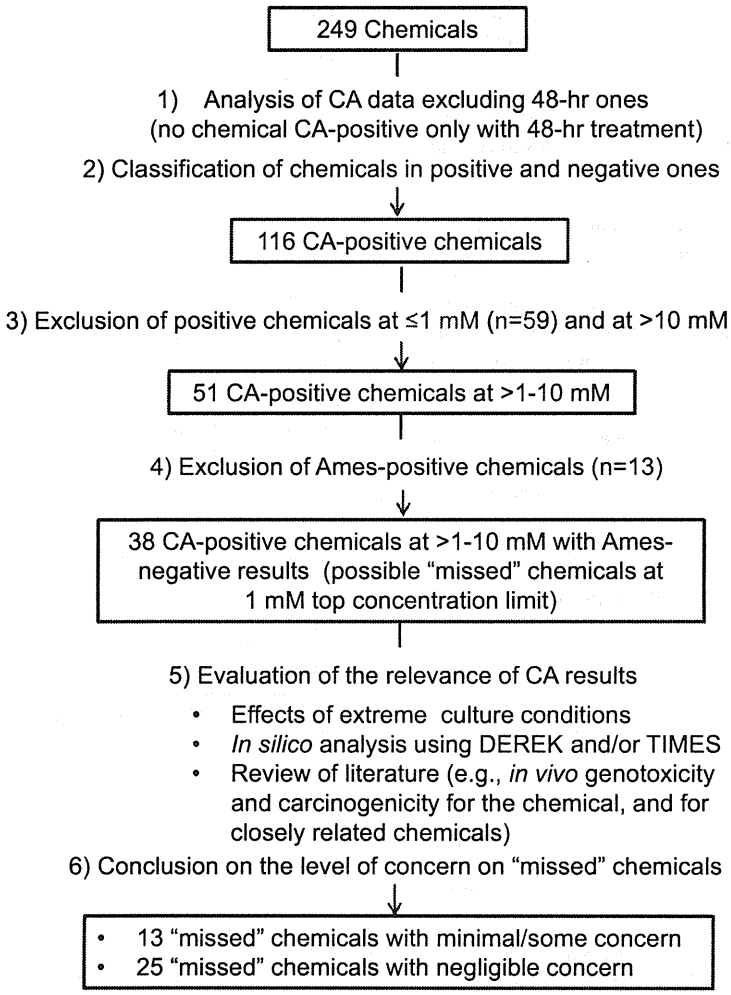


Fig. 1. Flow chart of the different steps of the analysis (weight of evidence approach).

cells with CAs, respectively. CA-induction by the latter chemical was considered to be due to low pH, so equivocal was assigned in the original report [18]. In addition, we regarded 3 chemicals, 2-amino-5-methylbenzenesulfonic acid (ID81), 4-hydroxybenzoic acid (ID83), and methyl acetoacetate (ID84) (Table 4), as positive in this analysis; their original "call" were negative, but the frequencies of CAs observed by the 3 chemicals were more than 10%. Clastogenicity induced by them was considered to be due to low pH, and neutralization of culture medium gave negative results. Thus a negative call was assigned for these chemicals in the original reports [16–18,54].

Among the 249 chemicals tested that had been subjected to the *in vitro* CA test, 116 (46.6%) were positive (Tables 1–4), and 133 (53.4%) were negative (Table 5). No chemicals were CA-positive only with a 48-h treatment. Almost all chemicals were also Ames tested, although we took some Ames data from other literature sources including US National Toxicology Program (NTP, http://ntp-apps.niehs.nih.gov/ntp_tox/) [55–57]. The Ames data are included in Tables 1–5.

Based on the steps of the analysis (Fig. 1), the 249 chemicals in the *in vitro* CA test were divided into five groups; (1) 59 chemicals positive at ≤1 mM (Table 1); (2) six chemicals positive at >10 mM which are considered negative following the criteria given in the current guidelines (Table 2); (3) 13 chemicals positive at >1–10 mM with Ames-positive results which were not "missed" by the test battery (Table 3); (4) 38 chemicals positive at >1–10 mM with Ames-negative results which would be missed in top concentration of 1 mM (possible "missed" chemicals, Table 4); and (5) 133 chemicals negative in the *in vitro* CA test (Table 5).

3.2. Evaluation of the relevance of in vitro CA results

Thirty eight chemicals were chemicals that would be missed if 1 mM was employed as the top concentration limit (*i.e., in vitro* CA negative at >1–10 mM and Ames-negative). The relevance of *in vitro* CA results was evaluated based on a weight of evidence approach including analysis of effects of extreme culture conditions (low pH, high toxicity, and precipitation), *in silico* structural alert analysis using DEREK and/or TIMES, and review of literature for *in vivo* genotoxicity and carcinogenicity tests and the genotoxicity/carcinogenicity of closely related chemicals (Table 4).

On measuring cytotoxicity, there were cases where even though there was apparently, for example 50% relative cell growth, measured by cell counts or confluence, there were insufficient mitotic cells to score.

3.2.1. Possible effects of extreme culture conditions (15 chemicals)

3.2.1.1. Low pH (seven chemicals). A low pH effect was defined as responsible for CA induction when the medium pH was 6.0 or below at the beginning of or just after treatment. Morita et al. reported that initial pH 6.2 or below in 6-h treatment with S9 mix, and initial pH 5.5 or below in 24-h treatment without S9 mix, were clastogenic

Table 1
59 Japanese high production volume chemicals positive at ≤1 mM[a]) in the *in vitro* chromosomal aberration test with CHL cells at (1994–2006, $n = 249$).

ID no.	Chemical name	CAS	MW	LEC (mM)	LEC (mg/mL)	Ames test	Reference
1	Acenaphthene	83-32-9	154.2	1.0	0.2	–	[17]
2	3-Aminophenol	591-27-5	109.1	0.3	0.03	–	[21]
3	4-Aminophenol	123-30-8	109.1	0.02	0.003	–	[17]
4	4,4′-Biphenyldiol	92-88-6	186.2	0.2	0.03	–	[25]
5	1,2-Bis(2-chloroethoxy)ethane	112-26-5	187.1	0.3	0.06	+	[24]
6	Bis(1-methylethyl)naphthalene	38640-62-9	212.3	0.7	0.14	–	[20]
7	N-tert-Butyl-2-benzothiazolesulfenamide	95-31-8	238.4	0.8	0.2	–	[17]
8	*o-sec*-Butylphenol	89-72-5	150.2	0.1	0.02	–	[19]
9	6-*tert*-Butyl-*m*-cresol	88-60-8	164.3	0.05	0.01	–	[19]
10	2-*tert*-Butylphenol	88-18-6	150.2	0.05	0.01	–	[20]
11	*p-tert*-Butylphenol	98-54-4	150.2	0.2	0.03	–	[16]
12	Cadmium nitrate tetrahydrate	10022-68-1	308.5	0.02	0.01	–	[22]
13	1-Chloro-2-(chloromethyl)benzene	611-19-8	161.0	0.6	0.1	+	[19]
14	4-Chloro-*o*-cresol	1570-64-5	142.6	0.6	0.1	–	[16]
15	Chloropentabromocyclohexane	87-84-3	513.1	0.06	0.03	–	[16]
16	4-Chlorophenol	106-48-9	128.6	0.4	0.05	–	[20]
17	Chromic acid disodium salt dihydrate	7789-12-0	297.8	0.002	0.001	+	[23]
18	2,4-Diamino-6-phenyl-*s*-triazine	91-76-9	187.2	0.4	0.08	–	[19]
19	1,3-Dibromopropane	109-64-8	201.9	0.3	0.06	+	[23]
20	2,4-Di-*tert*-butylphenol	96-76-4	206.3	0.04	0.01	–	[20]
21	3,4-Dichloro-1-butene	760-23-6	125.0	0.1	0.01	+	[16]
22	1,2-Dichloro-3-nitrobenzene	3209-22-1	192.0	0.6	0.1	–	[13,34]
23	1,4-Dichloro-2-nitrobenzene	89-61-2	192.0	0.8	0.15	+	[15]
24	*N,N*-Dicyclohexyl-2-benzothiazolesulfenamide	4979-32-2	346.6	0.6	0.2	–	[15]
25	*O,O′*-Diethyl dithiophosphate	298-06-6	186.2	0.6	0.12	+	[25]
26	Diethyl fumarate	623-91-6	172.2	0.1	0.01	–	[14]
27	2-(Dimethylamino)ethyl acrylate	2439-35-2	143.2	0.4	0.05	+	[17]
28	*N*-(1,3-Dimethylbutyl)-*N′*-phenyl-*p*-phenylenediamine	793-24-8	268.4	0.02	0.005	–	[19]
29	Diphenyl cresyl phosphate	26444-49-5	340.3	0.1	0.04	–	[14]
30	Disperse Yellow 42	5124-25-4	369.4	0.2	0.08	–	[22]
31	2,3-Epoxypropyl methacrylate	106-91-2	142.2	0.2	0.02	+	[17,35]
32	4-Ethoxybenzeneamine (*p*-Phenetidin)	156-43-4	137.2	0.4	0.05	–	[13]
33	2-Ethylanthraquinone	84-51-5	236.3	0.6	0.16	–	[20]
34	3-Ethylphenol	620-17-7	122.2	0.4	0.05	–	[21]
35	4-Ethylphenol	123-07-9	122.2	0.3	0.04	–	[20]
36	Hydrazine monohydrate	7803-57-8	50.1	1.0	0.06	+	[23]
37	2-Hydroxybenzaldehyde	90-02-8	122.1	0.8	0.1	–	[16]
38	Methacrylonitrile (methyl acrylonitrile)	126-98-7	67.1	1.0	0.07	–	[20]
39	Methoxymethanol	4461-52-3	62.1	0.3	0.02	+	[14]
40	1-Methoxynaphthalene	2216-69-5	158.2	0.1	0.02	–	[16]
41	4,4′-Methylenebis(2-chloroaniline)	101-14-4	267.2	0.1	0.04	+	[25]
42	Methylenediphenol	1333-16-0	200.2	0.05	0.01	–	[21]
43	4,4′-Methylenediphenol	620-92-8	200.2	1.0	0.2	–	[25]
44	4-(1-Methylethenyl)phenol	4286-23-1	134.2	0.5	0.06	–	[23]
45	Methyl isothiocyanate	556-61-6	73.1	0.03	0.003	–	[25]
46	3-Methyl-4-nitrophenol	2581-34-2	153.2	0.3	0.04	–	[14]
47	3-Methylphenol (*m*-Cresol)	108-39-4	108.1	0.2	0.03	–	[20]
48	2-(4-Morpholinyldithio)benzothiazole	95-32-9	284.4	0.3	0.1	–	[24]
49	4-Nitro-*o*-anisidine	97-52-9	168.2	0.5	0.08	+	[17]
50	2-Pentylanthraquinone	13936-21-5	278.4	0.2	0.06	+	[26]
51	*N*-Phenylmaleimide	941-69-5	173.2	0.02	0.01	+	[21]
52	*N*-Phenyl-*N′*-isopropyl-*p*-phenylenediamine	101-72-4	226.3	0.01	0.01	–	[23]
53	Phosphoric acid, dodecyl ester, sodium salt	50957-96-5	288.3	0.16	0.05	–	[26]
54	3a,4,7,7a-Tetrahydro-1*H*-indene	3048-65-5	120.2	0.8	0.004	–	[17]
55	Thymol	89-83-8	150.2	0.5	0.002	–	[16]
56	2,4,6-Tribromophenol	118-79-6	330.8	0.2	0.05	–	[19]
57	1,3,5-Trihydroxybenezene	108-73-6	126.1	1.0	0.1	–	[22]
58	2,3,6-Trimethylphenol	2416-94-6	136.2	0.4	0.05	–	[19]
59	2-Vinylpyridine	100-69-6	105.2	0.1	0.01	+	[17]

(–): Negative; (+): positive; MW: molecular weight; LEC: lowest effective concentration.
[a] As lowest effective concentration.

Table 2
6 Japanese high production volume chemicals positive at >10 mM[a]) in the *in vitro* chromosomal aberration test with CHL cells at (1994–2006, $n = 249$).

ID no.	Chemical name	CAS	MW	LEC (mM)	LEC (mg/mL)	Ames test	Reference
60	o-Acetoacetotoluidine	93-68-5	191.2	13.1	2.5	–	[19]
61	Benzyltrimethylammonium chloride[b]	56-93-9	185.7	10.2	1.9	–	[16]
62	3-Methylzenzoic acid	99-04-7	136.2	11.0	1.5	–	[19]
63	3-Nitrobenzenamine	99-09-2	138.1	11.6	1.6	+	[13]
64	Phthalimide	85-41-6	147.1	17.0	2.5	–	[19]
65	2,2,6,6-Tetramethyl-4-hydroxypiperidine	2403-88-5	157.3	12.7	2.0	–	[18]

(–): Negative; (+): positive; MW: molecular weight; LEC: lowest effective concentration.
[a] As lowest effective concentration.
[b] The original "call" was equivocal [16]. However, it regarded as positive in this analysis because the effect was reproducible.

Table 3
13 Japanese high production volume chemicals positive at >1–10 mM[a] in the *in vitro* chromosomal aberration test with CHL cells with Ames-positive results (1994–2006, $n=249$).

ID no.	Chemical name	CAS	MW	LEC (mM)	LEC (mg/mL)	Ames test	Reference
66	*N*-(Aminoethyl)ethanolamine	111-41-1	104.2	9.6	1.0	+	[16,55]
67	2-Amino-1-naphthalenesulfonic acid	81-16-3	223.3	4.9	1.1	+	[15]
68	1-Bromo-3-chloropropane	109-70-6	157.4	1.6	0.3	+	[20]
69	2-(Dimethylamino)ethyl methacrylate	2867-47-2	157.2	4.0	0.6	+/−	[18,53]
70	2,3-Dimethylaniline	87-59-2	121.2	5.0	0.6	+	[17]
71	2,6-Dimethylaniline (2,6-Xylidine)	87-62-7	121.2	2.5	0.3	+	[25]
72	3,5-Dimethylaniline (3,5-Xylidine)	108-69-0	121.2	7.4	0.9	+	[17,56]
73	Disperse Red 206	26630-87-5	580.1	4.3	2.5	+	[23]
74	3-Methoxybenzeneamine	536-90-3	123.2	6.1	0.8	+	[13]
75	4,4′-Oxybis(benzenesulfonylhydrazide)	80-51-3	358.4	1.6	0.6	+	[24]
76	Thiourea dioxide	4189-44-0	108.1	5.5	0.6	+	[19]
77	Tolylene diisocyanate (Toluene diisocyanate)	26471-62-5	174.2	1.8	0.3	+	[21]
78	2,4,6-Trinitrophenol (Picric acid)	88-89-1	229.1	7.0	1.6	+	[20]

(−): Negative; (+): positive; MW: molecular weight; LEC: lowest effective concentration.
[a] As lowest effective concentration.

to CHO-K1 cells [58,59], and stable pH 6.5 or below for 24 h, or stable pH 5.8 or below for 6 h without S9 mix were clastogenic to CHL cells [60]. Therefore, pH 6.0 or below, regardless of fluctuation or stability, in the culture medium might cause CAs both with and without S9 mix.

ID79. 3-Aminobenzenesulfonic acid (CAS no. 121-47-1) [molecular weight (MW) = 173]: 3-Aminobenzenesulfonic acid induced CAs with S9 mix (5.0 and 16.5% at 2.4 and 4.8 mM (0.83 mg/mL), respectively) [14]. The pH of the medium was 5.8 or 6.3 at the beginning of the 6-h treatment and 6.2 or 6.5 just after it at 4.8 or 2.4 mM, respectively. Relative cell growth, as measured by monolayer confluence, was about 100% or 90% at 4.8 or 9.5 mM, respectively. However, there were no metaphases at 9.5 mM. Without S9 mix, no CA induction was observed after 6- or 24-h treatment. The reason for this will be due to the short duration in low pH culture condition; the pH of the medium without S9 mix was 5.8 or 6.5 at the beginning of the 6-h treatment and 6.6 or 6.9 just after it at 4.8 or 2.4 mM, respectively. Initial pHs of the medium were similar, but the pHs after the treatment without S9 mix were higher than that with S9 mix. The window of the induction of CAs by low pH is narrower without S9 mix than that with S9 mix, generally; the same effect was observed for hydrochloric acid and sulfuric acid [58]. 3-Aminobenzenesulfonic acid does not possess any DEREK structural alerts. In addition, an *in vivo* MN test was negative for a related structural analogue, 2-amino-5-methylbenzenesulfonic acid (CAS no. 88-44-8, ID81) [51]. The CAs observed are considered as irrelevant as they were only seen at low pH, and it is supported by all other available data. Thus the level of concern is negligible.

ID80. 2-Amino-5-chloro-4-methylbenzenesulfonic acid (CAS no. 88-53-9) [MW = 222]: 2-Amino-5-chloro-4-methylbenzenesufonic acid induced CAs (7.5% and 50.0%) at 9 mM (2 mg/mL) after 24-h treatment without S9 mix, and at 10 mM (2.2 mg/mL) after 6-h treatment with S9 mix, respectively. The pH of the medium was 6.4–6.6 without S9 mix and 5.5–5.8 with S9 mix in the beginning of the treatment [13]. Relative cell growth, as measured by monolayer confluence, was about 100% at 10 mM with S9 mix. Continuous low pH (6.5 or below) condition for 24 h without S9 mix was known to induce structural CAs in CHL cells [60]. No structural alerts were identified by DEREK. An *in vivo* MN test was negative for a related structural analogue, 2-amino-5-methylbenzenesulfonic acid (CAS no. 88-44-8, ID81) [51]. The CAs observed are considered as irrelevant due to low pH, and it is supported by all other available data. Thus the level of concern is negligible.

ID81. 2-Amino-5-methylbenzenesulfonic acid (CAS no. 88-44-8) [MW = 187]: 2-Amino-5-methylbenzenesulfonic acid induced CAs (7.0%) at 5.1 mM (1.0 mg/mL) after 6-h treatment with S9 mix, and relative cell growth, as measured by monolayer confluence, was about 40%. Only 5 metaphases were analyzed at 10 mM (1.9 mg/mL) due to severe cytotoxicity. The pH of the medium was 5.8 at the beginning of the treatment and 6.3 at the end [16]. No CAs were observed up to 10 mM when the pH of the culture medium was adjusted to about pH 7 by adding 1 N NaOH [16,54]. No structural alerts were identified by DEREK. Furthermore, an *in vivo* mouse bone marrow MN test was negative after oral administration up to 5000 mg/kg [51]. The CAs observed are considered as irrelevant due to low pH, and it is supported by all other available data. Thus the level of concern is negligible.

ID82. Glycerol triacetate (CAS no. 102-76-1) [MW = 218]: Glycerol triacetate induced CAs (42.0%) at 10 mM (2.2 mg/mL) with S9 mix, in which relative cell growth, as measured by monolayer confluence, was about 30%. The color of the medium became yellow only in the treatment with S9 mix. A confirmation study was conducted at neutral pH culture condition (adjusted to pH 6.9 by adding 1 N NaOH at the beginning of the treatment, but the pH value just after the addition of test chemicals was not described) [18]. However, the pH of the medium decreased to 4.9 after 6-h treatment with S9 mix [18,40]. Acidic metabolite(s) might be generated at high concentration with S9 mix, resulting in the induction CAs. No CA induction was observed at the lower concentration of 2.5 mM or 5 mM; relative cell growth was about 100% or 90%, respectively. The effect of the generation of acidic metabolite(s) will be *in vitro* specific at high concentration in the presence of S9 mix. No structural alerts were identified by DEREK. The CAs observed are considered as irrelevant due to low pH, and thus the level of concern is negligible.

ID83. 4-Hydroxybenzoic acid (CAS no. 99-96-7) [MW = 138]: 4-Hydroxybenzoic acid induced CAs (27.5% or 26.5%) at 5.1 mM (0.7 mg/mL) after 24-h treatment without S9 mix or 6-h treatment with S9 mix (181 cells analyzed); relative cell growth, as measured by monolayer confluence, was 58% or 28%, respectively. Severe cytotoxicity was observed at 10 mM (1.4 mg/mL) in all treatments. The pH of the medium was 6.1 or 5.8 at the beginning of the treatment and 6.6 or 6.2 at the end after 24-h treatment without S9 mix or 6-h treatment with S9 mix, respectively [17]. Culture medium with continuous low pH (6.5 or below) was known to induce CAs in CHL cells with S9 mix [60]. No CA induction was observed up to 10 mM when the pH of the culture medium was adjusted to about pH 7.5 by adding 1 N NaOH [17,52,54]. No structural alerts were identified by DEREK. The CAs observed are considered as irrelevant due to low pH, and thus the level of concern is negligible.

ID84. Methyl acetoacetate (CAS no. 105-45-3) [MW = 116]: Methyl acetoacetate induced CAs (11.0%) at 10 mM (1.2 mg/mL) after 6-h treatment with S9 mix; relative cell growth, as measured by monolayer confluence, was 88%. Though the color of the medium became yellow, no pH measurement was conducted [18]. No CA induction

Table 4
Analysis for the relevance of CA results in 38 "missed" chemicals which are CA-positive at >1–10 mM with Ames-negative results.

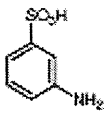
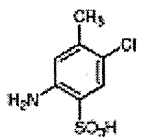
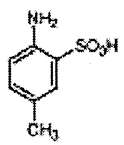
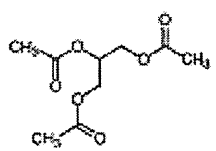
ID no.	Chemical name	CAS	Molecular structure	MW	LEC (mM)	LEC (mg/mL)	DEREK[a]	TIMES[b]	Ames test [Ref.]	*In vivo* MN (CA) [Ref.]	Note
1. Possible effects of extreme culture conditions (n = 15)											
1.1. Low pH (7 chemicals)											
79	3-Aminobenzenesulfonic acid[e]	121-47-1		173.2	2.4	0.4	No alert	ND	– [14]	<–> [51]	Irrelevant due to low pH; negative in *in vivo* MN test for a related structural analogue, 2-mino-5-methylbenzenesulfonic acid (ID81) [51]; negligible concern.
80	2-Amino-5-chloro-4-methylbenzenesulfonic acid	88-53-9		221.5	9.0	2.0	No alert	ND	– [13]	<–> [51]	Irrelevant due to low pH; negative in *in vivo* MN test for a related structural analogue, 2-mino-5-methylbenzenesulfonic acid (ID81) [51]; negligible concern.
81	2-Amino-5-methylbenzenesulfonic acid[c,e]	88-44-8		187.2	5.1	1.0	No alert	ND	– [16]	– [51]	Irrelevant due to low pH; no increase in CAs by neutralization of culture medium; negative in *in vivo* MN test; negligible concern.
82	Glycerol triacetate[d,e]	102-76-1		218.2	10.0	2.2	No alert	ND	– [25]		Irrelevant due to low pH; possible generation of acidic metabolite(s) in *in vitro* specific condition with S9 mix; negligible concern.

Table 4 (*Continued*)

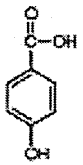
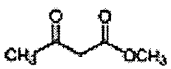
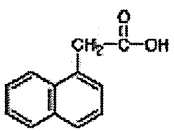
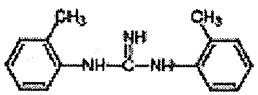
ID no.	Chemical name	CAS	Molecular structure	MW	LEC (mM)	LEC (mg/mL)	DEREK[a]	TIMES[b]	Ames test [Ref.]	*In vivo* MN (CA) [Ref.]	Note
83	4-Hydroxybenzoic acid[c]	99-96-7		138.1	5.1	0.7	No alert	ND	– [17,52]		Irrelevant due to low pH; no increase in CAs by neutralization of culture medium; negligible concern.
84	Methyl acetoacetate[c,e]	105-45-3		116.1	10.0	1.2	No alert	ND	– [18]		Not concluded irrelevant due to low pH; lack of information on pH in the initial phase and after the treatment; no increase in CAs by neutralization of culture medium; minimal concern.
85	1-Naphthylacetic acid	86-87-3		186.2	9.1	1.7	No alert	ND	– [14]	– [61]	Irrelevant due to low pH; negative in *in vivo* MN test; negligible concern.
1.2. High toxicity (6 chemicals)											
86	1,3-Bis(2-methylphenyl)guanidine[e]	97-39-2		239.3	2.5	0.6	No alert	ND	– [24]	– [42]	Not concluded irrelevant due to high toxicity; insufficient of negative in bone marrow MN test because *in vitro* CA-positive only with S9 mix; minimal concern.

Table 4 (*Continued*)

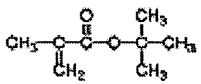
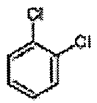
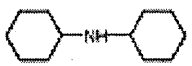
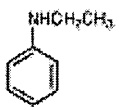
ID no.	Chemical name	CAS	Molecular structure	MW	LEC (mM)	LEC (mg/mL)	DEREK[a]	TIMES[b]	Ames test [Ref.]	*In vivo* MN (CA) [Ref.]	Note
87	*tert*-Butyl-methacrylate	585-07-9		142.2	2.8	0.4	CA-induction due to alpha, beta-unsaturated ester or thioester	ND	– [16]	<–> [44,53,62]	Maybe associated with cytotoxicity; negative in *in vivo* MN test for other methacrylates, 2-(dimethylamino)ethyl methacrylate (ID69) [53], 2-hydroxyethyl methacrylate (ID91) [44], and methyl methacrylate [62]; no evidence of carcinogenic potential for methyl methacrylate in rats and mice [62]; negligible concern.
88	*o*-Dichlorobenzene[c]	95-50-1		147.0	1.6	0.2	Carcinogenicity due to polyhalogenated aromatic	ND	– [20]	–, (–) [41]	Irrelevant due to high toxicity; negative in *in vivo* MN and CA tests, and in *in vivo* carcinogenicity tests; negligible concern.
89	Dicyclohexylamine	101-83-7		181.3	3.3	0.6	No alert	ND	– [18]	<(–)> [43]	Not considered to be irrelevant due to high toxicity; negative in *in vivo* CA test for a closely related structural analogue, *N*-methyl dicyclohexylamine [43]; negligible concern.
90	*N*-Ethylaniline	103-69-5		121.2	9.1	1.1	No alert	ND	– [15]		Maybe irrelevant due to high toxicity; no supporting evidence to reduce the level of concern; minimal concern.

Table 4 (*Continued*)

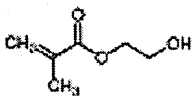
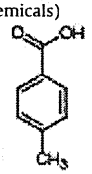
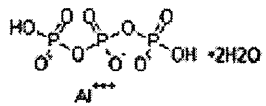
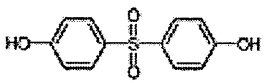
ID no.	Chemical name	CAS	Molecular structure	MW	LEC (mM)	LEC (mg/mL)	DEREK[a]	TIMES[b]	Ames test [Ref.]	*In vivo* MN (CA) [Ref.]	Note
91	2-Hydroxyethyl methacrylate	868-77-9		130.2	5.0	0.7	CA-induction due to alpha, beta-unsaturated ester or thioester	ND	– [17]	– [44]	Maybe associated with cytotoxicity; negative in *in vivo* MN test; no evidence of carcinogenic potential for methyl methacrylate in rats and mice [62]; negligible concern.
1.3. Precipitation coupled with high toxicity (2 chemicals)											
92	4-Methylbenzoic acid	99-94-5		136.2	8.8	1.2	No alert	ND	– [24]	– [45]	Irrelevant due to precipitation and following high toxicity; precipitation at all concentrations tested; negative in *in vivo* MN test; negligible concern.
93	Triphosphoric acid aluminium salt[c]	13939-25-8		317.9	6.3	2.0	No alert	ND	– [23]		Irrelevant due to precipitation and following high toxicity; precipitation at all concentrations tested; negligible concern.
2. Weak evidence for a positive ($n=2$)											
94	4,4′-Sulfonyldiphenol	80-09-1		250.3	1.6	0.4	No alert	ND	– [19]		Low biological significance; statistically significant, but not tested for confirmation of reproducibility; negligible concern.

Table 4 (*Continued*)

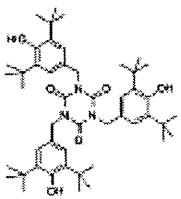
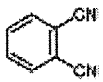
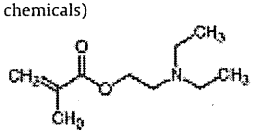
ID no.	Chemical name	CAS	Molecular structure	MW	LEC (mM)	LEC (mg/mL)	DEREK[a]	TIMES[b]	Ames test [Ref.]	*In vivo* MN (CA) [Ref.]	Note
95	1,3,5-Tris(3,5-di-*tert*-butyl-4-hydroxybenzyl)isocyanuric acid	27676-62-6		784.1	3.2	2.5	No alert	ND	– [24]		Maybe low biological significance; statistically significant increase only in polyploidy based on the analysis of 800 cells per group, and outside of the historical range for negative controls; minimal concern.
3. Possible other factors (n = 21)											
3.1. Induction of polyploidy only (1 chemical)											
96	1,2-Dicyanobenzene	91-15-6		128.1	2.5	0.3	No alert	ND	– [20]	– [46]	Induction of polyploidy only; negative in *in vivo* MN test; mode of action by non-DNA target; negligible concern.
3.2. Selected chemical class with DNA reactivity (4 chemicals)											
97	2-(Diethylamino)ethyl methacrylate[e]	105-16-8		185.3	3.2	0.6	CA-induction due to alpha, beta-unsaturated ester or thioester	CA-induction due to possible metabolite(s): unsaturated aldehydes can interact with topoisomerases/proteins.	– [18]	<–> [44,53,62]	Maybe associated with the DNA reactivity and/or cytotoxicity; negative in *in vivo* MN test for other methacrylates, 2-(dimethylamino)ethyl methacrylate (ID69) [53], 2-hydroxyethyl methacrylate (ID91) [44], and methyl methacrylate [62]; no evidence of carcinogenic potential for methyl methacrylate in rats and mice [62]; negligible concern.

Table 4 (*Continued*)

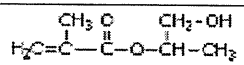
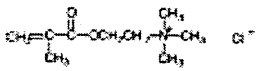
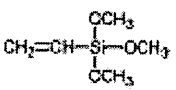
ID no.	Chemical name	CAS	Molecular structure	MW	LEC (mM)	LEC (mg/mL)	DEREK[a]	TIMES[b]	Ames test [Ref.]	*In vivo* MN (CA) [Ref.]	Note
98	Methacrylic acid, monoester with propane-1,2-diol	27813-02-1		144.2	5.0	0.7	CA-induction due to alpha,beta-unsaturated ester or thioester	CA-induction due to parent chemical and possible metabolite(s): acrylates can interact with DNA and topoisomerases/proteins; unsaturated aldehydes can interact with topoisomerases/proteins.	– [16]	<–> [44,53,62]	Maybe associated with the DNA reactivity and/or cytotoxicity; negative in *in vivo* MN test for other methacrylates, 2-(dimethylamino)ethyl methacrylate (ID69) [53], 2-hydroxyethyl methacrylate (ID91) [44], and methyl methacrylate [62]; no evidence of carcinogenic potential for methyl methacrylate in rats and mice [62]; negligible concern.
99	(Methacryloyloxyethyl) trimethylammonium chloride	5039-78-1		207.7	10.0	2.1	CA-induction due to alpha,beta-unsaturated ester or thioester	CA-induction due to parent chemical: alkylamido betaines, theoretically interact with DNA or topoisomerases/proteins.	– [21]	<–> [44,53,62]	Maybe associated with the DNA reactivity; negative in *in vivo* MN test for other methacrylates, 2-(dimethylamino)ethyl methacrylate (ID69) [53], 2-hydroxyethyl methacrylate (ID91) [44], and methyl methacrylate [62]; no evidence of carcinogenic potential for methyl methacrylate in rats and mice [62]; negligible concern.
100	Ethenyltrimethoxysilane[c]	2768-02-7		148.2	5.0	0.8	No alert	CA-induction due to possible metabolite(s): epoxides aziridines can interact with topoisomerases/proteins.	– [26]	<–> [65]	Maybe associated with the DNA reactivity of metabolite(s); negative in *in vivo* MN test for an alkoxy silane, [3-(methacryloxy)propyl] trimethoxysilane [65], but insufficient of negative in *in vivo* MN test because *in vitro* CA-positive only with S9 mix; minimal concern.

Table 4 (*Continued*)

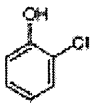
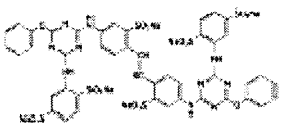
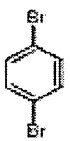
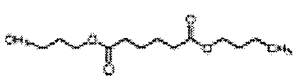
ID no.	Chemical name	CAS	Molecular structure	MW	LEC (mM)	LEC (mg/mL)	DEREK[a]	TIMES[b]	Ames test [Ref.]	*In vivo* MN (CA) [Ref.]	Note
3.3. Others (16 chemicals)											
101	2-Chlorophenol	95-57-8		128.6	2.0	0.3	CA-induction due to halophenol	No alert	– [20]	– [67]	Negative in *in vivo* MN test; not found to be carcinogenic in rats [68]. Negligible concern.
102	C.I. Fluorescent brightner 271	41267-43-0		1347.1	3.7	5.0	No alert	Not applied due to too large molecule	– [26]		Negative in *in vivo* CA test for other 3 structural related compounds (C.I. fluorescent brightner 24, 225 and 260) [64]; no *in vivo* supporting evidence of a reduced level of concern; minimal concern.
103	1,4-Dibromobenzene[c]	106-37-6		235.9	2.3	0.6	No alert	CA-induction due to possible metabolite(s): epoxides and aziridines can interact with topoiso-merases/proteins.	– [14]	<–> [69]	Negative in *in vivo* MN test for a closely related structural analogue, 1,4-dichlorobenzenen (non-genotoxic carcinogen) [69]; negligible concern.
104	Dibutyl adipate[c]	105-99-7		258.4	2.5	0.7	No alert	No alert	– [15]		No supporting evidence of a reduced level of concern; some concern.

Table 4 (*Continued*)

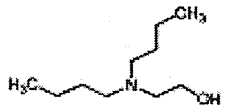
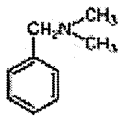
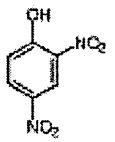
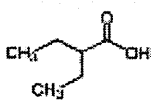
ID no.	Chemical name	CAS	Molecular structure	MW	LEC (mM)	LEC (mg/mL)	DEREK[a]	TIMES[b]	Ames test [Ref.]	*In vivo* MN (CA) [Ref.]	Note
105	2-(Di-*n*-butylamino)ethanol	102-81-8		173.3	1.9	0.3	No alert	No alert	– [25]	<–> [70,71]	Negative in *in vivo* MN test for related structural analogues, 2-aminoethanol [70] and 2-diethylaminoethanol [71]; negligible concern.
106	N,N-Dimethylbenzylamine[e]	103-83-3		135.2	2.8	0.4	No alert	CA-induction due to possible metabolite(s): benzylamines theoretically can interact with DNA.	– [17]	– [72]	Insufficient of negative in *in vivo* MN test because *in vitro* CA-positive only with S9 mix; minimal concern.
107	2,4-Dinitrophenol	51-28-5		184.1	6.5	1.2	Carcinogenicity due to aromatic nitro; CA-induction due to polynitrophenol or precursor.	CA-induction due to parent chemical and possible metabolite(s): nitro compounds can interact with DNA; amines, aminophenols and phenylenediamines, aminophenols, or hydroxylamines can interact with DNA and topoisomerases/proteins.	– [20]		Metabolic poison (uncouples oxidative phosphorylation, the mechanism with a threshold); Positive *in vitro* CA at cytotoxic levels in CHO and TK cells [73]; clastogenicity by indirect mechanism (energy depletion) [74]; negligible concern
108	2-Ethylbutyric acid	88-09-5		116.2	3.4	0.4	No alert	ND	– [22]	– [25,49]	Negative in *in vivo* MN test; negligible concern

Table 4 (*Continued*)

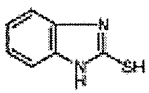
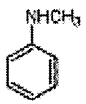
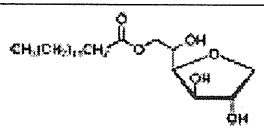
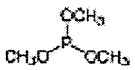
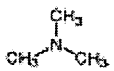
ID no.	Chemical name	CAS	Molecular structure	MW	LEC (mM)	LEC (mg/mL)	DEREK[a]	TIMES[b]	Ames test [Ref.]	*In vivo* MN (CA) [Ref.]	Note
109	Ferrous sulfate heptahydrate	7782-63-0	$FeSO_4 \cdot 7H_2O$	278.0	1.8	0.5	No alert	ND	– [23]	– [47,76]	Negative *in vivo* digestive tract MN assay including stomach, duodenum and colon; No increase in tumor incidence for ferric chloride [47]; negligible concern.
110	2-Hydroxypropanenitrile	78-97-7		71.1	10.0	0.7	No alert	No alert	– [13]		No supporting evidence of a reduced level of concern; some concern.
111	2-Mercaptobenzimidazole[c]	583-39-1		150.2	5.3	0.8	Mutagenicity due to benzimidazole; CA-induction due to 2-thio-benzimidazole or -benzothiazole	CA-induction due to parent chemical and possible metabolite(s): thiols can interact with topoiso-merases/proteins.	– [15]	– [15,77]	Negative in Ames test and *in vivo* 13-week inhalation MN test; insufficient of negative in long term erythrocyte MN test by inhalation because of possibility of poor systemic exposure and *in vitro* CA-positive only with S9 mix; minimal concern.
112	*N*-Methylaniline	100-61-8		107.2	5.5	0.6	No alert	CA-induction due to possible metabolite(s): hydroxyl amines interact with DNA.	– [15]	<+> [79]	*N*-Methylaniline yields aniline in rats or rabbits [78], which is carcinogen and induces MN in mice and rats [79]; some concern.
113	*p*-Nitrophenol sodium salt	824-78-2		161.1	3.8	0.6	No alert	CA-induction due to parent chemical and possible metabolite(s): nitro compounds can interact with DNA; amines, aminophenols, or phenyleneamines can interact with DNA or topoiso-merases/proteins; hydroxylamines can interact with DNA.	– [21]	<–> [82]	Negative in *in vivo* MN test for free base, *p*-nitrophenol [82]; negligible concern.

Table 4 (*Continued*)

ID no.	Chemical name	CAS	Molecular structure	MW	LEC (mM)	LEC (mg/mL)	DEREK[a]	TIMES[b]	Ames test [Ref.]	*In vivo* MN (CA) [Ref.]	Note
114	Sorbitan monooctadecanoate[e]	1338-41-6		430.6	2.5	1.1	No alert	No alert	– [16]		No evidence of carcinogenic potential for sorbitan monostearate in rats and mice [83]; negligible concern.
115	Trimethoxyphosphine	121-45-9		124.1	10.0	1.2	No alert	ND	– [19]		No supporting evidence of a reduced level of concern; some concern.
116	Trimethylamine	75-50-3		59.1	6.4	0.4	No alert	No alert	– [20]	<(–)> [84]	Negative in *in vivo* 15- or 90-day inhalation CA test for closely related structural analogue, dimethylamine [84]; insufficient of negative in long term bone marrow CA test by inhalation because of possibility of poor systemic exposure; minimal concern.

(–): Negative; (+): positive; <–>: negative in a related structural analogue; <+>: positive in a related structural analogue; MW: molecular weight; LEC: lowest effective concentration; CA: chromosomal aberration; MN: micronucleus; ND: not done.

[a] Structure alert for mutagenicity, chromosome damage and carcinogenicity.

[b] Structure alert for CA.

[c] The original call was "negative" because the effect was considered as due to low pH [16–18,54]. These induced more than 10% aberrant cells, but did not induce CA when the pH of the culture medium was adjusted to pH 7–8 by adding 1 N NaOH. These were regarded as positive in this analysis.

[d] The original "call" was equivocal [18]. However, it was regarded as positive in this analysis because the effect was reproducible.

[e] CA-positive only with S9 mix (IDs 79, 81, 82, 84, 86, 88, 93, 97, 100, 103, 104, 106, 111 and 114).

Table 5
133 Japanese high production volume chemicals negative in the *in vitro* chromosomal aberration test with CHL cells (1994–2006, $n = 249$).

ID no.	Chemical name	CAS	MW	Ames test	Reference
117	1-Aminoanthraquinone	82-45-1	223.2	+	[15]
118	2-Amino-2-ethyl-1,3-propanediol	115-70-8	119.2	–	[25]
119	7-Amino-4-hydroxy-2-naphthalenesulfonic acid	87-02-5	239.3	+	[16]
120	2,2′-Azobis(2-methylpropionitrile)	78-67-1	164.2	–	[17]
121	1,3-Bis(aminomethyl) benzene	1477-55-0	136.2	–	[15]
122	1,1-Bis(*tert*-butyldioxy)-3,3,5-trimethylcyclohexane	6731-36-8	302.5	–	[21]
123	3,3-Bis(*p*-dimethylaminophenyl)-6-dimethylaminophthalide	1552-42-7	415.5	–	[23]
124	Bis(2-ethylhexyl) azelate	103-24-2	412.7	–	[24]
125	Bis(1-methyl-1-phenylethyl) peroxide	80-43-3	270.4	–	[20]
126	1,2-Bis(staeroylamino) ethane	110-30-5	593.0	–	[20]
127	1,2-Butanediol	584-03-2	90.1	–	[13]
128	1,4-Butanediol	110-63-4	90.1	–	[17]
129	2-*tert*-Butoxyethanol	7580-85-0	118.2	–	[22]
130	2-*sec*-Butyl-4,6-dinitrophenol	88-85-7	240.2	–	[25]
131	Butyl methacrylate	97-88-1	142.2	–	[18]
132	6-tert-Butyl-2,4-xylenol	1879-09-0	178.3	–	[15]
133	*N*-(Carboxymethy)-*N*,*N*-dimethyl-1-dodecanaminium, inner salt	683-10-3	271.4	–	[26]
134	1-Chlorobutane	109-69-3	92.6	–	[14]
135	C.I.Pigment Red 22	6448-95-9	426.4	+	[23]
136	C.I.Pigment Yellow 53	8007-18-9	488.6	–	[22]
137	Cyanoguanidine	461-58-5	84.1	–	[18]
138	3-Cyanopyridine	100-54-9	104.1	–	[17]
139	Cyclohexene	110-83-8	82.1	–	[22]
140	*N*-Cyclohexyl-2-benzothiazolesulfenamide	95-33-0	246.3	–	[17]
141	D&C Red No.7	5281-04-9	424.3	–	[14]
142	Diacetone alcohol	123-42-2	116.2	–	[17]
143	2,3-Dibromosuccinic acid	526-78-3	275.9	–	[14]
144	Dibutyl phosphate	107-66-4	210.2	–	[14]
145	2,4-Dichloro-1-methylbenzene	95-73-8	161.0	–	[13]
146	2,4-Dichloronitrobenzene	611-06-3	192.0	+	[15,36]
147	2,6-Dichlorotoluene	118-69-4	161.0	–	[22]
148	1,3-Dicyanobenzene	626-17-5	128.1	–	[16]
149	1,4-Dicyanobenzene	623-26-7	128.1	–	[15]
150	Dicyclohexylcarbodiimide	538-75-0	206.3	–	[13]
151	Dicyclopentadiene	77-73-6	132.2	–	[15,37]
152	Dicyclopentylsilanediol	211495-85-1	200.4	–	[21]
153	1,4-Diethylbenzene	105-05-5	134.2	–	[14]
154	Diethylbiphenyl	28575-17-9	210.3	–	[26]
155	*N*,*N*-Diethyl-*m*-toluamide	134-62-3	191.3	–	[24]
156	Diheptyl phthalate	3648-21-3	362.5	–	[16]
157	2,3-Dihydroxypropyl 9-*cis*-octadecenoate	111-03-5	356.5	–	[26]
158	Diisopropylbenzene	25321-09-9	162.3	–	[18]
159	3,4-Dimethylaniline (3,4-Xylidine)	95-64-7	121.2	+	[15,57]
160	Dimethyl 2,6-naphthalenedicarboxylate	840-65-3	244.3	–	[17]
161	1,4-Dimethyl-2-(1-phenylethyl) benzene	6165-51-1	210.3	–	[22]
162	2,2-Dimethyl-1,3-propandediol	126-30-7	104.2	–	[13]
163	1,3-Diphenylguanidine	102-06-7	211.3	–	[20]
164	Diphenyl disulfide	882-33-7	218.3	–	[25]
165	Diphenyl 2-ethylhexyl phosphate	1241-94-7	362.4	–	[17]
166	Disodium succinate hexahydrate	6106-21-4	270.1	–	[22]
167	Ditridecyl phthalate	119-06-2	530.8	–	[18]
168	Divinylbenzene	1321-74-0	130.2	–	[18]
169	Docosanoic acid	112-85-6	340.6	–	[18]
170	4-Ethylbiphenyl	5707-44-8	182.3	–	[19]
171	2-Ethylhexyl methacrylate	688-84-6	198.3	–	[18]
172	2-Ethyl-2-hydroxymeth-1,3-propanediol	77-99-6	134.2	–	[13]
173	5-Ethylidene-2-norbornene	16219-75-3	120.2	–	[18]
174	Ethyl methyl ketoxime	96-29-7	87.1	–	[16]
175	4-Ethylmorpholine	100-74-3	115.2	–	[24]
176	2,2,4,4,6,8,8-Heptamethylnonane	4390-04-9	226.5	–	[13]
177	*n*-Hexadecane	544-76-3	226.5	–	[13]
178	2-Hydro-4-(octyloxy)benzophenone	1843-05-6	326.4	–	[16]
179	2-(2′-Hydroxy-3′,5′-di-*tert*-butylphenyl) benzotriazole	3846-71-7	323.4	–	[23]
180	2-Imidazolidinethione	96-45-7	102.2	+	[25]
181	Isocyanuric acid	108-80-5	129.1	–	[17,38]
182	4,4′-Isopropylidenebis (2,6-dibromophenol)	79-94-7	543.9	–	[20]
183	Lithium bromide	7550-35-8	86.9	–	[23]
184	Methacrylamide	79-39-0	85.1	–	[19]
185	4-Methoxybenzaldehyde	123-11-5	136.2	–	[20]
186	3-Methoxy-3-methyl-1-butanol	56539-66-3	118.2	–	[23]
187	4-Methylbenzenesulfonamid	70-55-3	171.2	–	[13]
188	Methyl dodecanoate	111-82-0	214.4	–	[16]
189	1-Methylethenylbenzene	98-83-9	118.2	–	[15]
190	4-(1-Methylethyl) aniline	99-88-7	135.2	+	[19]
191	2-(1-Methylethoxy) ethanol	109-59-1	104.1	–	[23]
192	2-Methyl-5-nitrobenzenesulfonic acid	121-03-9	217.2	+	[18]

Table 5 (*Continued*)

ID no.	Chemical name	CAS	MW	Ames test	Reference
193	3-Methyl-1,5-pentanediol	4457-71-0	118.2	–	[17]
194	4-Methy-1-pentene	691-37-2	84.2	–	[26]
195	4-(1-Methyl-1-phenylethyl) phenol	599-64-4	212.3	–	[21]
196	4-(1-Methylpropyl)phenol	99-71-8	150.2	–	[14]
197	Monosodium 4-amino-5-hydroxy-2,7-naphthalenedisulfonate	5460-09-3	341.3	–	[13]
198	1-Naphthol-4-sulfonic acid sodium salt	6099-57-6	160.2	–	[22]
199	2,2′-Nethylenebis(6-*tert*-butyl-*p*-cresol)	119-47-1	340.5	–	[16]
200	Nickel(II) carbonate hydroxide tetrahydrate	39430-27-8	376.2	–	[26]
201	Nonylphenol	25154-52-3	220.4	–	[16]
202	1-Octanethiol	111-88-6	146.3	–	[24]
203	*p*-*tert*-Octylphenol	140-66-9	206.4	–	[13]
204	*n*-Pentadecane	629-62-9	212.4	–	[13]
205	Pentaerythritol	115-77-5	136.2	–	[15]
206	Pentaerythritol tetra(2-ethylhexanoate)	7299-99-2	640.9	–	[26]
207	3-Phenoxytoluene	3586-14-9	184.3	–	[17]
208	Phthalocyanine Blue	147-14-8	576.1	–	[13]
209	Pigment Green No.7 (Hexadecachloro)	14832-14-5	1127.2	–	[14]
210	Pigment Green No.7 (Plychloro, unspecified)	1328-53-6	1127.2	–	[21]
211	Pigment Orange 16	6505-28-8	620.7	–	[21]
212	Potassium 7-hydroxy-1,3-naphthalenedisulfonate	842-18-2	380.5	–	[18]
213	Propylene glycol monomethyl ether acetate	108-65-6	132.2	–	[18]
214	Silicone nitride	12033-89-5	140.3	–	[23]
215	Sodium 4-amino-1-naphthalenesuifonate	130-13-2	245.2	–	[18]
216	Sodium 1-methoxycarbonylpentadecane-2-sulfonate	4016-24-4	372.5	–	[22]
217	Sodium 2-naphthol-3,6-disulfonate	135-51-3	348.3	–	[18]
218	Sodium 3-nitrobenzenesulfonate	127-68-4	225.2	–	[18]
219	Sodium *p*-toluenesulfonate	657-84-1	194.2	–	[21]
220	Tetrabromoethane	79-27-6	345.7	–	[23]
221	Tetrahydrofurfuryl alcohol	97-99-4	102.1	–	[24]
222	Tetrahydromethyl-1,3-isobenzofuranedione	11070-44-3	166.2	–	[17]
223	Tetrahydrothiophene 1,1-dioxide	126-33-0	120.6	–	[16]
224	Tetramethylammonium hydroxide	75-59-2	91.2	–	[20]
225	Tetrasodium monosilicate hydrate	13472-30-5	180.0	–	[26]
226	4,4′-Thiobis(6-*tert*-butyl-*m*-cresol)	96-69-5	358.5	–	[16]
227	3,3′-Thiobispropanoic acid	111-17-1	178.2	–	[24]
228	Thiophene	110-02-1	84.1	–	[16]
229	*o*-Toluenesulfonamide	88-19-7	171.2	–	[19]
230	*m*-Toluidine	108-44-1	107.2	–	[14]
231	Trifluoromethylbenzene	98-08-8	146.1	–	[16]
232	Triisobutylene	7756-94-7	168.3	–	[14]
233	1,2,3-Trimethylbenzene	526-73-8	120.2	–	[16]
234	1,2,4-Trimethylbenzene	95-63-6	120.2	–	[16]
236	2,2,4-Trimethyl-1,3-pentanediol diisobutyrate	6846-50-0	286.5	–	[14]
237	Trimethyl phosphate	512-56-1	140.1	–	[15,39]
238	Trimethylsilanol	1066-40-6	90.2	–	[18]
239	Trioctylbenzene-1,2,4-tricarboxylate	89-04-3	546.8	–	[22]
240	Triphenylchloromethane	76-83-5	278.8	–	[22]
241	Tripropylene glycol	24800-44-0	192.3	–	[14]
242	Tris(2-butoxyethyl) phosphate	78-51-3	398.5	–	[17]
243	Tris(*p*-cumenyl) phosphate	26967-76-0	452.6	–	[14]
244	Tris(2-ethylhexyl) 1,2,4-benzenetricarboxylate	3319-31-1	546.9	–	[16]
245	Tris(2-ethylhexyl) phosphate	78-42-2	434.6	–	[14]
246	1,3,5-Tris(2-hydroxyethyl)-1,3,5-triazine-2,4,6-(*1H,3H,5H*)-trione	839-90-7	261.2	–	[21]
247	1,1,1-Tris(hydroxymethyl)ethane	77-85-0	120.2	–	[18]
248	1,3,5-Tris(2-propenyl) isocyanuric acid	1025-15-6	249.3	–	[24]
249	Undecane	1120-21-4	156.3	–	[16]

(–): Negative; (+): positive; MW: molecular weight.

was observed at 10 mM when the pH of the culture medium was adjusted to about pH 7.0 by adding 1 N NaOH [18,54]. No structural alerts were identified by DEREK. The clastogenic effects observed might be due to low pH. As no information provided on pH in the culture medium in the initial phase and after the treatment, it cannot be concluded that the CAs observed are irrelevant due to low pH. The weight of evidence suggests that the level of concern is minimal.

ID85. 1-Naphthylacetic acid (CAS no. 86-87-3) [MW = 186]: 1-Naphthylacetic acid induced CAs (9.0% and 6.5%) at 9.1 mM (1.7 mg/mL) after 6-h treatment with and without S9 mix, and relative cell growth, as measured by monolayer confluence, was about 40% or 45%, respectively. The pH of the medium was 5.9 at the beginning of the treatment and 5.0 or 5.1 at the end [14]. No CA induction was observed at lower concentration of 2.5 or 4.8 mM; relative cell growth was about 100% or 70–80%, respectively. No structural alerts were identified by DEREK. A mouse bone marrow MN test was negative after intraperitoneal administration [61]. The CAs observed are considered to be irrelevant due to low pH, and it is supported by all other available data. Thus the level of concern is negligible.

3.2.1.2. High toxicity (six chemicals). A high toxicity effect was defined as responsible for CA induction only under severe cytotoxic conditions (*i.e.*, less than 50% relative cell growth compared to the negative control). Cytotoxicity measurements, in terms of relative cell growth, were based on confluence or on cell counts. Note that confluence is rather variable and inaccurate, and cell counts can underestimate toxicity. Information on the number of metaphases analyzed was presented, if available.

ID86. 1,3-Bis(2-methylphenyl) guanidine (CAS no. 97-39-2) [MW=239]: In two independent experiments, 1,3-Bis(2-methylphenyl) guanidine induced CAs weakly (7.0% or 9.0%) with S9 mix at the highest concentration of 2.5 mM (0.6 mg/mL) in which the relative cell growth, as measured by monolayer confluence, was about 44% or 35%, respectively [24]. It was negative in an *in vivo* mouse MN test after oral administration [42] and did not produce any DEREK structural alerts. However, a negative result in *in vivo* MN test in the bone marrow is not usually considered to be sufficient weight of evidence to overcome an *in vitro* CA positive result with S9 mix, as sufficient concentrations of reactive metabolite(s) may not reach the bone marrow to induce micronuclei. A genotoxicity test in the liver would be needed in addition to cover the detection of locally produced genotoxic metabolite(s). The data does not explain that the CAs observed *in vitro* are irrelevant due to high toxicity. There is insufficient weight of evidence to classify this as negligible level of concern, so we concluded that it is in the category of minimal level of concern.

ID87. tert-Butyl-methacrylate (CAS no. 585-07-9) [MW=142]: *tert*-Butyl methacrylate induced CAs (10.5%) without S9 mix at the highest concentration of 2.8 mM (0.4 mg/mL) at which the relative cell growth, as measured by survival cell count, was 40% [16]. DEREK showed a structural alert for alpha, beta-unsaturated esters or thioesters for chromosome damage. However, other methacrylates, 2-hydroxyethyl methacrylate (CAS no. 868-77-9, ID91) and 2-(dimethylamino)ethyl methacrylate (CAS no. 2867-47-2, ID69) were negative in the *in vivo* rat or mouse bone marrow MN test [44,53]. Methyl methacrylate (CAS no. 80-62-6) also was negative in the *in vivo* mouse bone marrow MN test by gavage and a mouse dominant lethal test by inhalation [62]. There is no evidence that methyl methacrylate administered by inhalation to rats and mice is carcinogenic [62] (see analysis on IDs91, 97, 98, 99). CA-induction by *tert*-butyl methacrylate might be associated with cytotoxicity. The level of concern is negligible.

ID88. o-Dichlorobenzene (CAS no. 95-50-1) [MW=147]: In two independent experiments, *o*-dichlorobenzene induced CAs weakly (6.5% or 5.5%) with S9 mix at the highest concentration of 1.6 mM (0.2 mg/mL) at which relative cell growth, as measured by monolayer confluence, was about 20% [20]. An *in vivo* CA test in rat bone marrow and DNA damage studies in rats were negative. A positive *in vivo* MN test in mouse bone marrow was not confirmed in a more recent, well-conducted study. Note that the negative *in vivo* bone marrow MN test may not necessarily define the lack of clastogenicity of this compound given that the *in vitro* result was S9-dependent and thus reactive metabolite(s) may not have reached the bone marrow at detectable concentrations. In a 2-year oral study in rats and mice, *o*-dichlorobenzene was considered not to be carcinogenic [41]. DEREK showed a structural alert for the carcinogenicity of polyhalogenated aromatics. A weak positive in CAs for this chemical can be outweighed by the lack of *in vivo* carcinogenicity, regardless of DEREK alerts. The CAs observed *in vitro* are considered to be irrelevant as they were only seen at high toxicity, and it is supported by all other available data. Thus the level of concern is negligible.

ID89. Dicyclohexylamine (CAS no. 101-83-7) [MW=181]: Dicyclohexylamine induced CAs (9.5%) after 6-h treatment without S9 mix at the highest concentration of 3.3 mM (0.6 mg/mL), at which the relative cell growth, as measured by monolayer confluence, was 34%. With S9 mix, CAs (13.5% at 4.4 mM (0.8 mg/mL) and 35.0% at 5.5 mM (1.0 mg/mL)) were induced at a relative cell growth of 60% and 35%, respectively [18]. No valid data are available on *in vivo* genotoxicity but a closely related structural analogue, *N*-methyl dicyclohexylamine, was negative in a battery of *in vivo* genotoxicity tests (CA test in rats, dominant lethal test in rats, sperm abnormality test in mice, and Drosophila sex-linked recessive lethal test). Dicyclohexylamine is not mutagenic but clastogenic *in vitro* and, based on *in vivo* data from a structurally closely related analogue, is anticipated to be non-genotoxic *in vivo* [43]. The chemical did not produce any DEREK structural alerts. There was considerable toxicity associated with the aberration induction. Overall the weight of evidence suggests the level of concern is negligible.

ID90. N-Ethylaniline (CAS no. 103-69-5) [MW=121]: *N*-Ethylaniline induced CAs (25.0%) after 6-h treatment without S9 mix at the highest concentration of 9.1 mM (1.1 mg/mL), at which the relative cell growth, as measured by monolayer confluence, was 40% [15]. The lower concentrations did not induce CAs (0.5% at 2.5 mM, 2.0% at 5 mM); relative cell growths at both concentrations were about 100%. It did not produce any DEREK structural alerts. The CAs observed might be due to high toxicity. However, there is no supporting evidence to reduce the level of concern; thus minimal concern still exists.

ID91. 2-Hydroxyethyl methacrylate (CAS no. 868-77-9) [MW=130]: 2-Hydroxyethyl methacrylate induced CAs after 24-h treatment without S9 mix (4.0% at 5 mM and 63.3% at 10 mM (1.3 mg/mL)). The relative cell growth, as measured by monolayer confluence, was 65% or 47% at 5 mM or 10 mM, respectively. However, only 177 cells were analyzed at 10 mM due to cytotoxicity. In the preliminary cell growth inhibition test, there was a steep dose response for inhibition between 0 and 2.5 mM (100–55%) and stable inhibition of about 50% between 2.5 and 10 mM. The cytotoxicity assessment is possibly not very exact. With S9 mix, CAs (11.0%) were induced at 10 mM; the relative cell growth was 58% [17]. The chemical did not induce MN in rat bone marrow up to the maximum tolerated dose [44]. Thus, the chemical is not genotoxic *in vivo*. DEREK showed a structural alert of alpha, beta-unsaturated esters or thioesters for chromosome damage. Many methacrylates induced CA *in vitro*, but not MN *in vivo* (see IDs 87, 97, 98, 99). Methyl methacrylate (CAS no. 80-62-6) was not carcinogenic to rats and mice [62]. Many methacrylates cause aberrations that seem to be associated with cytotoxicity. The level of concern is negligible.

3.2.1.3. Precipitation coupled with high toxicity (two chemicals). A precipitation effect was defined for these compounds as CA induction was only seen under precipitating concentrations at the end of treatment. Solubility of the test chemical is now a limiting factor for selecting the upper test concentration. It is agreed currently that not more than one precipitating concentration should be tested [12,63].

ID92. 4-Methylbenzoic acid (CAS no. 99-94-5) [MW=136]: 4-Methylbenzoic acid induced CAs (7.0% at 8.8 mM (1.2 mg/mL) and 13.0% at 10.3 mM) after 6-h treatment with S9 mix; the relative cell growth, as measured by survival cell count, was 34% or 24% [24]. Without S9 mix, CAs (11.0% or 9.5%) were induced at concentrations above 10 mM after 6- or 24-h treatment; the relative cell growth was 14% or 8%, respectively. Precipitation of the test chemical was observed at ≥1.8 mM (≥0.25 mg/mL, *i.e.*, at all concentrations tested) with S9 mix or at ≥7.4 mM (1.0 mg/mL) without S9 mix at the end of the treatment; this is not desirable as persistent precipitations may cause additional cytotoxicity. The test concentrations used were not suitable as defined by recent guidance [12,63]. An *in vivo* mouse MN test was negative up to 2000 mg/kg after oral administration. The chemical is not likely to be genotoxic *in vivo* [45]. It did not produce any DEREK structural alerts. Thus, the CAs observed are considered to be irrelevant due to precipitation and following high toxicity. The level of concern is negligible.

ID93. Triphosphoric acid aluminium salt (CAS no. 13939-25-8) [MW=318]: In the two independent experiments, triphosphoric acid aluminium salt induced CAs (33.0% or 11.0%) with S9 mix at the highest concentration of 6.3 mM (2 mg/mL); the relative cell growth, as measured by survival cell count, was 10% or 38%, respectively [23]. Precipitation was observed at 3.2 mM (1 mg/mL) or

more, *i.e.*, at all concentrations tested, at the end of the treatment. It did not produce any DEREK structural alerts. The test concentrations used were not suitable as defined by recent guidance [12,63]. The CAs observed are considered to be irrelevant due to precipitation and following high toxicity. The level of concern is negligible.

3.2.2. Weak evidence for a positive (two chemicals)

Very weak (less than 5% cells with CAs) but statistically significant increase in CAs were observed for some chemicals. There were cases that were positive in the original "call" would be assigned as positive even if there were less than 5% cells with CAs (*i.e.*, within negative criteria). These positive "calls" were outside the normal criteria, but were based on a dose relation and/or reproducibility. The relevancy of CA-induction and biological significance should be considered, taking into account historical control data.

ID94. 4,4′-Sulfonyldiphenol (CAS no. 80-09-1) [MW=250]: 4,4′-Sulfonyldiphenol induced CAs weakly (4.5%) after the 24-h treatment without S9 mix at the highest concentration of 1.6 mM (0.4 mg/mL) in which the relative cell growth, as measured by monolayer confluence, was 38% [19]. The effect at 1.6 mM was statistically significant, but at lower concentration it was not (1% at 0.8 mM). It did not produce any DEREK structural alerts. The effect of CA-induction might meet the minimal criteria for a positive; however, a confirmation test for reproducibility was not conducted. The CAs observed are not considered to be of biological significant, and thus the level of concern is negligible.

ID95. 1,3,5-Tris(3,5-di-tert-butyl-4-hydroxybenzyl)isocyanuric acid (CAS no. 27676-62-6) [MW=784]: 1,3,5-Tris(3,5-di-*tert*-butyl-4-hydroxybenzyl)isocyanuric acid did not induce any structural CAs but it did induce slight but statistically significant ($p < 0.01$, based on 800 cells per concentration) increases in polyploidy (1.1–3.4% at 3.2-6.4 mM) after 6- (1.1% or 3.4% at 3.2 or 6.4 mM (5 mg/mL)) or 24-h (1.4% or 3.1% at 3.2 or 6.4 mM) treatment without S9 mix [24]. No severe cytotoxicity was observed at any treatment (68–99% of relative cell growth measured by monolayer confluence). On the other hand, no polyploidy induction was observed after 6-h treatment with S9 mix up to 6.4 mM. Though no information was given on the historical control range of polyploidy in the test laboratory, Sofuni [64] reported that it is less than 1% in general (0.5–1.8% in the different treatment length) in CHL cells. As 800 cells per concentration were analyzed for polyploidy in this study, statistical power is higher than that of analysis of 100–200 cells. The mode of action of polyploidy induction will be through a non-DNA target. Biological significance of this very weak effect might be low. However, polyploidy induction may be evidence that aneuploidy could be induced. The suggestion might be that it would be valuable to carry out a micronucleus test *in vitro* to see if indeed aneuploidy is induced. Although this effect will have a threshold, it could still be a significant genotoxic effect. Thus, the level of concern is minimal.

3.2.3. Possible other factors (21 chemicals)

3.2.3.1. Induction of polyploidy only (one chemical). One chemical showed a relatively high frequency of polyploidy (>20%) without induction of structural CAs. *In vivo* relevance of polyploidy induction was evaluated based on review of the literature.

ID96. 1,2-Dicyanobenzene (CAS no. 91-15-6) [MW=128]: 1,2-Dicyanobenzene did not induce structural CAs but caused polyploidy in the 24- (1.6% or 4.1% at 3.2 or 6.3 mM (0.8 mg/mL), respectively) or 6-h treatment (1.1%, 8.6% or 9.5% at 2.5, 5 or 10 mM (1.3 mg/mL), respectively) without S9 mix, and in the 6-h treatment (5.1%, 26.1% or 24.4% at 2.5, 5 or 10 mM, respectively) with S9 mix [20]. The frequencies of polyploidy shown were statistically significant ($p < 0.01$, based on 800 cells per concentration). Relative cell growth, as measured by monolayer confluence, was 44% to 88% at the above mentioned concentrations. No structural alerts were identified by DEREK, but it is not clear whether DEREK is effective in predicting polyploidy induction or not. However, 1,2-dicyanobenzene did not show any cytogenetic effects in the *in vivo* MN test. The negative result of *in vivo* MN test demonstrates that the genotoxic potential or risk would be low in rodents [46]. The mode of action of polyploidy induction will be through a non-DNA target. The weight of evidence suggests that the level of concern is negligible.

3.2.3.2. Selected chemical class with DNA reactivity (four chemicals). Clastogenicity *in vitro* of methacrylate or alkoxy silane was considered to be the DNA reactivity and/or cytotoxicity based on the data from structurally related chemicals or literature, with some exceptions.

ID97. 2-(Diethylamino)ethyl methacrylate (CAS no. 105-16-8) [MW=185]: 2-(Diethylamino)ethyl methacrylate induced CAs only with S9 mix (24.0% at 3.2 mM (0.6 mg/mL)); relative cell growth, as measured by monolayer confluence, was 50% [18]. DEREK showed a structural alert of alpha, beta-unsaturated ester or thioester for chromosome damage, and TIMES showed an alert of CA induction by a metabolite(s); unsaturated aldehyde can interact with topoisomerases/proteins, which is not a DNA-reactive mechanism and thus may have a threshold. Other methacrylates including *tert*-butyl-ethacrylate (CAS no. 585-07-9, ID87) and 2-hydroxyethyl methacrylate (CAS no. 868-77-9, ID91) are discussed in the section on high toxicity (see 3.2.1.2.), and the latter was negative in the *in vivo* rat bone marrow MN test [44]. Butyl methacrylate (CAS no. 97-88-1, ID131) and 2-ethylhexyl methacrylate (CAS no. 688-84-6, ID171) were negative in the *in vitro* CA test (Table 5). Although 2-(dimethylamino)ethyl methacrylate (CAS no. 2867-47-2, ID69), is a closely related analogue of this compound (ID97), it was positive in the Ames test (for only TA 1537 at 2500 μg/plate without S9 mix) and *in vitro* CA tests when tested as part of the Japanese HPV chemicals project. Other Ames tests for ID69 carried out in accordance with OECD test guidelines and to GLP standards, was negative and two *in vivo* mouse bone marrow MN tests by intraperitoneal or oral administration gave negative results [53]. Thus, 2-(dimethylamino)ethyl methacrylate (ID69) is not considered to be genotoxic *in vivo* [53]. Many methacrylate induced CAs *in vitro* but not MN *in vivo* (see IDs 87, 91, 98, 99), and methyl methacrylate (CAS no. 80-62-6) was not carcinogenic to rats and mice [62].So, CA-induction might be associated with the DNA reactivity *in vitro* and/or cytotoxicity of methacrylate. The level of concern of this chemical (ID97) is negligible, based on the weight of evidence.

Note that a methacrylate compound, 2,3-epoxypropyl methacrylate (CAS no. 106-91-2, ID31) was positive in the Ames, *in vitro* CA, and *in vivo* MN tests [35]. The positive results are likely to be due to the epoxy moiety.

ID98. Methacrylic acid, monoester with propane-1,2-diol (CAS no. 27813-02-1) [MW=144]: Methacrylic acid, monoester with propane-1,2-diol, induced CAs after 24-h treatment without S9 mix (16.5% at 5 mM (0.7 mg/mL)) and after 6-h treatment with S9 mix (21.0 and 68.5% at 5 and 10 mM, respectively) [16], in which it was expressed as 2-hydroxypropyl methacrylate, CAS no. 923-26-2]. Relative cell growth, as measured by monolayer confluence, was about 60% or 35% at 5 or 10 mM with S9 mix, respectively. DEREK showed a structural alert for alpha, beta-unsaturated ester or thioester for chromosome damage. TIMES also showed two alerts for CA-induction for both the parent chemical and metabolite(s): (1) acrylates interact with DNA and topoisomerases/proteins, and (2) unsaturated aldehydes interact with topoisomerases/proteins. Many methacrylate induce CAs *in vitro* (see IDs 87, 91, 97, 99), so the CA-induction seen might be associated with the DNA reactivity *in vitro* and/or cytotoxicity of methacrylate. TIMES showed alerts for DNA-reactive mode of action, however, other

methacrylates, 2-hydroxyethyl methacrylate (CAS no. 868-77-9, ID91) and 2-(dimethylamino)ethyl methacrylate (CAS no. 2867-47-2, ID69) and methyl methacrylate (CAS no. 80-62-6) were negative in the *in vivo* rat or mouse bone marrow MN test [44,53,62]. Methyl methacrylate was not carcinogenic to rats and mice [62]. Thus, the weight of evidence suggests that the level of concern is negligible for this methacrylate (ID98).

ID99. (Methacryloyloxyethyl)trimethylammonium chloride (CAS no. 5039-78-1) [MW = 208]: (Methacryloyloxyethyl) trimethylammonium chloride induced CAs (10.5%) at 10 mM (2.1 mg/mL) after 24-h treatment without S9 mix; relative cell growth, as measured by monolayer confluence, was 86% [21]. DEREK showed a structural alert for alpha, beta-unsaturated ester or thioester for chromosome damage. TIMES showed an alert for CA-induction for both the parent chemical and metabolite(s): alkylamino betaines theoretically interact with DNA or topoisomerases/proteins (see ID97, 2-(diethylamino)ethyl methacrylate). Many methacrylates induce CAs *in vitro* (see IDs 87, 91, 97, 98), so the CA-induction seen might be associated with the DNA reactivity of methacrylate. Other two methacrylates (IDs 69, 91) and methyl methacrylate (CAS no. 80-62-6) were negative in the *in vivo* rat or mouse bone marrow MN test [44,53,62], and methyl methacrylate was not carcinogenic to rats and mice [62], as described in the above section. The weight of evidence suggests that the level of concern is negligible.

ID100. Ethenyltrimethoxysilane (CAS no. 2768-02-7) [MW = 148]: Ethenyltrimethoxysilane induced CAs at 5 and 10 mM (1.5 mg/mL) only with S9 mix (31.5% and 58.0%, respectively) [26]. No cytotoxicity was observed; relative cell growth, as measured by survival cell count, was about 100%. TIMES showed an alert of CA-induction due to possible epoxide and aziridine metabolite(s); these can interact with topoisomerases/proteins. Many epoxides and aziridines can react also with DNA. DEREK showed no structural alert. Another alkoxy silane, [3-(methacryloxy)propyl]trimethoxysilane (CAS no. 2530-85-0), did not induce mutations in bacteria. In the CA test *in vitro*, the substance (CAS no. 2530-85-0) was weakly clastogenic without S9 mix and produced concentration-dependent clastogenic effects with S9 mix. It was negative in an *in vivo* erythrocyte MN test [65]. However, negative *in vivo* MN test will be not sufficient evidence for in *vitro* CA-positive only with S9 mix. Another alkoxy silane, methyltriethyoxylsilane (CAS no. 2031-67-6), was non-mutagenic in bacteria; the compound induces CAs weakly *in vitro* with and without S9 mix [66], maybe reflecting the DNA reactivity of metabolite(s) of alkoxy silane. The weight of evidence suggests the level of concern is minimal.

3.2.3.3. Others (16 chemicals). Remaining 16 chemicals were not categorized for possible factors of effects to the relevance of CA results.

ID101. 2-Chlorophenol (CAS no. 95-57-8) [MW = 129]: 2-Chlorophenol induced CAs at 3.9 mM (0.5 mg/mL) after 6-h treatment without S9 mix (34.0%) and at 2.0 and 3.9 mM after 6-h treatment with S9 mix (12.0% and 38.6%, respectively) [20]. Relative cell growth measured by survival cell count was 25% at 3.9 mM without S9 mix, and 90% or 30% at 2 or 3.9 mM with S9 mix, respectively. DEREK showed a halophenol structural alert for chromosome damage, but TIMES did not. A mouse bone marrow MN test was negative after oral administration [67], and tumor incidence, latency, and type did not differ between rats given the chemical in drinking water and controls [68]. This chemical will be a good example of a strong *in vitro* CA inducer without carcinogenicity. The level of concern is negligible.

ID102. C.I. Fluorescent brightener 271 (CAS no. 41267-43-0) [MW = 1347]: C.I. Fluorescent brightener 271 weakly induced 4.0% and 9.0% CAs after 6-h treatment without S9 mix at 1.9 and 3.7 mM (5 mg/mL), respectively. Relative cell growth, as measured by monolayer confluence, was 82% at 3.7 mM [26]. No CAs were observed up to 5 mg/mL with S9 mix; relative cell growth was 60%. Structural related compounds, C.I. fluorescent brighteners 24 (CAS no. 12224-02-1), 225 (CAS no. 24019-80-5) and 260 (CAS no. 16090-02-1), were negative in *in vitro* CA tests using CHL cells [64]. However, there is no supporting evidence in *in vivo* to reduce level of concern, thus minimal concern still remains.

ID103. 1,4-Dibromobenzene (CAS no. 106-37-6) [MW = 236]: 1,4-Dibromobenzene induced CAs only with S9 mix (9.0%, 6.0%, and 17.3% at 2.3, 4.7, and 9.3 mM (2.2 mg/mL), respectively) [14]. Relative cell growth, as measured by monolayer confluence, was about 55%, 65% or 50% at 2.3, 4.7 or 9.3 mM, respectively. However, the number of cells analyzed was only 127 at 9.3 mM due to cytotoxicity. TIMES indicated an alert of CA-induction due to possible epoxide and aziridine metabolite(s); these can interact with topoisomerases/proteins, which cause CAs. Many epoxides and aziridines react also with DNA. DEREK did not show any structural alert. In addition, an *in vivo* MN test was negative for a closely related structural analogue, 1,4-dichlorobenzene (CAS no. 106-46-7, a non-genotoxic carcinogen) [69]. The weight of evidence suggests the level of concern is negligible.

ID104. Dibutyl adipate (CAS no. 105-99-7) [MW = 258]: Dibutyl adipate induced CAs only with S9 mix [15,48]. The response was weak without a clear concentration relationship (5.5%, 11.0%, and 3.0% at 2.5, 5, and 10 mM (2.6 mg/mL), respectively). Relative cell growth, as measured by monolayer confluence, was about 95%, 55% or 45% at 2.5, 5, or 10 mM, respectively. No structural alerts were shown by DEREK and TIMES. However, there is no supporting evidence to reduce the level of concern, thus some concern remains.

ID105. 2-(Di-n-butylamino)ethanol (CAS no. 102-81-8) [MW = 173]: 2-(Di-n-butylamino)ethanol induced CA (23.5%) after 6-h treatment without S9 mix at the highest concentration of 10 mM (1.7 mg/mL); relative cell growth, as measured by monolayer confluence, was 44%. CAs were also induced with S9 mix (7.0%, 30.0%, and 96.0% at 1.9, 3.8, and 7.5 mM, respectively); relative cell growth was 85%, 67%, and 50%, respectively [25]. No structural alerts were shown by DEREK and TIMES. A structurally related analogue, 2-aminoethanol (CAS no. 141-43-5), was negative in the Ames test, SHE cell transformation assay, and the *in vivo* mouse MN test with oral administration [70]. Other structurally related analogue, 2-diethylaminoethanol (CAS no. 100-37-8), was also negative in the Ames test, HPRT mutation assay with V79 cells, and the *in vivo* mouse MN test with oral administration [71]. 2-Diethylaminoethanol was not carcinogenic to rats by feed in a limited 2-year study from the 1960s [71]. The weight of evidence suggests that the level of concern is negligible.

ID106. N,N-Dimethylbenzylamine (CAS no. 103-83-3) [MW = 135]: *N,N*-Dimethylbenzylamine induced CAs only with S9 mix (53.0% and 56.5% at 2.8 and 5.6 mM (0.75 mg/mL), respectively); relative cell growth, as measured by monolayer confluence, was about 85% or 50% at 2.8 or 5.6 mM, respectively [17]. TIMES showed an alert for CA-induction due to possible benzylamine metabolite(s) which theoretically interact with DNA. DEREK did not show any structural alerts, however. No genotoxic activity was observed in the mouse MN test [72]. However, negative *in vivo* MN test will be not sufficient evidence for in *vitro* CA-positive only with S9 mix. The weight of evidence suggests the level of concern is minimal.

ID107. 2,4-Dinitrophenol (CAS no. 51-28-5) [MW = 184]: 2,4-Dinitrophenol induced CAs after 6-h treatment without S9 mix (11.5% and 23.0% at 6.5 and 8.2 mM (1.5 mg/mL), respectively) and with S9 mix (17.0%, 22.5% and 18.0% at 6.5, 8.2 and 10 mM, respectively) [20]. Relative cell growth, as measured by monolayer confluence, was 45% or 39% at 6.5 or 8.2 mM without S9 mix, and 49%, 35% or 26% at 6.5, 8.2 or 10 mM with S9 mix, respectively. DEREK showed structural alerts for carcinogenicity

by aromatic nitro compounds and CA-induction by polynitrophenol or precursor. TIMES showed two alerts for CA-induction by both parent chemical and metabolite(s): (1) nitro compounds interact with DNA, and (2) amines, aminophenols, phenyleneamines or hydroxylamines interact with DNA and topoisomerases/proteins. 2,4-Dinitrophenol acts as a metabolic poison by uncoupling oxidative phosphorylation, and this mechanism will have a threshold. It reduced ATP level and induced CAs in CHO and TK cells at cytotoxic concentrations *in vitro* [73]. Dinitrophenol is recognized as a chemical which shows clastogenicity by indirect mechanism, *i.e.*, energy depletion [74]. The weight of evidence suggests the level of concern is negligible.

ID108. 2-Ethylbutyric acid (CAS no. 88-09-5) [MW = 116]: 2-Ethylbutyric acid induced CAs after 24-h treatment without S9 mix (5.5%, 5.0%, and 17.0% at 3.4, 6.9, and 10.3 mM (1.2 mg/mL), respectively); relative cell growth, as measured by survival cell count, was 94%, 83% or 62%, respectively [21]. A mouse bone marrow MN test was negative [22]. These data indicate that this chemical is not mutagenic *in vivo* [35]. The level of concern is negligible.

ID109. Ferrous sulfate heptahydrate (CAS no. 7782-63-0) [MW = 278]: In the two independent experiments, ferrous sulfate heptahydrate induced CAs after 6-h treatment without S9 mix (19.0% and 39.0% at 5.4 mM (1.5 mg/mL)); relative cell growth, as measured by survival cell count, was 45% and 12%, respectively. Reproducible CA-induction was also observed in the treatments with S9 mix (in the first test, 9.0% and 72.5% at 1.8 (0.5 mg/mL) and 3.6 mM in which relative cell growth was 82% and 45%, respectively; in the second test, 23.0–85.5% at 3.2-5.4 mM in which relative cell growth was 59–19%, respectively) [23]. DEREK did not show any structural alerts. Iron salts are known to induce genotoxicity due to the Fenton reaction and production of oxygen radicals, a mechanism with a threshold [75]. *In vivo*, ferrous sulfate heptahydrate and the other iron salt, ferric chloride hexahydrate (CAS no. 10025-77-1), did not induce micronuclei in the digestive tract including stomach, duodenum and colon after oral administration [47,76]. A mouse bone marrow MN test for ferrous chloride was negative after intraperitoneal injection [47]. No increase in tumor incidence was reported for rats ingesting ferric chloride in drinking water for 2 years [47]. The weight of evidence suggests the level of concern is negligible.

ID110. 2-Hydroxypropanenitrile (CAS no. 78-97-7) [MW = 71]: 2-Hydroxypropanenitrile induced CAs weakly (10.0% and 9.5%) after 6-h treatment with and without S9 mix at 10 mM (0.7 mg/mL), respectively [13,50]. Relative cell growth, as measured by monolayer confluence, was about 65% at 10 mM with S9 mix. No structural alerts were shown by DEREK and TIMES. There is no supporting evidence for a reduced level of concern, so some concern still remains.

ID111. 2-Mercaptobenzimidazole (CAS no. 583-39-1) [MW = 150]: 2-Mercaptobenzimidazole induced CAs only with S9 mix (11.0% and 11.5% at 5.3 and 10 mM (1.5 mg/mL), respectively) [15]. Relative cell growth, as measured by monolayer confluence, was about 85–95% at 2.5–10 mM. DEREK showed a structural alert for mutagenicity due to a benzimidazole moiety, but that chemical was negative in the Ames test. An alert for CA-induction due to 2-thiobenzimidazole or -benzothiazole was also shown. TIMES showed an alert for CA-induction for both parent chemical and metabolite(s): thiols interact with topoisomerases/proteins. There was no evidence of MN induction in the mouse peripheral blood MN test in a 13-week inhalation study [77]. However, *in vivo* long term MN test by inhalation route will not have resulted in much systemic exposure, compared to an acute MN test by oral or intraperitoneal routes. In addition, the *in vivo* erythrocyte MN test is not definitive as the *in vitro* result was S9-dependent and thus reactive metabolite(s) may not have reached the bone marrow in sufficient concentrations to elicit an effect. The level of concern is minimal.

ID112. N-Methylaniline (CAS no. 100-61-8) [MW = 107]: *N*-Methylaniline induced CAs after 24-h treatment without S9 mix (15.0% and 18.2% at 5.5 and 10 mM (1.1 mg/mL), respectively) and after 6-h treatment with S9 mix (12.4% at 10 mM) [15]. Relative cell growth, as measured by monolayer confluence, was about 50% at 10 mM with S9 mix. However, the number of cells analyzed were only 177 or 148 at 10 mM with or without S9 mix, respectively. DEREK did not show any structural alerts, but TIMES showed an alert for CA induction due to possible formation of hydroxyl amine metabolite(s), which can interact with DNA. *N*-Methylaniline yields aniline (CAS no. 62-53-3) in rat and rabbit [78], and aniline induces MN in mice and rats [79]. Aniline is assigned to carcinogen category 2 in the Globally Harmonised System of Classification and Labeling of Chemicals (GHS) classification by the EU regulation [80]. Though *N*-ethylaniline (CAS no. 103-69-5, ID90), a closely related structural analogue, was discussed in a section of the effect of high toxicity (see Section 3.2.1.2.), the definition is not suitable for *N*-methylaniline. Thus, the some level of concern remains. Note that there is a question as to whether aniline is a genotoxic carcinogen, and MN induction may be secondary to methemoglobinemia and regenerative anemia [81].

ID113. p-Nitrophenol sodium salt (CAS no. 824-78-2) [MW = 161]: *p*-Nitrophenol sodium salt induced CAs after 6-h treatment without S9 mix (7.5% and 28.0% at 5 and 7.5 mM (1.2 mg/mL), respectively) and with S9 mix (11.5%, 19.0%, 33.5%, and 48.0% at 3.8, 5.0, 6.3, and 7.5 mM, respectively) [21]. Relative cell growth, as measured by monolayer confluence, was 66% or 35% at 5 or 7.5 mM without S9 mix, and 80%, 80%, 61% or 42% at 3.8, 5, 6.3, or 7.5 mM, respectively. TIMES showed three structural alerts for CA-induction for both parent chemical and possible metabolite(s): (1) nitro compounds interact with DNA, (2) amines, aminophenols, or phenyleneamines interact with DNA or topoisomerases/proteins, (3) hydroxylamines interact with DNA. These alerts should be also Ames-positive but *p*-nitrophenol is Ames-negative. DEREK did not show any structural alerts. In addition, *p*-nitrophenol (CAS no. 100-02-7, free base of the chemical) was negative in an *in vivo* mouse bone marrow MN test with intravenous treatment [82]. The weight of evidence suggests the level of concern is negligible.

ID114. Sorbitan monooctadecanoate (CAS no. 1338-41-6) [MW = 431]: Sorbitan monooctadecanoate induced CAs with S9 mix (21.0%, 26.0%, and 45.5% at 2.5, 5, and 10 mM (4.3 mg/mL), respectively) in which relative cell growth, as measured by monolayer confluence, was about 85%, 80% or 70%, respectively [16]. No structural alerts were shown by DEREK and TIMES. There was no evidence of carcinogenic potential in rats and mice [83]. The weight of evidence suggests the level of concern is negligible.

ID115. Trimethoxyphosphine (CAS no. 121-45-9) [MW = 124]: Trimethoxyphosphine induced CAs at the highest concentration of 10 mM (1.2 mg/mL) with 24-h treatment without S9 mix (4.5%) and with 6-h treatment with S9 mix (7.0%) [19]. Relative cell growth, as measured by survival cell count, was about 85%, 80% or 70%, respectively. No structural alerts were shown by DEREK and TIMES. There is no supporting evidence for a reduced level of concern. Thus, the some level of concern remains.

ID116. Trimethylamine (CAS no. 75-50-3) [MW = 59]: Trimethylamine induced CAs after 6-h treatment without S9 mix (9.0%, 22.5%, and 22.5% at 6.4, 8, and 10 mM (0.6 mg/mL), respectively) and with S9 mix (2.0%, 5.5%, and 45.0% at 6.4, 8, and 10 mM, respectively) [20]. Relative cell growth, as measured by monolayer confluence, was 42%, 23% or 6% without S9 mix, or 52%, 42% or 17% with S9 mix, respectively. Extremely toxic doses (less than 25% relative cell growth) increased the frequencies of CAs. A close analogue, dimethylamine (CAS no. 124-40-3), was negative in the standard Ames test, *in vitro* CA test with CHL cells, and *in vivo* rat bone marrow CA test by inhalation for 3 months, examined 15 and 90 days after the end of exposure [84]. However, *in vivo* long term bone

Table 6
Evaluation of level of concern for human health risk assessment on 38 "missed" chemicals.

Possible factors of irrelevant positives	Number of chemicals with different level of concern (Chemical ID)		
	Negligible	Minimal	Some
1. Possible effects of extreme culture conditions (n=15)			
1.1 Low pH (n=7)	6 (IDs 79,80,81,82,83,85)	1 (ID 84)	0
1.2 High toxicity (n=6)	4 (IDs 87,88,89,91)	2 (IDs 86,90)	0
1.3 Precipitation coupled with high toxicity (n=2)	2 (IDs 92,93)	0	0
2. Weak evidence for a positive (n=2)	1 (ID 94)	1 (ID 95)	0
3. Possible other factors (n=21)			
3.1 Induction of polyploidy only (n=1)	1 (ID 96)	0	0
3.2 Selected chemical class with DNA reactivity (n=4)	3 (IDs 97,98,99)	1 (ID 100)	0
3.3 Others (n=16)	8 (IDs 101,103,105, 107,108,109,113,114)	4 (IDs 102,106,111,116)	4 (IDs 104,110,112,115)
Total (n=38)	25	9	4

marrow CA test by inhalation route may not have given much systemic exposure, compare than acute CA test by oral or intraperitoneal route. The level of concern is minimal.

3.3. *Level of concern for human health risk assessment on 38 "missed" chemicals*

The result of evaluation of the level of concern was summarised in Table 6. Among 38 missed chemicals, four were considered to be of some concern, or nine were considered to be of minimal concern, and remaining 25 were considered to be of negligible concern. Note that the "of some concern" classification is in most cases due to the absence of relevant additional data, and not to available data that suggest a real concern.

3.4. *Application of different top concentrations to the "missed" chemicals*

The results of application of several top concentration limits to the missed chemicals are shown in Table 7. It would be preferable that the top concentration limit detects the 13 missed chemicals with minimal or some concern and does not detect the 25 missed chemicals with negligible concern. The numbers of chemicals detected at 1 mM or 0.5 mg/mL, whichever is higher, 2 mM or 1 mg/mL, whichever is higher, 4 mM or 2 mg/mL, whichever is lower, and 10 mM or 2 mg/mL, whichever is lower were 2, 8, 3 and 11 for 13 chemicals with some or minimal concern, and 9, 17, 14 and 23 for 25 chemicals with negligible concern, respectively. The top concentration of 2 mM or 1 mg/mL, whichever is higher is the most effective concentration, *i.e.*, relatively higher (8/13) or lower (17/25) detection number among 13 or 25 chemicals, respectively. On the other hand, 1 mM or 0.5 mg/mL, whichever is higher, was not effective (2/13) for detection of 13 chemicals with concern for this data set. The highest concentration of 10 mM or 2 mg/mL, whichever is lower, was good detection (11/13) of 13 chemicals with concern; however, it detected almost all (23/25) of 25 chemicals with negligible concern. Other top concentration employed of 4 mM or 2 mg/mL, whichever is lower, was not effective (3/13) for detection of 13 chemicals with concern.

4. Discussion

In this analysis of 249 HPV chemicals tested in the *in vitro* CA test with CHL cells in accordance with Japanese or OECD test guidelines, we singled out 38 chemicals that were positive for CAs at >1 mM but negative at ≤1 mM and negative in the Ames test—chemicals that would be missed in the standard genotoxicity test battery if the highest concentration tested were 1 mM. Based on weight of evidence approach, including evaluations of effects of extreme culture conditions (low pH, high toxicity, or precipitation), *in silico* structural alert analysis, *in vivo* genotoxicity and carcinogenicity test data, mode of action, or information from closely related chemicals, we evaluated the level of concern for human health risk assessment on 38 "missed" chemicals. After an exhaustive review, we identified four chemicals with some concern, nine with minimal concern, and remaining 25 with negligible concern. Several proposals to reduce the top concentration in *in vitro* mammalian cell genotoxicity tests have been made [4,5,12]. Those are as follows: (1) 1 mM or 0.5 mg/mL, whichever is lower, (2) 1 mM or 0.5 mg/mL, whichever is higher, (3) 4 mM or 2 mg/mL, whichever is lower, and (4) 10 mM or 2 mg/mL, whichever is lower. Item (1) is for pharmaceuticals, but the following note is also added; for pharmaceuticals with unusually low molecular weight (*e.g.*, less than 200) higher test concentrations should be considered [12]. The other items are for industrial chemicals. Note that a large percentage of these industrial chemicals had molecular weights of ≤200, with some notable exceptions. On the other hand, such a reduction runs the risk of eliminating genotoxic agents in the hazard identification stage [2]. Thus, several top concentration limits including 2 mM or 1 mg/mL, whichever is higher, were applied to 38 missed chemicals. It will be preferable that the top test concentration allows the detection of 13 chemicals with minimal or some concern, but cannot detect 25 chemicals with negligible concern. The top concentration of 2 mM or 1 mg/mL, whichever is higher, is most effective, *i.e.*, relatively higher (8/13) or lower (17/25) detection among 13 or 25 chemicals, respectively. Other top concentration, 1 mM or 0.5 mg/mL, whichever is higher [4], was not effective (2/13) for detecting chemicals with concern, but good (*i.e.*, low, 9/25) for chemicals with negligible concern. The other two top concentrations (4 mM or 2 mg/mL, whichever is lower, and 10 mM or 2 mg/mL, whichever is lower) did not show enough response to one of both groups of chemicals; 10 mM or 2 mg/mL, whichever is lower, detected almost all (23/25) chemicals with negligible concern, and 4 mM or 2 mg/mL, whichever is lower, was not effective (3/13) for 13 chemicals with concern. Therefore, we propose 2 mM or 1 mg/mL, whichever is higher, as the top concentration limit for industrial chemicals. If the top concentration were reduced to 2 mM or 1 mg/mL, whichever is higher, the percent of positives would be reduced to 37.8% (94/249) in the dataset of 249 HPV chemicals; current percent of positives was 46.6% (116/249) including 6 chemicals positive at >10 mM. Approximately 80% (204/249) of the analyzed chemicals had molecular weight <300; this means that more than 3.3 mM will be selected as top concentration of 1 mg/mL for majority of chemicals in the dataset (Table 8). In case of chemicals with molecular weight of >1000, top concentration of more than 2 mg/mL will be selected.

Conclusion from our analysis is not based on the carcinogenicity data, unlike in the case of analysis by Parry or Kirkland [3,4]; unfortunately, our dataset did not contain sufficient