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[e]	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

Table 7
Application of different top concentrations to 38 missed chemicals (13 with minimal or some concern and 25 with negligible concern).

ID no.	Chemical name	CAS	MW	LEC (mM)	LEC (mg/mL)	Detection at different top concentration limit			
						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	10 mM or 2 mg/mL, whichever is lower
13 missed chemicals with minimal or some concern									
84	Methyl acetoacetate	105-45-3	116.1	10.0	1.2	No	No	No	Yes
86	1,3-Bis(2-methylphenyl)guanidine	97-39-2	239.3	2.5	0.6	No	Yes	Yes	Yes
90	*N*-Ethylaniline	103-69-5	121.2	9.1	1.1	No	No	No	Yes
95	1,3,5-Tris(3,5-di-*tert*-butyl-4-hydroxybenzyl)isocyanuric acid	27676-62-6	784.1	3.2	2.5	No	No	No	No
100	Ethenyltrimethoxysilane	2768-02-7	148.2	5.0	0.8	No	Yes	No	Yes
102	C.I. Fluorescent brightner 271	41267-43-0	1347.1	3.7	5.0	No	No	No	No
104	Dibutyl adipate	105-99-7	258.4	2.5	0.7	No	Yes	Yes	Yes
106	*N*,*N*-Dimethylbenzylamine	103-83-3	135.2	3.8	0.4	Yes	Yes	Yes	Yes
110	2-Hydroxypropanenitrile	78-97-7	71.1	10.0	0.7	No	Yes	No	Yes
111	2-Mercaptobenzimidazole	583-39-1	150.2	5.3	0.8	No	Yes	No	Yes
112	*N*-Methylaniline	100-61-8	107.2	5.5	0.6	No	Yes	No	Yes
115	Trimethoxyphosphine	121-45-9	124.1	10.0	1.2	No	No	No	Yes
116	Trimethylamine	75-50-3	59.1	6.4	0.4	Yes	Yes	No	Yes
	Number of chemicals detected among the 13 chemicals					2	8	3	11
25 missed chemicals with negligible concern									
79	3-Aminobenzenesulfonic acid	121-47-1	173.2	2.4	0.4	Yes	Yes	Yes	Yes
80	2-Amino-5-chloro-4-methylbenzenesulfonic acid	88-53-9	221.5	9.0	2.0	No	No	No	Yes
81	2-Amino-5-methylbenzenesulfonic acid	88-44-8	187.2	5.1	1.0	No	Yes	No	Yes
82	Glycerol triacetate	102-76-1	218.2	10.0	2.2	No	No	No	No
83	4-Hydroxybenzoic acid	99-96-7	138.1	5.1	0.7	No	Yes	No	Yes
85	1-Naphthylacetic acid	86-87-3	186.2	9.1	1.7	No	No	No	Yes
87	*tert*-Butyl-methacrylate	585-07-9	142.2	2.8	0.4	Yes	Yes	Yes	Yes
88	*o*-Dichlorobenzene	95-50-1	147.0	1.6	0.2	Yes	Yes	Yes	Yes
89	Dicyclohexylamine	101-83-7	181.3	3.3	0.6	No	Yes	Yes	Yes
91	2-Hydroxyethyl methacrylate	868-77-9	130.2	5.0	0.7	No	Yes	No	Yes
92	4-Methylbenzoic acid	99-94-5	136.2	8.8	1.2	No	No	No	Yes
93	Triphosphoric acid aluminium salt	13939-25-8	317.9	6.3	2.0	No	No	No	Yes
94	4,4′-Sulfonyldiphenol	80-09-1	250.3	1.6	0.4	Yes	Yes	Yes	Yes
96	1,2-Dicyanobenzene	91-15-6	128.1	2.5	0.3	Yes	Yes	Yes	Yes
97	2-(Diethylamino)ethyl methacrylate	105-16-8	185.3	3.2	0.6	No	Yes	Yes	Yes
98	Methacrylic acid, monoester with propane-1,2-diol	27813-02-1	144.2	5.0	0.7	No	Yes	No	Yes
99	(Methacryloyloxyethyl) trimethylammonium chloride	5039-78-1	207.7	10.0	2.1	No	No	No	No
101	2-Chlorophenol	95-57-8	128.6	2.0	0.3	Yes	Yes	Yes	Yes
103	1,4-Dibromobenzene	106-37-6	235.9	2.3	0.6	No	Yes	Yes	Yes
105	2-(Di-*n*-butylamino)ethanol	102-81-8	173.3	1.9	0.3	Yes	Yes	Yes	Yes
107	2,4-Dinitrophenol	51-28-5	184.1	6.5	1.2	No	No	No	Yes
108	2-Ethylbutyric acid	88-09-5	116.2	3.4	0.4	Yes	Yes	Yes	Yes
109	Ferrous sulfate heptahydrate	7782-63-0	278.0	1.8	0.5	Yes	Yes	Yes	Yes
113	*p*-Nitrophenol sodium salt	824-78-2	161.1	3.8	0.6	No	Yes	Yes	Yes
114	Sorbitan monooctadecanoate	1338-41-6	430.6	2.5	1.1	No	No	Yes	Yes
	Number of chemicals detected among the 25 chemicals					9	17	14	23

Table 8
Comparison of selection of top test concentration for chemicals with different molecular weight in 2 mM or 1 mg/mL, whichever is higher.

Molecular weight	Selection of 2 mM or 1 mg/mL (whichever is higher)		
100	2 mM (0.2 mg/mL)	<	1 mg/mL (10 mM)
300	2 mM (0.6 mg/mL)	<	1 mg/mL (3.3 mM)
500	2 mM (1 mg/mL)	=	1 mg/mL (2 mM)
800	2 mM (1.6 mg/mL)	>	1 mg/mL (1.3 mM)
1000	2 mM (2 mg/mL)	>	1 mg/mL (1 mM)

Underlines show concentration to be selected.

carcinogenicity information, so we determined the biologic relevancy of *in vitro* CA induction based on the weight of evidence approach. Results from *in vitro* CA test with CHL cells only might lead to biased conclusions. However, the strength of our study is the high reliability of the test results due to the fact that all data were generated according to national or international test guideline under GLP conditions. Therefore, our analysis would be helpful to discuss on top concentration issues. In this analysis, many "irrelevant" positives by extreme culture conditions (low pH, high toxicity, and precipitation) were also identified. Note that CHL cells are often described as among the most sensitive cells, *i.e.*, effects observed at lower concentrations as compared to the other cell lines. The recently suggested improvements in testing are important to reduce irrelevant positives, in addition to defining the top concentration. Data from *in vitro* mammalian genotoxicity tests, using the criteria defined by this paper, should be helpful in genotoxic hazard identification.

Conflict of interest

There are no conflicts of interest.

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Industrial Health 2011, 49, 559–565 **Original Article**

Expert Review for GHS Classification of Chemicals on Health Effects

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Abstract: Intoxication as a result of chemical accidents is a major issue in industrial health. The Globally Harmonized System of Classification and Labelling of Chemicals (GHS) provides a framework for hazard communication on chemicals using labelling or safety data sheets. The GHS will be expected to reduce the number of chemical accidents by communicating the hazards posed and prompting safety measures to be taken. One of the issues which may be a barrier to effective implementation of the GHS results from discrepancies in GHS classifications of chemicals across countries/regions. The main reasons are the differences in information sources used and in the expertise of people making the classification (Classifiers). The GHS requests expert judgment in a weight of evidence (WOE) approach in the application of the criteria of classification. A WOE approach is an assessment method that considers all available information bearing on the determination of toxicity. The quality and consistency of the data, study design, mechanism or mode of action, dose-effect relationships and biological relevance should be taken into account. Therefore, expert review should be necessary to classify chemicals accurately. However, the GHS does not provide any information on the required level of expertise of the Classifiers, definition of who qualifies as an expert, evaluation methods of WOE or data quality, and the timing of expert judgment and the need for updating/re-classification as new information becomes available. In this paper, key methods and issues in expert reviews are discussed. Examples of expert reviews and recommendations for harmonized classification are also presented.

Key words: GHS, Expert review, Weight of evidence, Data quality, Classification, Health effects

Introduction

Intoxication caused by chemicals, including organic solvents, is one of the major issues in industrial health. More than 50 selected case examples per year are reported by the Japanese Ministry of Health, Labour and Welfare to illustrate the need for prevention of chemical accidents, and the occurrence factors including lack of recognition of hazards, insufficient education of safety and health, and non-use of personal protective equipment, etc[1]. Implementation of the Globally Harmonized System of Classification and Labelling of Chemicals (GHS) will make improvements to these situations. The GHS is a scheme recommended by the United Nations issued in 2003, which aims to enhance the protection of human health and the environment by providing an internationally comprehensible system for hazard communication[2]. The classification and labeling of chemicals are key elements of industrial health to reduce the number of chemical accidents. Many efforts for implementation of the GHS are being made at national and international levels, since 2003. The efforts in Japan include issue of regulations (e.g., Revised Industrial Safety and Health Law), provisions of information for industries (e.g., Guidance on Consumer Product Risk Assessment for GHS Labeling, GHS Classification Guidance for Enterprises, or Support

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Tools for GHS Classification), education for GHS audiences (*e.g.*, holding of workshops or seminars, provision of education tools), or provision of GHS classification results of chemicals[3].

One of the issues on implementation of the GHS is discrepancies in GHS classifications of chemicals across countries/regions. As the GHS is a globally harmonized system, uniform GHS classification criteria are applied to each health hazard. However, different results of classification can be obtained for the same chemicals[4, 5]. The main reason is the difference in datasets (*i.e.*, information sources) used. The other reasons are adaptation of a building block approach[6] and the differences of expertise/experience of Classifiers[7]. The GHS is designed as a self-classification system, and it requires expert judgment in a weight of evidence (WOE) approach in the application of the criteria. The hazard classification process under the GHS is highly technical in nature, and it requires a certain background and level of expertise to perform it accurately. If a Classifier lacks understanding of the GHS classification criteria, the effort should be repeated by an expert or reviewed carefully before finalization of the results[7]. Therefore, an expert review process is important for GHS classification. However, the GHS does not provide any information on the necessary expertise of Classifiers, definition of the required expertise, evaluation methods for the WOE approach or data quality, and the timing of expert judgment. The authors have been involved in the review system for GHS classification projects for the Organization for Economic Co-operation and Development (OECD)[5] or in Japan[8]. Based on these experiences, key methods, examples, and recommendations on the application of expert review are presented in this paper.

Expert Review

Expert review for GHS classification in health effect is defined as evaluation process based on scientific evidence, expertise, experience, knowledge, and judgment in a WOE approach. Main targets of the evaluations are information sources, data quality, and WOE of the data. The experts should be people who have scientific knowledge, experimental skill and expertise in toxicology or industrial hygiene. They should understand well the classification criteria in the GHS and the regulatory sciences including test protocols. They should also recognize that the classification will be conducted based on hazard identification, not on risk assessment for humans.

Evaluation of information sources/datasets

One of the major factors of the different classifications for individual chemicals was the different sources used[4, 5]. Therefore, evaluation of information sources is an important factor for reliable classification. Experts know where to find the information necessary for classification and, more importantly, how to correctly interpret these data. Several types of information sources are available. These include review documents, peer-reviewed papers, industry based reports, abstracts, or databanks, etc. The most reliable source is international or national review documents in terms of the quality, availability and suitability of information that has to be used in decision making. Peer-reviewed papers and industry based reports have high quality and suitability, but low availability. Databanks have high availability, but low quality. Abstracts should not be used for classification without any supportive information. The age of the data differs among these sources. Newer information will be available from more recent documents, and this information could result in changed assessment of chemicals. Classification based on old or limited information will possess lower reliability. The evaluation of test results in each information source should be checked with multiple sources of information, if available. Original peer-reviewed papers are the best source for assessing difficult and comprehensive test results: these should be included in information collection, if possible.

Evaluation of data quality

Even for chemicals with testing data, inherent differences among test protocols and the interpretation of test data may confound hazard evaluation[9]. The determination of the quality of test data is a critical point for the classification. Therefore the evaluation of data quality has to be done by an expert. The evaluation of data quality includes assessment of three basic elements, *i.e.*, reliability, relevance and adequacy. Definitions of these terms are shown in Table 1[10].

In order to evaluate the reliability of the data, the following are examples of key points in an expert review[11]:

- Were the data obtained from the test using a standardized method (accordance with recent OECD test guideline or internationally recognized methods)?
- Was the test conducted in compliance with the principles of Good Laboratory Practice (GLP) or equivalent standards?
- Was purity or the physicochemical properties of the test chemical suitable for the test?
- Were the findings clear and plausible?
- Was the reporting information sufficient to make a judgment?

For regulatory purposes, a GLP study, in accordance

Table 1. Three basic elements of the evaluation of data quality

Element	Explanation
Reliability	Evaluating the inherent quality of a test report or publication relating to preferably standardised methodology and the way the experimental procedure and results are described to give evidence of the clarity and plausibility of the findings.
Relevance	Covering the extent to which data and tests are appropriate for a particular hazard identification or risk characterisation.
Adequacy	Defining the usefulness of data for hazard/risk assessment purposes. Where there is more than one study for each endpoint, the greatest weight is attached to the studies that are the most relevant and reliable.

Table 2. A scoring system to assess the reliability of toxicological data

Reliability of data	Explanation
Reliable without restrictions	Data generated according to generally valid and/or internationally accepted testing guidelines (preferably performed according to GLP) or in which the test parameters documented are based on a specific (national) testing guideline or in which all parameters described are closely related/comparable to a guideline method.
Reliable with restrictions	Data (mostly not performed according to GLP), in which the test parameters documented do not totally comply with the specific testing guideline, but are sufficient to accept the data or in which investigations are described which cannot be subsumed under a testing guideline, but which are nevertheless well documented and scientifically acceptable.
Not reliable	Data in which there were interferences between the measuring system and the test substance or in which organisms/test systems were used which are not relevant in relation to the exposure (e.g. unphysiological pathways of application) or which were carried out or generated according to a method which is not acceptable, the documentation of which is not sufficient for assessment and which is not convincing for an expert judgment.
Not assignable	Data which do not give sufficient experimental details and which are only listed in short abstracts or secondary literature (books, reviews, etc.).

with standardized methods, has a high level of reliability in toxicology. On the other hand, a research oriented study may be of low reliability. A scoring system to assess the reliability of toxicological data is shown in Table 2[10, 11].

Examples of key points of evaluation of the relevance of the data are as follows[11]:

- Was the study design suitable? It should include vehicle, animal species, route of administration, doses or concentration used, parameters examined, etc.
- Were there dose-effect relationships?
- Was the effect of statistical and biological significant?
- What test system was used (*e.g.*, *in vitro*, *in vivo*, or human)?

The level of relevance of toxicological findings will be higher usually in the following order: i) human data (meta-analysis, randomized controlled trial, case control study, cross-sectional study, and case report); ii) animal data (primate, rodent, other mammals, non-mammals); iii) *in vitro* data (mammalian cells, microorganisms, biochemical reactions).

Examples of key points of the evaluation of the data adequacy are as follows[11]:

- Recognition of the strengths and weaknesses of the test method (*e.g.*, sensitivity, specificity, accuracy).
- What was the key study?
- Was the finding supported by other data?
- What kind of mechanisms or mode of action was involved?

The above key points are important for *in vitro* data, *in silico* data, or human data. The level of adequacy of any toxicological findings will be higher usually in the following order: i) similar findings in more than single study; ii) the findings obtained with a validated test method; iii) the finding is supported by the other data; iv) single study; v) *in silico* data.

Evaluation of WOE among the data

Generally, three objectives of the WOE approach are suggested for regulatory decision-making: i) provision of a "clear and transparent framework" for evaluation of the evidence in risk determination; ii) offer of a consistent and standardized approach to evaluating toxic substances submitted to regulatory agencies; and iii) help of identification of the discretionary assumptions in risk determinations from experts[12–14]. The GHS defines WOE as follows[2]: "All available information bearing on the determination of toxicity is considered together, including the results of valid *in vitro* tests, relevant animal data, and human experience such as epidemiological and clinical studies and well-documented case reports and observations. Both positive and negative test results are assembled together in the weight of evidence determination. However, a single positive study performed according to good scientific principles and with statistically and biologically significant positive

results may justify classification." When multiple data for one endpoint exist, the WOE approach must be applied by experts. Toxicology experts must consider all available data (both positive and negative), weigh it with respect to validity, and finally reach a conclusion. In a WOE approach, quality and consistency of the data, study design, mechanism or mode of action, dose-effect relationships, reproducibility, biological relevance, strength of the evidence, and purity of the test substance should be taken into account. It is noticed that any discrepancy in classification will be based on the different weighting evidence used from expert to expert. Harmonization of expert judgment is not easy, and is not static[7].

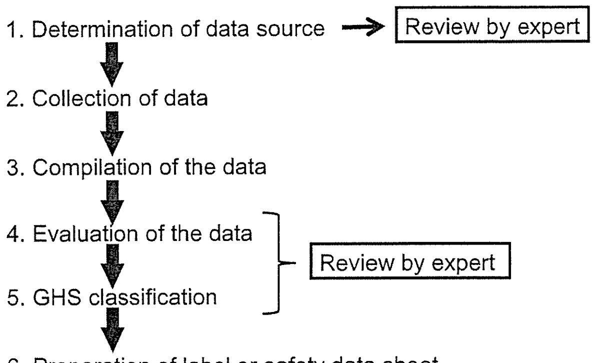

Fig. 1. Timing of expert review.

Issues in Expert Review

Expert review should promote and reflect the objective consideration of the full weight of evidence from alternative information sources, taking into account quality of data (*i.e.*, reliability, relevance and adequacy)[9]. The issues in expert review include: i) a single opinion from an expert might be low on transparency and high on subjectivity; ii) consistency of the judgment is unclear on between experts.

Transparency and objectivity

A WOE approach is one of key elements of expert review. Therefore, issues in applying it are also ones that might confound an expert review. The term WOE does neither constitute a scientifically well-defined term nor an agreed formalized concept characterized by defined tools and procedures. It is not clear which methods may be used, how they may be applied to the scientific evidence, what the results might be and how these may be used to make decisions in a specific hazard identification[14, 15]. The issues of a WOE approach in GHS classification include: i) application of WOE depends on the expertise of experts; ii) there are no canonical frameworks for weighting scientific evidence; iii) a process methodology is low on transparency and high on subjectivity; iv) WOE is usually applied in the case where there is no conclusive single study in demonstrating a cause-effect relationship; v) WOE looks like a 'seat-of-the pants' qualitative assessment. Without an explanation of how evidence is "weighed" or "weighted", the WOE approach may be to be a "black box" of scientific judgment[13]. To keep transparency of expert reviews, the review should be objective and taken into consideration of evidence based toxicology[16].

Consistency

Consistency of the results of expert review on similar subjects is a fundamental principal for hazard classification of chemicals. The outcome from an expert should be consistent in between experts and in chronological order if no new data or scientific evidence is available.

Timing of expert review

The practical application of an expert review (*i.e.*, how and when that review should be applied) is not mentioned in the GHS. This can result in ineffective hazard classification, hazard communication and chemical management[9]. Based on the experiences from the GHS classification projects, suitable timing of expert review is proposed (Fig. 1). General steps of GHS classification are as follows: Data collectors gather available data with a certain list. Classifiers read, select, compile and evaluate the information (*i.e.*, data), and then classify the chemical based on GHS criteria. Information gathering is the first step of hazard classification of chemicals. Different information source sets result in different classification results. It is important for GHS classification to establish useful and effective information source set. Experts should review suitability of the information sources at the beginning of GHS classification work, and should provide sufficient list of data sources. Examples of the sources are provided by Japan Ministry of Economy, Trade and Industry (METI)[17] or European Chemicals Agency[18]. Experts will review the necessity of additional source(s). Next timing of expert review is at time of evaluation of the data and following assignment of classification by Classifiers. Experts should review the relevance of the classification assigned by classifiers based on data quality of key studies and total weight of evidence of the findings[7].

Examples of Expert Reviews Where Re-classification Was Needed

Followings are examples of expert review in Japanese

GHS classification projects. Details of some examples are available from the web site of METI[19]. Examples for germ cell mutagenicity are given in a previous article[7].

Antimony trioxide [1309-64-4]

The original classification for this compound was Category 2B in regard to eye irritation, based on a mild irritation seen in rabbits[20]. An expert pointed out that draft EU Risk Assessment Report evaluates this compound as non-irritant based on the result from a new GLP study. The draft is not available yet, but the original test report is available through the OECD[21]. As the result is now non-irritating in the rabbit, "Not classified" was re-assigned in the review. This case suggests the importance of data collection.

4,4'-Thiobis(6-tert-butyl-m-cresol) (TBBC) [96-69-5]

The original classification was Category 1 in skin sensitization based on two patients with positive patch tests to TBBC who developed contact dermatitis to TBBC-containing latex gloves[22]. An expert questioned the reliability and relevancy of this information. The American Conference of Governmental Industrial Hygienists summarized that sufficient data were not available to recommend sensitization notation[22]. Therefore, "Classification not possible" was assigned by expert review. The point of debate was the evaluation of data quality.

p-Dichlorobenzene [106-46-7]

The original classification was Category 2 for germ cell mutagenicity based on a negative result from a dominant lethal test and a positive result in a micronucleus test[23]. However, a reviewing expert noticed that both positive and negative results existed for micronucleus tests of this compound. The positive result was not confirmed by additional tests including tests using a similar protocol to the first test. A positive result in a kidney micronucleus test was considered of low reliability and relevancy. Another 5 or more micronucleus tests showed negative results. Based on WOE, "Not classified" was assigned by the expert. The reasons for the changed classification were that multiple negative results had more weight than a single positive result and also an evaluation of data quality for the original positive result.

Styrene [100-42-5]

The original classification was Category 2 for carcinogenicity based on the classification in Group 2B by evaluation of International Agency for Research on Cancer[24]. A reviewing expert suggested that a recent analysis revealed that lymphatic and haematopoietic neoplasms seen in humans exposed to styrene are likely to be due to concomitant exposure to butadiene[25]. Mouse specific mode of action (MOA) exists in the induction of mouse lung tumor[26]. Opinions on the interpretation of the cancer data were different among experts. Finally, after much discussion resulted in a "Not classified" Category instead of Category 2. Thus the change in Classification resulted from the recent re-evaluation and analysis of the MOA.

Ethylene glycol [107-21-1]

The original classification was Category 1B for reproductive toxicity based on reduced skeletal ossification and malformations of the skeleton, which were observed without maternal toxicity[27]. A reviewing expert pointed out that the above effects were seen in rats at 1,500 mg/kg (over the limit dose of 1,000 mg/kg) or mice at 500 mg/kg. The expert introduced a recent evaluation document[28] which mentions that ethylene glycol is not directly responsible for developmental toxicity, but that this toxicity is due to the accumulation of glycolic acid (a metabolic breakdown of ethylene glycol). The saturation level of this compound is lower in humans than that in rodents. There is negligible concern (at current human exposure levels) for reproductive toxicity in humans. Therefore, "Not classified" was assigned by an expert review. The relevant points for the change in classification were the effective dose for toxicity shown in animal experiments which was not relevant to human exposure and the findings of different metabolism in human compared to rats for this compound.

Hydroquinone [123-31-9]

The original classification was Category 1B for reproductive toxicity based on an increase in foetal resorptions[29]. A reviewing expert pointed out that the above finding was based on old (1955–1964) studies. Recent evaluations generated negative results in rat and rabbit developmental tests and a rat two generation fertility test[30, 31]. These tests were conducted in accordance with recent guidelines, giving more reliability. Therefore, "Not classified" was assigned by the expert review for reproductive toxicity.

With respect to specific target organ toxicity (single exposure), the original classification was Category 1 (central nerve system and kidney) based on the appearance of tremor, vomiting and cyanosis which was observed in exposed humans and the observation of kidney damage in rats[29]. However, a reviewing expert pointed out that the human findings were based on exposure to mixtures containing hydroquinone plus other substances. Symptoms observed after exposure to

hydroquinone alone were transient central nerve system effects.

The rat is unique in susceptibility to kidney effects following hydroquinone exposure[31]. Based on the new evaluation document, Category 3 (narcotic effect) was assigned by an expert review. The relevant points for the change in classification were insufficient information gathering in the first instance, careful review of the documents and use of recent evaluation using more reliable data.

Ferric Chloride [7705-08-0]

The original classification was Category 1 for aspiration hazards based on the following finding[32]; a woman presented with vomiting after ingestion of 200 ml ferric chloride solution (pH 1.0). Three hours after her ingestion she presented with drowsy consciousness, tachycardia and protracted vomiting. Aspiration pneumonia was also noted. A reviewing expert noticed that the aspiration pneumonia was observed after vomiting of a corrosive solution, which does not necessarily indicate an aspiration hazard. In addition, the findings did not fit the GHS criteria for aspiration hazard. Therefore, "Classification not possible" was assigned by the expert review. The relevant points were recognitions of the definition in the GHS text and the effect by ingestion of a corrosive solution.

Recommendations

Consideration of different information sources can result in different GHS classification results. Judgments of data quality and weight given to findings will vary among experts. To minimize these variations, the following approaches will be needed for harmonized classification: i) Development of an internationally-constructed and maintained information database for GHS classification; ii) Provision of rationales of selection of (key) studies and a classification derived from them for maintaining transparency; iii) Discussion on how to apply expert judgment and how to assess the quality of data from limited studies; iv) Establishment of a GHS classification data bank which collects GHS classification results including related information; v) International review system of classification on specific chemicals; and vi) Consultation system for companies/Institutes without experts. These will be help the harmonization and transparency of GHS classifications. Duplication of classification will be also avoided.

Conclusions

It is clear that suitable classification depends on the correct interpretation of the data, the application of the weight of evidence approach and basing judgments only on high quality data. Toxicologists or industrial hygienists, as experts, play an important role in assigning supportable classifications. They should consider data quality, and should review critically several authoritative documents including original articles to support the classification of chemicals.

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REVIEW

Micronucleus assays in rodent tissues other than bone marrow

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This report updates previous reviews that were conducted as part of the third and fourth International Workshops on Genetic Toxicology Testing of micronucleus (MN) assays in rodent tissues other than bone marrow. Tissues discussed here are liver, lung, skin, colon, spleen, testes and foetal/neonatal tissues with transplacental exposure. Previous reviews have been updated to include literature published after 2000. In addition to the previously described tissues, MN assays in bladder, buccal mucosal cells, stomach and vagina are also included. MN assays using tissues other than bone marrow are critical for risk assessments, for *in situ* evaluation and for studies of systemic genotoxic effects and modes of action. Protocols for the majority of assays in tissues other than bone marrow have not yet been well standardised and validated for regulatory application, and further development is needed to support regulatory studies.

Introduction

Evaluation of genetic toxicity is an important component of the safety assessment of chemicals, including pharmaceuticals, agricultural chemicals, food additives and industrial chemicals. Up to the present time, genotoxicity has been regulated mainly on the basis of qualitative outcomes of hazard identification assays, i.e. decisions are often based on classification as positive or negative for genotoxic potential. Recently, the field is moving towards quantitative risk assessments and with more reliance on weight of evidence (WOE) approaches (1–3). *In vivo* assays are critical components of both WOE analysis and of quantitative risk assessments. The *in vivo* micronucleus (MN) assay using rodent haematopoietic cells from bone marrow or peripheral blood is widely used for the assessment of clastogenicity/aneugenicity of chemicals, both as a hazard screening assay and as a component of exposure-based risk assessments. The assay is usually incorporated into the standard genotoxicity test battery as a representative *in vivo* assay together with an *in vitro* gene mutation assay using bacteria and an *in vitro* chromosomal aberration assay using cultured mammalian cells (4). The MN assay was originally established using rodent erythrocytes as a simple method of assessing cytogenetic damage, taking advantage of two important characteristics of the haematopoietic system: (i) the ease of scoring MN in the newly formed anucleate reticulocytes derived from bone marrow and (ii) the ability to identify the newly formed erythrocytes that had completed their last division during or shortly after exposure to the test agent as those staining positive for RNA content (5,6). These important advantages initially led to an almost exclusive focus on the haematopoietic cells as the target. However, in principle, MN can be scored in any dividing cell population, and the assay was soon extended to tissues other than bone marrow.

One of the early extensions of the assay was to male germ cells, which are a key cell population for genetic studies and which undergo continuous active division (7,8). The assay was also extended to liver by stimulating regeneration and hence cell replication, by partial hepatectomy (9–12) or pretreatment with hepatotoxicants (13,14). Later, colon, skin and young rodent liver were studied extensively and shown to be suitable for MN analysis. Studies using human tissues or cell preparations include corneal cells and exfoliated cells from urinary bladder, oesophagus, cells of the nasal cavity and oral buccal mucosa (15,16). Rodent embryonic cells have also been used to evaluate effects of transplacental application of test chemicals. In addition, human lymphocytes have been used to assess cytogenetic damage in epidemiology studies of environmentally or occupationally exposed individuals, as summarised previously. The MN assay using peripheral lymphocytes or other human tissues is reviewed separately in this special issue.

In regulatory testing, the rodent erythrocyte MN assay is the most common first choice among *in vivo* assays used for subsequent testing when *in vitro* genotoxicity test(s) are found to be positive. The unscheduled DNA synthesis assay using rat liver has also been used in this situation, but its sensitivity has been questioned (17). The single-cell gel electrophoresis (comet) assay and gene mutation assays using transgenic animals are frequently considered for use as follow-up assays when *in vitro* gene mutation assay(s) are positive. It is important to evaluate cytogenetic damage in those cell populations or tissues that are the relevant sites of distribution and metabolism *in vivo*, and so bone marrow haematopoietic cells alone cannot satisfy this need.

Scientific considerations as well as the animal welfare movement, which emphasises the reduction, replacement and/or refinement of animal use (the '3Rs'), have contributed to the implementation of protocols that allow the integration of genotoxicity end points into general toxicological tests. Assays that have been shown to be appropriate for integration with repeat-dose general toxicology studies include the bone marrow and peripheral blood erythrocyte MN assays, the peripheral blood lymphocyte assay and a new MN assay method using rat liver after an extended period of repeated dosing (18). The liver is a key site of xenobiotic metabolism

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