1	144.2	+	1.44	0.7	4.9	47	+	+	- (+)
80	(Methacryloyloxyethyl)trimethylammonium chloride	5039-78-1	207.7	+	2.08	2.1	10.0	47	+	-	-
81	Methacrylonitrile (Methyl Acrylonitrile)	126-98-7	67.1	+	0.67	0.07	1.0	47	+	+	+
82	3-Methoxybenzeneamine	536-90-3	123.2	+	1.23	0.8	6.5	47	+	+	- (+)
83	Methoxymethanol	4461-52-3	62.1	+	0.62	0.02	0.3	47	+	+	+
84	1-Methoxynaphthalene	2216-69-5	158.2	+	1.58	0.02	0.1	47	+	+	+
85	Methyl acetoacetate	105-45-3	116.1	+	1.16	1.2	10.0	47	+	+	- (+)
86	N-Methylaniline	100-61-8	107.2	+	1.07	0.6	5.6	47	+	+	- (+)
87	3-Methylbenzoic acid	99-04-7	136.2	+	1.36	1.5	11.0	47	-	-	-
88	4-Methylbenzoic acid	99-94-5	136.2	+	1.36	1.2	8.8	47	+	+	- (+)
89	4,4'-Methylenebis(2-chloroaniline)	101-14-4	267.2	+	2.67	0.04	0.1	47	+	+	+
90	Methylenediphenol	1333-16-0	200.2	+	2.00	0.01	0.05	47	+	+	+
91	4,4'-Methylenediphenol	620-92-8	200.2	+	2.00	0.2	1.0	47	+	+	+
92	4-(1-Methylethenyl)phenol	4286-23-1	134.2	+	1.34	0.06	0.4	47	+	+	+
93	Methyl isothiocyanate	556-61-6	73.1	+	0.73	0.003	0.03	47	+	+	+
94	3-Methyl-4-nitrophenol	2581-34-2	153.2	+	1.53	0.04	0.3	47	+	+	+
95	3-Methylphenol (m-Cresol)	108-39-4	108.1	+	1.08	0.03	0.3	47	+	+	+
96	2-(4-Morpholinyldithio)benzothiazole	95-32-9	284.4	+	2.84	0.1	0.3	47	+	+	+
97	1-Naphthylacetic acid	86-87-3	186.2	+	1.86	1.7	9.1	47	+	+	- (+)
98	4-Nitro-o-anisidine	97-52-9	168.2	+	1.68	0.08	0.5	47	+	+	+
99	3-Nitrobenzenamine	99-09-2	138.1	+	1.38	1.6	11.6	47	-	-	-
100	p-Nitrophenol sodium salt	824-78-2	161.1	+	1.61	0.6	3.7	47	+	+	- (+)
101	4,4'-Oxybis(benzenesulfonylhydrazide)	80-51-3	358.4	+	3.58	0.6	1.7	47	+	+	-
102	2-Pentylanthraquinone	13936-21-5	278.4	+	2.78	0.06	0.2	47	+	+	+
103	N-Phenylmaleimide	941-69-5	173.2	+	1.73	0.01	0.02	47	+	+	+
104	N-Phenyl-N'-isopropyl-p-phenylenediamine	101-72-4	226.3	+	2.26	0.01	0.01	47	+	+	+
105	Phosphoric acid, dodecyl ester, sodium salt	50957-96-5	288.3	+	2.88	0.05	0.16	47	+	+	+
106	Phthalimide	85-41-6	147.1	+	1.47	2.5	17.0	47	-	-	-
107	Sorbitan monooctadecanoate	1338-41-6	430.6	+	4.31	1.1	2.5	47	+	+	-

Table 2 (*Continued*)

JEC ID	Chemical name	CAS No.	MW	CA (original call)	Equiv. to 10 mM (mg/mL)	LEC (mg/mL)	LEC (mM)	Ref.	1997-OECD[a]	r-OECD[b]	ICH[c]
									CA	CA	CA
108	4,4'-Sulfonyldiphenol	80-09-1	250.3	+	2.50	0.4	1.6	47	+	+	-
109	*3a,4,7,7a-Tetrahydro-1H-indene*	*3048-65-5*	*120.2*	+	*1.20*	*0.004*	*0.8*	*47*	+	+	+
110	2,3,4,4'-Tetrahydroxybenzophenone	31127-54-5	246.2	+	2.46	0.0148	0.06	47	+	+	+
111	*2,2,6,6-Tetramethyl-4-hydroxypiperidine*	*2403-88-5*	*157.3*	+	*1.57*	*2.0*	*12.7*	*47*	-	-	-
112	*Thiourea dioxide*	*4189-44-0*	*108.1*	+	*1.08*	*0.6*	*5.5*	*47*	+	+	- (+)
113	*Thymol*	*89-83-8*	*150.2*	+	*1.50*	*0.002*	*0.01*	*47*	+	+	+
114	*Tolylene diisocyanate (Toluene diisocyanate)*	*26471-62-5*	*174.2*	+	*1.74*	*0.3*	*1.8*	*47*	+	+	- (+)
115	2,4,6-Tribromophenol	118-79-6	330.8	+	3.31	0.05	0.2	47	+	+	+
116	*1,3,5-Trihydroxybenezene*	*108-73-6*	*126.1*	+	*1.26*	*0.1*	*1.0*	*47*	+	+	+
117	*2,4,6-Trimercapto-S-triazine*	*638-16-4*	*177.3*	+	*1.77*	*0.8*	*4.5*	*47*	+	+	- (+)
118	*Trimethoxyphosphine*	*121-45-9*	*124.1*	+	*1.24*	*1.2*	*10.0*	*47*	+	+	- (+)
119	*Trimethylamine*	*75-50-3*	*59.1*	+	*0.59*	*0.4*	*6.8*	*47*	+	+	- (+)
120	*2,3,6-Trimethylphenol*	*2416-94-6*	*136.2*	+	*1.36*	*0.05*	*0.4*	*47*	+	+	+
121	2,4,6-Trinitrophenol (Picric acid)	88-89-1	229.1	+	2.29	1.6	7.0	47	+	+	-
122	Triphosphoric acid aluminium salt	13939-25-8	317.9	+	3.18	2.0	6.3	47	+	+	-
123	1,3,5-Tris(3,5-di-*tert*-butyl-4-hydroxybenzyl)isocyanuric acid	27676-62-6	784.1	+	7.84	2.5	3.2	47	+	-	-
124	*2-Vinylpyridine*	*100-69-6*	*105.2*	+	*1.05*	*0.01*	*0.1*	*47*	+	+	+

MW, Molecular weight; CA, Chromosomal aberration test; LEC, Lowest effective concentration;
Equivalaent to 10 mM means the equal concentration of weight per volume (mg/mL) to 10 mM.
+, positive; −, negative.
(+) shows positive after the appication of the r-OECD TG for the chemicals MW less than MW 200 (n = 41).
Italics means chemicals MW less than 200 (n = 85).
Highilight to the negative result by the re-evaluation.

[a] Current OECD test guideline adopted in 1997 (10 mM or 5 mg/mL whichever is lower)

[b] Draft revised OECD test guideline (10 mM or 2 mg/mL whichever is lower)

[c] ICH S2(R1) guideline (1 mM or 0.5 mg/mL whichever is lower);

Table 3
Sensitivity and specificity for carcinogenicity upon application of each test guideline for the dataset on 435 chemicals from the CGX database.

Test guideline	Dataset	CA-negative	CA-positive	Total	Calculation
Original call[a]	Carcinogen	107	210	317	Sensitivity, 66.2% (210/317)
	Non-carcinogen	61	57	118	Specificity, 51.7% (61/118)
	Total	168	267	435	
1997-OECD[b]	Carcinogen	117	200	317	Sensitivity, 63.1% (200/317)
	Non-carcinogen	70	48	118	Specificity, 59.3% (70/118)
	Total	187	248	435	
r-OECD[c]	Carcinogen	117	200	317	Sensitivity, 63.1% (200/317)
	Non-carcinogen	70	48	118	Specificity, 59.3% (70/118)
	Total	187	248	435	
ICH[d]	Carcinogen	173	144	317	Sensitivity, 45.4% (144/317)
	Non-carcinogen	86	32	118	Specificity, 72.9% (86/118)
	Total	259	176	435	
ICH (modified)[e]	Carcinogen	133	184	317	Sensitivity, 58.0% (184/317)
	Non-carcinogen	80	38	118	Specificity, 67.8% (80/118)
	Total	213	222	435	

[a] Call in CGX database [16], including 19 CA-positives (10 carcinogens and 9 non-carcinogens) at >10 mM.
[b] Current OECD test guideline adopted in 1997 (10 mM or 5 mg/mL whichever is lower).
[c] Draft revised OECD test guideline (10 mM or 2 mg/mL whichever is lower).
[d] ICH S2(R1) guideline (1 mM or 0.5 mg/mL whichever is lower).
[e] Applied to the r-OECD TG for the chemicals MW less than 200.

3.3. Evaluation of the relevance of in vitro CA results

Fifty-three chemicals showed different results between r-OECD and ICH TGs (*i.e.*, positive call and negative call, respectively) (Table 2). Thus, these 53 different chemicals were detected as positive in the *in vitro* CA test with r-OECD TG but not with the ICH TG, indicating that the 53 chemicals would be missed if the ICH TG had been used. The relevance of the *in vitro* CA results was evaluated on the basis of the weight-of-evidence approach, and the level of concern on "different" chemicals was defined.

The 53 different chemicals included 34 chemicals that had their appropriate levels of concern evaluated in our previous study (four of 'some concern', seven of 'minimal concern', and 23 of 'negligible concern') [9]. All 34 chemicals were negative in the Ames test [9,47].

The remaining 19 chemicals were evaluated as a new level of concern. Fifteen out of the 19 chemicals were positive in the Ames test. To reveal the weight of the Ames-positives, the *in vivo* genotoxicity and carcinogenicity assays were reviewed for the 15 chemicals (Table 5). Seven of these, *i.e.*, *N*-(aminoethyl)ethanolamine (JEC ID5), azodicarbonamide (JEC ID10), 2-(dimethylamino)ethyl methacrylate (JEC ID51), 2,6-dimethylaniline (JEC ID53), 4,4′-oxybis(benzenesulfonylhydrazide) (JEC ID101), tolylene diisocyanate (JEC ID114) and 2,4,6-trinitrophenol (JEC ID121), were negative in the *in vivo* micronucleus (MN) test [47,49,50]. However, two (JECD IDs 53 and 114) were categorized in the IARC Group 2B (possible human carcinogen) [50]. Two other chemicals, hydrazine monohydrate (JEC ID71) and 3-methoxybenzeneamine (JEC ID82), were positive in the *in vivo* MN test [47,50]; the former chemical (JEC ID71) was categorized in IARC's Group 2B [50]. No *in vivo* genotoxicity and/or carcinogenicity data were available for the remaining six chemicals. On the basis of these data, four chemicals (JEC IDs 53, 71, 82 and 114) can be considered to be of real concern as a possible human carcinogen or an *in vivo* genotoxin. Genotoxic effects could not be ruled out for Ames-positive chemicals, despite the negative results obtained in an *in vivo* MN test. Thus, the remaining 11 chemicals (five *in vivo* MN-negatives and six without *in vivo* genotoxicity data) were considered to be of some concern.

For the last four chemicals, two (JEC IDs 76 and 117) were of negligible concern, one (JEC ID1) was of minimal concern, and one (JEC ID73) was of some concern on the basis of the following evaluations:

JEC ID 1. Acenaphthene (CAS No. 83-32-9): Acenaphthene induced CAs (16.4%, 195 cells analyzed) at the highest concentration of 0.20 mg/mL (1.3 mM) only with S9-mix; the relative cell growth, as measured by monolayer confluence, was 28.0%. A lower concentration of 0.10 mg/mL showed a CA frequency of 4.5%, with 30.0% relative cell growth [47]. In a bacterial reverse-mutation assay (*i.e.*, Ames test), acenaphthene was negative with or without S9. No *in vivo* genotoxicity data were available. The data did not explain that the CAs observed *in vitro* were irrelevant due to their high toxicity. Acenaphthene was classified in Group 3 by IARC due to inadequate evidence in experimental animals for its carcinogenicity [50]. There was insufficient evidence to classify this finding as a negligible level of concern; thus, we concluded that it fell in the category of a minimal level of concern.

JEC ID 73. 4-Hydroxy-benzenesulfonic acid, tin (2+) tetrahydride (CAS No. 70974-33-3): 4-Hydroxy-benzenesulfonic acid, tin (2+) tetrahydride induced CAs (4.5%, 12.5% or 24.0% at 0.528 mg/mL (1.1 mM), 0.755 mg/mL or 1.078 mg/mL, respectively) after 6-h treatment without S9; the relative cell growth, as measured by ATP contents, was 85%, 64% or 53% [47]. With S9, CAs (14.0%) were induced at 2.2 mg/mL after 6-h treatment; the relative cell growth was 43%. Precipitation was observed at the end of the treatment period with S9. The Ames test provided negative results, with or without the S9 mix [47]. No *in vivo* genotoxicity data were available. There was no supporting evidence for a reduced level of concern, and thus some concern remains.

Table 4
Alterations of the number of 124 CA-positives from the JEC database after the application of each test guideline.

Dataset	Original call[a]	1997-OECD[b]	r-OECD[c]	ICH[d]	ICH (modified)[e]
JEC 124 CA-positives	124	118	113	60	101

[a] Call in JEC database [47], including 6 CA-positives) at >10 mM.
[b] Current OECD test guideline adopted in 1997 (10 mM or 5 mg/mL whichever is lower).
[c] Draft revised OECD test guideline (10 mM or 2 mg/mL whichever is lower).
[d] ICH S2(R1) guideline (1 mM or 0.5 mg/mL whichever is lower).
[e] Applied to the r-OECD TG for the chemicals MW less than 200.

Table 5
Summary of *in vivo* genotoxicity and carcinogenicity (in terms of the IARC classification) data on the 15 different chemicals that were positive in the Ames test.

JEC ID	Chemical name	CAS No.	Ames	*in vivo* MN	Carcinogenicity[a]	Ref.
5	*N*-(Aminoethyl)ethanolamine	111-41-1	+	–		[47,49]
7	2-Amino-1-naphthalenesulfonic acid	81-16-3	+			[47]
10	Azodicarbonamide	123-77-3	+	–		[47,49]
13	1,3-Bis(aminomethyl)cyclohexane (mixtures of cis-, trans-)	2579-20-6	+			[47]
17	1-Bromo-3-chloropropane	109-70-6	+			[47]
51	2-(Dimethylamino)ethyl methacrylate	2867-47-2	+	–		[44,47]
52	2,3-Dimethylaniline (2,3-Xylidine)	87-59-2	+			[47]
53	2,6-Dimethylaniline (2,6-Xylidine)	87-62-7	+	–	2B	[47,49,50]
54	3,5-Dimethylaniline (3,5-Xylidine)	108-69-0	+			[47]
71	Hydrazine monohydrate	7803-57-8	+	+[b]	2B[b]	[47,50]
82	3-Methoxybenzeneamine	536-90-3	+	+		[47]
101	4,4'-Oxybis(benzenesulfonylhydrazide)	80-51-3	+	–		[47]
112	Thiourea dioxide	4189-44-0	+			[47]
114	Tolylene diisocyanate (Toluene diisocyanate)	26471-62-5	+	–	2B	[47,50]
121	2,4,6-Trinitrophenol (Picric acid)	88-89-1	+	–		[47,49]

+, positive; –, negative; MN, micronucleus.
[a] In terms of the IARC classification.
[b] As hydrazine (CAS No. 302-01-2).

JEC ID 76. 3-Hydroxy-2-naphthalenecarboxylic acid (CAS No. 92-70-6): 3-Hydroxy-2-naphthalenecarboxylic acid induced CAs in Chinese hamster V79 cells after a 6-h or 18-h treatment with the highest test concentration (0.75 mg/mL, *i.e.*, 4.0 mM) without S9. With S9, no CAs were observed [49]. No information about the frequency of CAs or cytotoxicity was available. The Ames test was negative with or without S9 [49,51]. In an *in vivo* CA test in bone-marrow cells of hamsters, no clastogenic activity and no toxicity were observed at the maximum recommended dose of 2000 mg/kg. However, the test had severe limitations (only 50 metaphases were examined per animal and there was no indication that the target tissue was reached by the chemical). Still, recent *in vivo* mouse bone-marrow MN tests were negative after oral administration of up to 500 mg/kg/day for 2 days. One animal died at 700 mg/kg/day in a dose-range finding study [47]. The weight-of-evidence suggests that the level of concern is negligible.

JEC ID 117. 2,4,6-Trimercapto-S-triazine (CAS No. 638-16-4): 2,4,6-Trimercapto-S-triazine induced CAs at the highest concentration of 0.8 mg/mL (4.5 mM) after 6-h treatment with or without S9 (19.0% or 5.5%, respectively); the relative cell growth, as measured by monolayer confluence, was 73% or 55%, respectively. The pH of the medium at 1.2 mg/mL or more was approximately 6.0 or less. The pH at 0.8 mg/mL was not measured. In a confirmatory test in pH-adjusted medium with S9, the chemical induced CAs (31.5%) at the highest concentration of 1.2 mg/mL, and precipitation was observed at the beginning of the treatment; the relative cell growth was 77%. No CAs were observed up to 0.31 mg/mL after 24-h treatment without the S9 mix [47]. The Ames test was negative with or without S9 [47]. An *in vivo* mouse bone-marrow MN test was negative after oral administration of up to 1000 mg/kg/day for 2 days. One animal died at 2000 mg/kg/day in a dose-range finding study [47]. The weight-of-evidence suggests that the level of concern is negligible.

The results of the evaluation of the level of concern are summarized in Table 6. Of the 53 different chemicals, four chemicals were of 'real concern', 16 were of 'some concern', eight were of 'minimal concern', and the remaining 25 chemicals were of 'negligible concern'. Importantly, the 'of some concern' category in some cases was due to the absence of relevant additional data and not based available data suggesting real concern [9]. In this analysis, 15 Ames-positive chemicals were included in the 53 different (*i.e.*, missed by the application of the ICH TG) chemicals (Table 5). All of the Ames-positives were classified as of 'some concern' or of 'real concern' (Table 6). If the Ames-positive chemicals were excluded from the analysis due to detection by the test-battery system, 38 chemicals would be missed. Among the 38 chemicals, five were of 'some concern'; eight were of 'minimal concern'; and the remaining 25 chemicals were of 'negligible concern' (Table 6).

3.4. Distribution of chemical MWs

The distribution of the MWs of the 267 CA-positives from the CGX database or 124 CA-positives from the JEC database is presented in Table 7. The MWs of the majority of chemicals (71.9% in CGX, 84.7% in JEC) were between 100 and 300. Approximately half (141/267) of the 267 CA-positives from the CGX database had a MW below 200. Similar distributions in MWs have been shown in carcinogens and non-carcinogens. Approximately 70% (85/124) of the 124 CA-positives from the JEC data set, based on CSCL for industrial chemicals, had a MW of less than 200. These distributions indicate that 10 mM can be considered equivalent to 2 mg/mL for industrial chemicals.

4. Discussion

The present reduction in the top-concentration limit in the *in vitro* CA test is expected to reduce the number of false or misleading positives, and hopefully, it will not greatly affect the assay's sensitivity or specificity for rodent carcinogenicity. We investigated the effects of this reduction by means of two chemical data sets from the CGX and JEC databases, by applying three test guidelines, *i.e.*, the 1997-OECD [1], r-OECD [12] and ICH [11] TGs. The chemical dataset from the CGX [16] or JEC [47] databases consisted of a variety of chemical categories, including natural products, pharmaceuticals and pesticides or industrial chemicals. The sensitivity and specificity analysis of the 435 chemicals from the CGX database revealed that application of the r-OECD TG (10 mM or 2 mg/mL) did not affect the sensitivity (63.1%) or specificity (59.3%) against carcinogenicity compared with those (sensitivity 63.1%, specificity 59.3%) seen with the 1997-OECD TG (10 mM or 5 mg/mL). However, the ICH TG (1 mM or 0.5 mg/mL) showed a different outcome, *i.e.*, approximately a 18% decrease in sensitivity (45.4%) and a 14% increase in specificity (72.9%) (Table 3). These results indicate that the r-OECD TG demonstrated the same ability to detect rodent carcinogens as the 1997-OECD TG for chemicals in the CGX database. However, the ICH TG showed a low sensitivity (less than 50%) and was not useful for its detection. Analysis of the changes in the number of 124 CA-positives from the JEC database revealed a small reduction in the number induced under the r-OECD TG, and a remarkable reduction (about half) under the ICH TG (Table 4). These data indicate that application of ICH TG did not lead to an effective detection of rodent carcinogens among non-pharmaceuticals (*e.g.*, general

Table 6
Evaluation of level of concern for human health-risk assessment on the 53 different chemicals.

Level of concern	Number of chemicals with different result based on the different top-concentration limit between r-OECD and ICH TGs (chemical JEC ID)[a]	
Negligible	25	(JEC IDs 3, 4, 6, 19, 28, 33, 36, 39, 44, 45, 47, 57, 66, 69, 74, 75, 76*, 79, 88, 97, 100, 107, 108, 117*, 122)
Minimal	8	(JEC IDs 1*, 16, 55, 62, 64, 78, 85, 119)
Some	16	(JEC IDs 5*, 7*, 10*, 13*, 17*, 35, 51*, 52*, 54*, 73*, 77, 86, 101*, 112*, 118, 121*)
Real	4	(JEC IDs 53*, 71*, 82*, 114*)

Underlined: Ames-positive chemicals.
[a] Positive by the revised OECD test guideline (r-OECD), but negative by the ICH S2(R1) guideline (ICH).
* Evaluated in this paper. Other chemicals without asterisk were evaluated by Morita et al. [9].

Table 7
Distribution of the molecular weights of the 267 or 124 CA-positives from the CGX or JEC database, respectively.

Database	Dataset		Number of chemicals (%) in various ranges of molecular weight					
			<100	100–<200	200–<300	300–<400	400–<500	≥500
CGX	267 CA-positives	210 C	22(10.5)	92(43.8)	60(28.6)	30(14.3)	2(1.0)	4(1.7)
		57 NC	3(5.3)	25(43.9)	15(26.3)	6(10.5)	5(8.8)	3(5.3)
		Total	25(9.4)	117(43.8)	75(28.1)	36(13.5)	7(2.6)	7(2.6)
JEC	124 CA-positives		6(4.8)	79(63.7)	26(21.0)	7(5.6)	2(1.6)	4(3.2)

C, carcinogen; NC, non-carcinogen.

industrial chemicals). These data were supported by a relevance analysis of the *in vitro* CA results (Tables 5 and 6). Fifty-three chemicals, including 15 Ames-positives, were detected as CA-positive with the r-OECD TG, but not with the ICH TG. Twenty-five chemicals were considered to be of negligible concern; thus, a negative call upon the application of the ICH TG was not an issue in such cases. However, the remaining 28 chemicals, of which four chemicals were of real concern (*i.e.*, possible human carcinogens or *in vivo* genotoxins), were not detected as CA-positive under the ICH TG. These results indicate that the ICH TG will miss critical potential carcinogens. Importantly, 15 (*i.e.*, 11 of 15 chemicals of some concern and all four chemicals of real concern) of 28 chemicals of various concern levels were positive in the Ames test, and could be detected with the test-battery system, such as the ICH TG to detect genotoxic carcinogens. No or small changes in the sensitivity/specificity for carcinogenicity or alterations in the number of CA-positives with the r-OECD TG may be explained with the MW analysis of the chemical data set from the CGX and JEC databases. More than half (68.5%) of the CA-positive industrial chemicals had a MW of less than 200, and 90.3% had less than MW 300 in the JEC database (Table 7). Similar results (53.2% < MW 200, 81.3% < MW 300) were shown in the CA-positive data set from the CGX database, which included several pharmaceuticals. Because the MWs of the majority (84.7%) of industrial chemicals are between 100 and 300, 10 mM is considered to be equivalent to 2 mg/mL. Thus, the r-OECD TG showed effects similar to those of the 1997-OECD TG. The top-concentration limit in the ICH TG is 1 mM or 0.5 mg/mL, whichever is lower, although higher test concentrations should be considered for pharmaceuticals with unusually low MWs (*e.g.*, less than 200) [12]. However, no clear recommendation is provided in the ICH TG to determine exactly which 'higher concentrations' should be considered. In the CGX database, 142 chemicals (114 carcinogens and 28 non-carcinogens) had an MW < 200 (Table 7). Of the 142 chemicals, 65 compounds (50 carcinogens and 15 non-carcinogens) were CA-negative upon application of the ICH TG (Table 1). If r-OECD TG were applied to the 65 CA-negatives with MW < 200 (*i.e.*, application of modified ICH TG), 40 of 50 carcinogens and 6 of 15 non-carcinogens would be positive (Table 1). The sensitivity was increased to 58.0% from 45.4%, and the specificity was decreased to 67.8% from 72.9% (Table 3). These values were similar to those after the application of the r-OECD TG. In the JEC database, 85 chemicals were less than MW 200 (Table 7). Forty-seven of the 85 chemicals were negative in the CA test upon application of the ICH TG (Table 2). If r-OECD TG were applied to the 47 CA-negatives with MW < 200, 41 chemicals would be positive (Table 2). The number of CA-positives increased to 101 from 60 upon application of the modified ICH TG (Table 4). The number was similar to that found upon application of the r-OECD TG. This approach suggests the usefulness of applying the r-OECD TG for pharmaceutical substances with MW < 200. Recently, a simulation study performed by Brookmire et al. [10] suggested that lowering the highest concentration on the mg/mL scale to a value close to 2 mg/mL would result in an assay sensitivity close to the 10-mM limit; thus testing up to 5 mg/mL did not increase the sensitivity of the assay. The simulation study suggested also that lowering the current high concentration limit from 10 mM would dramatically impact the sensitivity of the assay. Our analysis with real data was consistent with this simulation study. We also revealed that the top concentration of 2 mg/mL did not decrease the specificity of the assay, although the simulation study did not dictate what the highest concentration should be, or address the specificity. In addition, the lack of significant changes in the sensitivity and specificity after the application of the r-OECD TG suggests that the new top-concentration limit proposed by the r-OECD TG would not affect the evaluation of chromosome damage in in-silico models.

In conclusion, the present analysis suggests that the application of the top-concentration limit (10 mM or 2 mg/mL, whichever is lower) proposed by the r-OECD TG will not affect the sensitivity or specificity of the detection of rodent carcinogens, indicating the validity of the guideline. Thus, the effects on the in-silico evaluation will also be small. However, the r-OECD TG has resulted in little or no reduction in the number of positive chemicals under the 1997-OECD TG, and nearly no improvements in reducing possible false positives for industrial chemicals have been made. Other approaches, *e.g.*, the consideration of the cell systems used, cytotoxicity measurements, non-physiological conditions or metabolic activation systems will be necessary to reduce the number of false positives [5].

Conflict of interests

There are no conflicts of interests.