2.2. Sources of genotoxicity test results

2.2.1. *Ames and* in vitro *CA tests*

Data for *in vitro* genotoxicity tests (*i.e.*, Ames and *in vitro* CA) were taken from the revised CGX dataset [17]. We have made some additional changes from the original CGX [10]. Examples of changes on the Ames and *in vitro* CA test results are as follows: The Ames test results for carbon tetrachloride (ID C173, 56-23-5) and chloroform (ID C160, 67-66-3) have been re-evaluated as positive (+) from negative (−) due to the positive data using gaseous exposures [18]. The CA result for DDT (ID C221, 50-29-3) has been changed to equivocal (E) from positive (+) due to the presence of one positive test in B14F28 cells and one negative test in V79 cells [19]. Data on the CA test result for *o*-phenylphenol sodium salt (ID C632, 132-27-4) have been added according to the review by Brusick [20]. Positive results in the Ames and CA tests have been added for aristolochic acid (ID C66, 313-67-7) due to a recent publication [21]. In addition, some data were identified during *in vivo* data collection as follows:

- Negative results in the CA or Ames test have been added for capsaicin (ID C130, 404-88-4) [22,23] and ICRF 159 (ID C416, 21416-87-5) [24], respectively.
- Negative results in the Ames and CA tests have been added for methyl clofenapate (ID C458, 21340-68-1) [25].
- Positive results in the Ames and CA tests have been added for 4-(methylnitrosamino)-1-(3-pyrridyl)-1-(butanone) (NNK, ID C478, 64091-91-4) [26].
- A negative result in the CA test has been added for procarbazine HCl (ID C645, 366-70-1) [27].

The revised Organisation for Economic Co-operation and Development (OECD) test guideline (TG) 473 [28] for *in vitro* CA test defines that the highest test concentration should correspond to 10 mM, 2 mg/mL or 2 μL/mL, whichever is the lowest. Morita et al. [29] investigated the lowest effective concentrations of chemicals in the *in vitro* CA test cited in the original CGX database. Therefore, with respect to the CA test results, the following 7 carcinogens and 5 non-carcinogens have been re-evaluated as (−) from (+) due to the positive findings only being found at concentrations of higher than both 10 mM and 2 mg/mL [29]:

- Carcinogens: hexanamide (ID C392, 628-02-4), nitrite sodium (ID C509, 7632-00-0), nitrobenzene (ID C514, 98-95-3), *N*-nitrosodiethylamine (ID C551, 55-18-5), saccharin sodium (ID C664, 128-44-9), trimethylphosphate (ID C734, 512-56-1) and urethane (ID C744, 51-79-6);
- Non-carcinogens: *o*-anthranilic acid (ID NC12, 118-92-3), benzyl alcohol (ID NC20, 100-51-6), 4-nitroanthranilic acid (ID NC124, 619-17-0), 1-phenyl-2-thiourea (ID NC144, 103-85-5) and resorcinol (ID NC152, 108-46-3).

2.2.2. In vivo *micronucleus test*

Test results from the *in vivo* rodent erythrocyte MN test were obtained from international chemical assessment documents (*i.e.*, European Union Risk Assessment Reports (EURAR), http://echa.europa.eu/web/guest/information-on-chemicals/information-from-existing-substances-regulation, OECD Screening Information Data Set (SIDS) documents, http://webnet.oecd.org/hpv/UI/Search.aspx, the International Programme on Chemical Safety (IPCS) Concise International Chemical Assessment Documents (CICAD), http://www.who.int/ipcs/publications/cicad/en/ or International Agency for Research on Cancer (IARC) Monographs on the Evaluation of Carcinogenic Risks to Humans, http://monographs.iarc.fr/ENG/Monographs/suppl7/index.php), several review papers on *in vivo* MN test data [30–33], several reports from large scale studies on the *in vivo* MN test [11,34–37] and the US National Toxicology Program (NTP) Chemical Effects in Biological Systems (CEBS) database (http://tools.niehs.nih.gov/cebs3/ui/). A PubMed literature search was also employed using search words of "CAS number or chemical name", "micronucle*" and "rodent" (http://www.ncbi.nlm.nih.gov/pubmed/).

2.2.3. In vivo *transgenic rodent mutation assay*

A detailed review paper on the TGR assay that was provided to OECD to support the development of a test guideline was used [16]. An earlier version of this review paper was also checked [15]. In addition, unpublished reports on the transgenic rodent mutation studies on food additives by the Japanese Ministry of Health Labor and Welfare (MHLW) were used [38–40].

2.3. Categories of the test results

Categories of the test results were based on the criteria by Kirkland et al. [10]; positive (+), negative (−), equivocal (E) and technically compromised (TC). Positive indicates a definitive positive response, either in a single publication or across the majority of publications with the chemical in question. Any negative results could be outweighed by overwhelming dominance of positive publications, or by viewing the data in detail and deciding that the negative test was not adequate. Negative indicates a clearly negative response in all publications found. Equivocal indicates the response is weak or not reproduced between experiments or between laboratories. Weak means that a dose-related increase in effect was noted close to the borderline of biological significance, but they were not biologically and/or statistically significant. Non-reproducible means that there were both positive and negative findings across different studies of apparent equal validity, and the weight of evidence did allow a clear positive or negative overall outcome to be concluded. If a published study was considered inconclusive, it was called E for convenience. TC indicates a test result that was questionable due to failure to meet essential standard criteria for an adequate study. An example for a TC classification in the *in vivo* MN test will be no proof of target cell exposure. Proof of target cell exposure can be shown by reduction in immature/mature erythrocytes ratio in the study or by proof of systemic exposure in other *in vivo* studies. However, many data sources of *in vivo* tests were international evaluation documents, published review papers or national databases. All of them do not provide information of target cell exposure in *in vivo* MN and TGR tests. Therefore, TC classification was not applied to the *in vivo* test in this analysis; it was employed to the only *in vitro* tests based on the previous classifications [10]. References for compounds with *in vivo* negative result for which no target cell exposure were noted in the review paper or database are marked (as "~") in the Appendix tables. E results were included in the total numbers of assays/chemicals evaluated, but not employed for calculations of the definitive performance of the assays. It was based on the clear positive or negative results. However, additional calculations of the performance have been made, in which E results were considered positive or negative. TC results were not included in the total numbers.

3. Results

Appendices A and B show each genotoxicity test result from the chemical dataset of the 756 carcinogens and 183 non-carcinogens, respectively.

Table 2
Summary performance of individual *in vitro* assays in detecting rodent carcinogens or non-carcinogens.

Carcinogenicity	Ames				*in vitro* CA			
	+	E	–	Total	+	E	–	Total
+	321	8	215	544	225	15	118	358
–	40	6	130	176	56	14	66	136
Total	361	14	345	720	281	29	184	494
Sensitivity[a]	59.0% (321/544)				62.8% (225/358)			
Specificity[a]	73.9% (130/176)				48.5% (66/136)			
Concordance[a]	62.6% (451/720)				58.9% (291/494)			

+, Positive; –, Negative; E, Equivocal.

[a] Equivocal (E) results were not counted either as positive or negative, but they were included in the total number. If E results are considered positive, the performanceis as follows: Ames: sensitivity, 60.5% (329/544); specificity, 73.9% (130/176), concordance, 63.8% (459/720). *In vitro* CA: sensitivity, 67.0% (240/358); specificity, 48.5% (66/136), concordance, 61.9% (306/494). If E results are considered negative, the performance is as follows: Ames: sensitivity, 59.0% (321/544); specificity, 77.3% (136/176), concordance, 63.5% (457/720). *In vitro* CA: sensitivity, 62.8% (225/358); specificity, 58.8% (80/136), concordance, 61.7% (305/494).

Table 3
Summary performance of individual *in vivo* assays in detecting rodent carcinogens or non-carcinogens.

Carcinogenicity	*in vivo* MN				TGR			
	+	E	–	Total	+	E	–	Total
+	120	11	162	293	55	0	21	76
–	24	10	52	86	0	0	4	4
Total	144	21	214	379	55	0	25	80
Sensitivity[a]	41.0% (120/293)				72.4% (55/76)			
Specificity[a]	60.5% (52/86)				Not calculated due to small numbers of non-carcinogens			
Concordance[a]	45.4% (172/379)				Not calculated			

+, Positive; –, Negative; E, Equivocal.

[a] Equivocal (E) results were not counted either as positive or negative, but they were included in the total number. If E results are considered positive, the performance is as follows: *In vivo* MN: sensitivity, 44.7% (131/293); specificity, 60.5% (52/86), concordance, 48.3% (183/379). If E results are considered negative, the performance is as follows: *In vivo* MN: sensitivity, 41.0% (120/293); specificity, 72.1% (62/86), concordance, 48.0% (182/379).

3.1. *Performance of* in vitro *assays*

The summary performances (sensitivity, specificity and concordance) of the Ames and *in vitro* CA tests in discriminating between rodent carcinogens and non-carcinogens are shown in Table 2. TC results were not included in the table.

3.1.1. *Ames test*

Ames test results were available for 544 carcinogens (excluding 1 TC result) and 176 non-carcinogens from the dataset. Of the 544 carcinogens, 321 clearly gave positive results (59.0%, sensitivity), 8 gave equivocal results, and 215 clearly gave negative results. Of the 176 non-carcinogens, 130 clearly gave negative results (73.9%, specificity), 6 gave equivocal results, and 40 gave positive results. Concordance was calculated as 62.6% (451/720) (Table 2). These performance indicators are consistent with those reported by Kirkland et al. (sensitivity, specificity and concordance were 58.8%, 73.9% and 62.5%, respectively) with the original CGX dataset [10]. If equivocal results are considered positive or negative, the sensitivity or specificity rises to 60.5% (329/544) or 77.3% (136/176), respectively.

3.1.2. In vitro *CA test*

CA results were available for 358 carcinogens (excluding 11 TC results) and 136 non-carcinogens (excluding 9 TC results) from the dataset. Of the 358 carcinogens, 225 clearly gave positive results (62.8%, sensitivity), 15 gave equivocal results, and 120 clearly gave negative results. Of the 136 non-carcinogens, 69 clearly gave negative results (48.5%, specificity), 14 gave equivocal results, and 52 gave positive results. Concordance was calculated as 58.9% (291/494) (Table 2). These performance indicators are also very similar to those reported by Kirkland et al. (sensitivity, specificity and concordance were 65.6%, 44.9% and 59.8%, respectively) with the original CGX dataset [10]. If equivocal results are considered positive or negative, the sensitivity or specificity rises to 67.0% (240/358) or 58.8% (80/136), respectively.

3.2. *Analysis of* in vivo *genotoxicity results with carcinogens*

3.2.1. In vivo *MN test*

MN results were available for 293 of the 756 carcinogens. Specific comments and evaluations assigned for the current analysis on the following 16 chemicals are as follows:

- ID C17, Acrylonitrile, 107-13-1 (negative)

Almost all studies showed negative results [11,41]. Positive results were obtained in rat bone marrow cells treated by intravenous (i.v.) injection, but not in the peripheral blood [11,35]. In mice treated by i.v. injection, negative results were obtained in both bone marrow cells and peripheral blood [11]. Acrylonitrile was regarded as negative based on the results with a relevant route of exposure.

- ID C179, Chlorpromazine hydrochloride, 69-09-0 (positive)

The reported positive finding is considered to be due to hypothermia [42]. The MN induction by hypothermia is due to an indirect genotoxic mode of action (*i.e.*, secondary effect) and threshold related, which indicates a genotoxic hazard. If core body temperature changes do not occur under conditions of human exposure, there is unlikely to be a genotoxic risk [43]. Therefore, a positive call was assigned.

- ID C197, C.I. Solvent yellow 3 (*o*-aminoazotoluene), 97-56-3 (positive)

Differences in species were found (positive in mice [11], but negative in rats [35]).

- ID C246, 1,2-Dibromoethane, 106-93-4 (equivocal)

Differences in route of exposure were found; negative by oral gavage [44,45] or by intraperitoneal (i.p.) injection [11], but positive by inhalation [35].

- ID C285, 3,3′-Dimethoxybenzidine 2HCl, 20325-40-0 (negative)

No *in vivo* MN data were available on this chemical. However, a negative result was obtained with the free base (3,3′-dimethoxybenzidine, 119-90-4) [11].

- ID C378, Haloperidol, 52-86-8 (positive)

The positive finding is considered to be due to hypothermia in mice [46]. On the other hand, rats showed a negative result [46].

- ID C439, Mercuric chloride, 7487-94-7 (positive)

Differences in species were found (positive in mice [47,48], but negative in rats [48]).

- ID C466, 4,4′-Methylenedianiline 2HCl, 13552-44-8 (positive)

This compound showed a positive result [34], however, there were negative results with the free base (4,4′-Methylenedianiline, 101-77-9) [11,49].

- ID C478, 4-(Methylnitrosamino)-1-(3-pyrridyl)-1-(butanone) (NNK), 64091-91-4 (equivocal)

There was one positive [26] and one negative [50] result. In addition a positive result was obtained in mice treated by subcutaneous injection for up to 52 weeks [51].

- ID C509, Nitrite, sodium, 7632-00-0 (equivocal)

There was one positive [30] and one negative [50] result. The OECD SIDS document evaluated this compound as positive [52].

- ID C631, Phenylhydrazine HCl, 59-88-1 (positive)

No *in vivo* MN data were available on this chemical. However, a positive result was obtained with the free base (phenylhydrazine, 100-63-0) [30].

- ID C657, Pyrimethamine, 58-14-0 (positive)

Differences in species were found (positive in rats [45,53], but negative in mice [51]).

- ID C660, Reserpine, 50-55-5 (positive)

The positive finding in mice might be due to hypothermia, whilst a negative result was obtained in rats [43].

- ID C672, Sodium dichromate, 10588-01-9 (positive)

A positive result was obtained by i.p. injection, but a negative result was obtained by oral administration (gavage) [32].

- ID C676, Styrene, 100-42-5 (positive)

Differences in species were found (positive in mice [30,32,50], but negative in rats [32]).

- ID C711, *o*-Toluidine, 95-53-4 (positive)

Differences in species were found (positive in rats [49,50], but negative in mice [11,50]).

3.2.2. TGR assay

TGR results were available for 76 of the 756 carcinogens. Specific comments on 6 chemicals (indicated as '+^' in Appendix A) which showed positive results in the non-target organ(s)/tissue(s) of carcinogenicity in rodents (mouse, rat and/or hamster) are as follows:

- ID C244, 1,2-Dibromo-3-chloropropane, 96-12-8

1,2-Dibromo-3-chloropropane showed a positive gene mutation result in the testis, but was negative in the liver. The target organs of carcinogenicity are the nasal cavity, oral cavity, stomach, adrenal gland and mammary gland in rats, and the lung, nasal cavity and stomach in mice [15].

- 1D C246, 1,2-Dibromoethane, 106-93-4

1,2-Dibromoethane showed a positive gene mutation result in the nasal mucosa and testes, but was negative in the lung and liver. The target organs of carcinogenicity are the nasal cavity, peritoneal cavity, pituitary gland, stomach, liver, lung and mammary gland [15].

- ID C340, Ethyl methanesulphonate, 62-50-0

Ethyl methanesulphonate showed positive gene mutation results in the bone marrow, epididymal sperm and liver, but was negative in the brain and small intestine. The target organs of carcinogenicity are the kidney, lung and thymus [15].

- ID C457, 3-Methylcholanthrene, 56-49-5

3-Methylcholanthrene showed a positive gene mutation result in the liver. The target organs of carcinogenicity are the lung, skin and mammary gland [15].

- ID C492, Mitomycin C, 50-07-7

Mitomycin C showed positive gene mutation results in the bone marrow and liver, but was negative in the small intestine and testis. The target organs of carcinogenicity are the intestine, mammary gland and peritoneal cavity [15].

- ID C702, Thio-tepa, 52-24-4

Thio-tepa showed a positive gene mutation result in splenic lymphocytes. The target organs of carcinogenicity are the ear/Zymbal's gland, haematopoietic system, skin, mammary gland and preputial gland [15].

Furthermore, specific comments on the 4 chemicals (indicated as '-^' in Appendix A) which showed negative results in the non-target organ/tissue(s) of carcinogenicity are as follows:

- ID C17, Acrylonitrile, 107-13-1

Acrylonitrile showed negative gene mutation results in the bone marrow, brain, lung, splenic lymphocytes and testicular germ cells [16]. The target organs of carcinogenicity are the ear/Zymbal's

gland, nervous system, oral cavity, small intestine, mammary gland and nasal cavity [15].

- ID C257, 1,2-Dichloroethane, 107-06-2

1,2-Dichloroethane showed negative gene mutation results in the liver and testis. The target organs of carcinogenicity are the stomach, subcutaneous tissue, vascular system, mammary gland, lung and uterus [15].

- ID C489, Metronidazole, 443-48-1

Metronidazole showed a negative gene mutation result in the stomach. The target organs of carcinogenicity are the pituitary gland, testes, liver, mammary gland, lung and haematopoietic system [15].

- ID C683, SX Purple, 2611-82-7

SX Purple showed negative gene mutation results in the liver and stomach [40]. No clear target organ of carcinogenicity was identified ("all tumor bearing animals") in the Carcinogenic Potency Database [54].

3.3. *Analysis of* in vivo *genotoxicity results with non-carcinogens*

3.3.1. In vivo *MN test*

In vivo MN results were available for 86 of the 183 non-carcinogens. Specific comments and evaluations assigned for the current analysis on the following 4 chemicals are as follows:

- ID NC8, dl-Amphetamine sulfate, 60-13-9 (positive)

The positive result assigned for the current analysis was based on the data obtained with the free base (dl-amphetamine, 300-62-9) [30].

- ID NC73, EDTA, trisodium salt trihydrate, 150-38-9 (negative)

The negative result assigned for the current analysis was from data obtained with the disodium salt (6381-92-6) [55]. Note that a positive result in mice after oral administration of the disodium salt was reported [56]. However, EURAR commented that the positive result seems to be of low reliability, and that EDTA does not induce micronuclei in bone marrow cells [55].

- ID NC91, Fluoride, sodium, 7681-49-4 (equivocal)

Kirkland et al. assigned this as positive based on a single positive result [33]. However, 3 negative studies were identified [50,57,58].

- ID NC138, Phenol, 108-95-2 (positive)

The positive finding is considered to be due to hypothermia [43,49].

3.3.2. *TGR assay*

TGR results were available for only 4 of the 183 non-carcinogens. Such limited data meant that specificity and concordance values could not be calculated.

3.4. *Performance of* in vivo *assays*

The summary performances of the *in vivo* MN and TGR assays in discriminating between rodent carcinogens and non-carcinogens are shown in Table 3.

3.4.1. *Performance of the* in vivo *MN test*

In vivo MN results were available for 293 of the 756 carcinogens and for 86 of the 183 non-carcinogens. Of the 293 carcinogens, 120 clearly gave positive results (41.0%, sensitivity), 11 gave equivocal results, and 162 clearly gave negative results. Of the 86 non-carcinogens, 52 clearly gave negative results (60.5%, specificity), 10 gave equivocal results, and 24 gave positive results. Concordance was calculated as 45.4% (172/379) (Table 3). If equivocal results are considered positive or negative, the sensitivity or specificity rises to 44.7% (131/293) or 72.1% (62/86), respectively.

3.4.2. *Performance of the TGR assay*

TGR results were available for 76 of the 756 carcinogens and for 4 of the 183 non-carcinogens. Of the 76 carcinogens, 55 clearly gave positive results (72.4%, sensitivity) and 21 clearly gave negative results (Table 3). All 4 non-carcinogens showed negative results. Specificity and concordance could not be calculated due to the very limited TGR data for non-carcinogens.

3.5. *Performance of the combination of two, three or four tests*

Based on the recent recommendations for genotoxicity test batteries or testing approaches as defined by advisory bodies and regulatory agencies (*e.g.* ICH, COM. ECHA, EFSA, FSC), the performances of the following combinations of tests were analyzed: Ames + *in vitro* CA, Ames + *in vivo* MN, Ames + TGR, Ames + *in vitro* CA + *in vivo* MN, Ames + *in vitro* CA + TGR, Ames + *in vivo* MN + TGR, Ames + *in vitro* CA + *in vivo* MN + TGR. The Ames test was included in all combinations employed due to it being the most basic genotoxicity test. The sensitivity calculations were based on at least one positive result in all of the genotoxicity tests in the combination among the carcinogens tested. The specificity calculations were based on there being negative results in all of the genotoxicity tests in the combination among the non-carcinogens tested. Performance of the combinations (*i.e.*, sensitivity and specificity) is described below. The specificity values for the combination of tests including the TGR assay were not calculated due to the very low number of non-carcinogens tested by TGR.

3.5.1. *Performance of Ames +* in vitro *CA*

Results were available from both Ames and *in vitro* CA tests for 350 carcinogens and 136 non-carcinogens. For the carcinogens, 149 or 111 chemicals were clearly positive (*i.e.*, equivocal results considered negative) in both tests or in one of the two tests when both were performed, respectively (Table 4). Therefore, the sensitivity value that was calculated from the results where both tests were performed was 74.3% (260/350). If equivocal results are considered positive, the sensitivity rises to 76.9% (269/350). For the non-carcinogens, clearly negative results were obtained in both tests for 51 of the 136 non-carcinogens (Table 5). Therefore, the specificity value that was calculated from the results where both tests were performed was 37.5% (51/136). If equivocal results are considered negative, the specificity rises to 47.8% (65/136).

3.5.2. *Performance of Ames +* in vivo *MN*

Results were available from both Ames and *in vivo* MN tests for 284 carcinogens and 86 non-carcinogens. For the carcinogens, 79 or 116 chemicals gave clearly positive in both tests or in one of the two tests when both were performed, respectively (Table 6). Therefore, the sensitivity value that was calculated from the results

Table 4
Performance of combinations of Ames and *in vitro* CA tests in detecting rodent carcinogens when both performed.

Ames	*in vitro* CA			
	+	E	–	Total
+	149	8	35	192
E	5	0	2	7
–	63	7	81	151
Total	217	15	118	350

No. of carcinogens tested in both tests (A): 350
No. (%) of clear positive results in both tests (B): 149 (42.6%)
No. (%) of clear positive results in only 1 of the two tests (C): 111 (31.7%)
Sensitivity (i.e., clearly positive in at least 1 test when both conducted ([B + C]/A)[a] : 74.3%

+, Positive; –, Negative; E, Equivocal
a: If E results are considered positive, the sensitivity is 76.9% (269/350)

Table 5
Performance of combinations of Ames and *in vitro* CA tests in detecting rodent non-carcinogens when both performed.

Ames	*in vitro* CA			
	+	E	–	Total
+	19	2	15	34
E	3	0	2	5
–	34	12	51	97
Total	56	14	66	136

No. of non-carcinogens tested in both tests (A): 136
No. of clear negative results in both tests (B): 51
Specificity (B/A)[a] : 37.5%

+, Positive; –, Negative; E, Equivocal
a: If E results are considered negative, the specificity is 47.8% (65/136)

Table 6
Performance of combinations of Ames and *in vivo* MN tests in detecting rodent carcinogens when both performed.

Ames	*in vivo* MN			
	+	E	–	Total
+	79	7	73	159
E	2	0	2	4
–	34	4	83	121
Total	115	11	158	284

No. of carcinogens tested in both tests (A): 284
No. (%) of clear positive results in both tests (B): 79 (27.8%)
No. (%) of clear positive results in only 1 of the two tests (C): 116 (40.8%)
Sensitivity (i.e., clearly positive in at least 1 test when both conducted ([B + C]/A)[a] : 68.7%

+, Positive; –, Negative; E, Equivocal
a: If E results are considered positive, the sensitivity is 70.8% (201/284)

where both tests were performed was 68.7% (195/284). If equivocal results are considered positive, the sensitivity rises to 70.8% (201/284). For the non-carcinogens, clearly negative results were obtained in both tests for 39 of the 86 non-carcinogens (Table 7). Therefore, the specificity value that was calculated from the results

Table 7
Performance of combinations of Ames and *in vivo* MN tests in detecting rodent non-carcinogens when both performed.

Ames	*in vivo* MN			
	+	E	–	Total
+	6	2	11	19
E	2	0	2	4
–	16	8	39	63
Total	24	10	52	86

No. of non-carcinogens tested in both tests (A): 86
No. of clear negative results in both tests (B): 39
Specificity (B/A)[a] : 45.3%

+, Positive; –, Negative; E, Equivocal
a: If E results are considered negative, the specificity is 60.0% (49/86)

Table 8
Performance of combinations of Ames and TGR tests in detecting rodent carcinogens when both performed.

Ames	TGR			
	+	E	–	Total
+	48	0	8	56
E	1	0	0	1
–	5	0	12	17
Total	54	0	20	74

No. of carcinogens tested in both tests (A): 74
No. (%) of clear positive results in both tests (B): 48 (64.9%)
No. (%) of clear positive results in only 1 of the two tests (C): 14 (18.9%)
Sensitivity (i.e., clearly positive in at least 1 test when both conducted ([B + C]/A)[a] : 83.8%

+, Positive; –, Negative; E, Equivocal
a: If E results are considered positive, the sensitivity is 83.8% (62/74)

where both tests were performed was 45.3% (39/86). If equivocal results are considered negative, the specificity rises to 60.0% (49/86).

3.5.3. *Performance of Ames + TGR*

Results were available from both Ames and TGR tests for 74 carcinogens. For the carcinogens, 48 or 14 chemicals gave clearly positive in both tests or in one of the two tests when both were performed, respectively (Table 8). Therefore, the sensitivity value that was calculated from the results where both tests were performed was 83.8% (62/74). Even if equivocal results are considered positive, the sensitivity is not changed. The sensitivities are not changed in other combinations including the TGR test.

3.5.4. *Performance of Ames* + in vitro *CA* + in vivo *MN*

Results in all three tests were available for 224 carcinogens and 75 non-carcinogens. For the carcinogens, 51 or 130 chemicals gave clear positive results in all three tests or in one or two of the tests when all three were performed (Table 9). Therefore, the sensitivity value that was calculated from the results where all three tests were performed was 80.8% (181/224). If equivocal results are con-

Table 9
Summary performance of three or four genotoxicity assays in detecting rodent carcinogens when all tests performed.

	Test combination			
	Ames + *in vitro* CA + *in vivo* MN	Ames + *in vitro* CA + TGR	Ames + *in vivo* MN + TGR	Ames + *in vitro* CA + *in vivo* MN + TGR
No. of carcinogens tested in all three or four test systems	224	64	64	56
No. (%) of clear positive results in all three or four test systems	51/224 (22.8%)	36/64 (56.3%)	31/64 (48.4%)	25/56 (44.6%)
No. (%) of clear positive results in one or two of the three assays	130/224 (58.0%)	21/64 (32.8%)	25/64 (39.1%)	Not applicable
No. (%) of clear positive results in one, two or three of the four assays	Not applicable	Not applicable	Not applicable	25/56 (44.6%)
Sensitivity (i.e., clearly positive in at least one assay when all three or four conducted)[a]	181/224 (80.8%)	57/64 (89.1%)	56/64 (87.5%)	50/56 (89.3%)

[a] If equivocal results are considered positive, the sensitivity of the combination of Ames + *in vitro* CA + *in vivo* MN is 83.0% (186/224). The sensitivities of other test combinations are not changed.

Table 10
Summary performance of three or four genotoxicity assays in detecting rodent non-carcinogens when all three or four tests performed.

	Test combination			
	Ames + *in vitro* CA + *in vivo* MN	Ames + *in vitro* CA + TGR	Ames + *in vivo* MN + TGR	Ames + *in vitro* CA + *in vivo* MN + TGR
No. of non-carcinogens tested in all three or four test systems (A)	75	4	3	3
No. (%) of clear negative results in all three or four test systems (B)	16	0	1	0
Specificity (B/A)[a]	16/75 (21.3%)	Not calculated	Not calculated	Not calculated

[a] If equivocal results are considered negative, the specificity is 29.3% (22/75).

Table 11
Concordance of Ames and TGR tests in detecting rodent carcinogens and non-carcinogens.

Ames	TGR			
	+ (C, NC)	E (C, NC)	– (C, NC)	Total
+	48 (48, 0)	0 (0, 0)	10 (8, 2)	58
E	1 (1, 0)	0 (0, 0)	0 (0, 0)	1
–	5 (5, 0)	0 (0, 0)	14 (12, 2)	19
Total	54 (54, 0)	0 (0, 0)	24 (12, 2)	78
Concordance[a]	79.5% (62/78)			

+, Positive; –, Negative; E, Equivocal; C, Carcinogens; NC, Non-carcinogens.
[a] Equivocal (E) results were not counted either as positive or negative, but they were included in the total number. If E results are considered positive, the concrdance is 80.8% (63/78). If E results are considered negative, the concrdance is 79.5% (62/78).

sidered positive, the sensitivity rises to 83.0% (186/224). For the non-carcinogens, clearly negative results were obtained in all three tests for 16 of the 75 non-carcinogens (Table 10). Therefore, the specificity value that was calculated from the results where all three tests were performed was 21.3% (16/75). If equivocal results are considered negative, the specificity rises to 29.3% (22/75).

3.5.5. *Performance of Ames* + in vitro *CA + TGR*

Results in all three tests were available for 64 carcinogens and 4 non-carcinogens. For the carcinogens, 36 or 21 chemicals gave clear positive results in all three tests or in one or two of the tests when all three were performed, respectively (Table 9). Therefore, the sensitivity value that was calculated from the results where all three tests were performed was 89.1% (57/64). For the non-carcinogens, none of the four chemicals were clearly negative in all three tests (Table 10).

3.5.6. *Performance of Ames* + in vivo *MN + TGR*

Results in all three tests were available for 64 carcinogens and 4 non-carcinogens. For the carcinogens, 31 or 25 chemicals gave clear positive results in all three tests or in one or two of the tests when all three were performed, respectively (Table 9). Therefore, the sensitivity value that was calculated from the results where all three tests were performed was 87.5% (56/64). For the non-carcinogens, none of the four chemicals were clearly negative in all three tests (Table 10).

3.5.7. *Performance of Ames* + in vitro *CA* + in vivo *MN + TGR*

Results in all four tests were available for 56 carcinogens and 3 non-carcinogens. For the carcinogens, 25 or another 25 chemicals gave clear positive results in all four tests or in one, two or three of the tests when all four tests were performed, respectively (Table 9). Therefore, the sensitivity value that was calculated from the results where all four tests were performed was 89.3% (50/56). For the non-carcinogens, none of the 3 chemicals were clearly negative in all four tests (Table 10).

3.6. *Comparison analysis between* in vitro *and* in vivo *tests with a similar endpoint*

Extrapolation of test results from *in vitro* to *in vivo* is one of the major issues in chemical risk assessment. The Ames and TGR tests can detect gene mutations, whereas the *in vitro* CA and *in vivo* MN tests can detect chromosome damage; the MN test can detect aneugenicity in addition to clastogencity. Therefore, concordance of these *in vitro* and *in vivo* tests in detecting rodent carcinogens and/or non-carcinogens was investigated. Though the results of *in vitro* MN tests were presented in the CGX database, the number (n = 89) of carcinogens with *in vitro* MN data in the dataset was quite small in comparison with that (n = 352) for the *in vitro* CA test [8]. Thus, for this evaluation the *in vitro* CA test was selected as the most appropriate *in vitro* test system that detects chromosome damage for comparison with the *in vivo* MN test.

Table 12
Incosistent results between Ames and TGR tests.

ID	Chemical	Chemical grouping	CAS	Carcinogenicity	Ames	TGR
C84	Benzene	Benzene	71-43-2	+	–	+
C384	Hexachlorobutadiene	Halogenated alkene	608-73-1	+	–	+
C605	Oxazepam	Aromatic amine or amide	604-75-1	+	–	+
C645	Procarbazine HCl	Mono- or di-alkylhydrazine	366-70-1	+	–	+
C742	Uracil	Substituted pyrimidine or purine	66-22-8	+	–	+
C17	Acrylonitrile	Alpha-, beta-unsaturated nitrile	107-13-1	+	+	–
C137	Carbon tetrachloride	Halogenated methane	56-23-5	+	+	–
C160	Chloroform	Halogenated methane	67-66-3	+	+	–
C257	1,2-Dichloroethane	vic-Dihalide	107-06-2	+	+	–
C395	Hydrazine sulphate	Hydrazine or monoacyl- or monosulphonyl-hydrazine	10034-93-2	+	+	–
C489	Metronidazole	Aromatic nitro compound	443-48-1	+	+	–
C509	Nitrite, sodium	Alkyl nitrite, nitrous acid or nitrite salt	7632-00-0	+	+	–
C622	Phenobarbital	(Thio)urea	50-06-6	+	+	–
NC52	2,6-Diaminotoluene 2HCl	Aromatic amine or amide	15481-70-6	–	+	–
NC126	1-Nitronaphthalene	Aromatic nitro compound	86-59-7	–	+	–

Table 13
Concordance of *in vitro* CA and *in vivo* MN tests in detecting rodent carcinogens and non-carcinogens.

in vitro CA	*in vivo* MN			
	+ (C, NC)	E (C, NC)	– (C, NC)	Total
+	82 (72, 10)	12 (6, 6)	91 (70, 21)	185
E	3 (1, 2)	0 (0, 0)	12 (7, 5)	15
–	27 (17, 10)	4 (3, 1)	69 (49, 20)	100
Total	112 (90, 22)	16 (9, 7)	172 (126, 46)	300
Concordance[a]	50.3% (151/300)			

+, Positive; –, Negative; E, Equivocal; C, Carcinogens; NC, Non-carcinogens.
[a] Equivocal (E) results not counted either as positive or negative, but they were included in the total number. If E results are considered positive, the concrdance is 55.3% (166/300). If E results are considered negative, the concrdance is 55.7% (167/300).

3.6.1. *Ames test and TGR tests*

Concordance of the Ames and TGR tests in detecting rodent carcinogens and non-carcinogens is shown in Table 11. Results were available from both Ames and TGR tests for 74 carcinogens and 4 non-carcinogens. Forty eight or 14 chemicals gave clearly positive or negative results in both tests, respectively. The concordance value calculated was 79.5% (62/78). Fifteen chemicals which showed inconsistent results are listed in Table 12. They consisted of 5 carcinogens with negative results in the Ames test but positive in the TGR test, 8 carcinogens with positive results in the Ames test but negative in the TGR test, and 2 non-carcinogens with positive results in the Ames test but negative in the TGR test.

3.6.2. In vitro *CA and* in vivo *MN tests*

Concordance of the *in vitro* CA and *in vivo* MN tests in detecting rodent carcinogens and non-carcinogens is shown in Table 13. Results were available from both *in vitro* CA and *in vivo* MN tests for 225 carcinogens and 75 non-carcinogens. Eighty two or 69 chemicals gave clearly positive or negative results in both tests, respectively. The concordance value calculated was 50.3% (151/300). There were many chemicals (70 carcinogens and 21 non-carcinogens) which showed positive results in the *in vitro* CA, but gave negative results in the *in vivo* MN. On the other hand, 27 chemicals (17 carcinogens and 10 non-carcinogens) showed negative results in the *in vitro* CA test, but were positive in the *in vivo* MN test. They are listed in Table 14.

4. Discussion

The *in vivo* MN and TGR tests are the most important *in vivo* genotoxicity tests for identification of chemical genotoxic hazard for regulatory bodies [1–5]. Therefore, performances (sensitivity and specificity) of these tests, alone and in combination with other assay(s), including *in vitro* tests, were investigated. The performance of the *in vivo* MN test was 41.0% for sensitivity or 60.5% for specificity. The sensitivity is lower than in both the Ames test (59.0%) and the *in vitro* CA test (62.8%), but the specificity fell between that for the two tests considered singly, *i.e.*, 73.9% and 48.5% (Table 15 and Fig. 1). The performance of the *in vivo* MN test previously reported by several investigators was wide ranging, namely 28–71% for sensitivity or 41–83% for specificity (Table 1). These variations might be due to different chemical sets, including IARC carcinogens by Morita et al. [11], NTP testing chemicals by Zeiger et al. [9] or Kim and Margolin [12], chemicals tested by TGR assay by Lambert et al. [15] or OECD [16] and ISSMIC chemicals by Benigni et al. [13,14]. Some of these references do not provide chemical names [9,13,14] or original data sources [15,16] in their texts. Therefore, it will be difficult to analyze the variations. Our investigation with a large number of chemicals gave sensitivity and specificity values that fell in the middle of the ranges given in those previous investigations. The *in vivo* MN test is recognized as demonstrating a relatively low sensitivity for the detection of carcinogens. Although the assay is sensitive to many different chemical classes, based on a study of IARC carcinogens (groups 1, 2A and 2B) [11], it is less sensitive to dialkyl type *N*-nitroso compounds, silica and metal compounds, aromatic amines excluding

Table 14
Incosistent results between *in vitro* CA (negative) and *in vivo* MN (positive) tests.

ID	Chemical	Chemical grouping	CAS	Carcinogenicity	*in vitro*CA	*in vivo*MN
C179	Chlorpromazine hydrochloride	Aromatic amine or amide	69-09-0	+	–	+a
C185	C.I. Direct black 38	Aromatic azo compound	1937-37-7	+	–	+
C198	C.I. Solvent yellow 14	Aromatic azo compound	842-07-9	+	–	+
C217	D&C Red 9	Aromatic azo compound	5160-02-1	+	–	+
C226	Decabromodiphenyl oxide	Polyhalogenated aromatic	1163-19-5	+	–	+
C240	Diazepam	Aromatic amine or amide	439-14-5	+	–	+
C277	3,4-Dihydrocoumarin	Glycidyl ether, amine, ester or amide	119-84-6	+	–	+
C305	Dimethylvinyl chloride	Halogenated alkene	513-37-1	+	–	+
C425	Isoprene	Alkene	78-79-5	+	–	+
C645	Procarbazine HCl	Mono- or di-alkylhydrazine	366-70-1	+	–	+
C660	Reserpine	Phenol or precursor	50-55-5	+	–	+b
C691	1,1,2,2-Tetrachloroethane	Gem-dihalide	79-34-5	+	–	+
C705	Titanium dioxide	Alkali, alkali earth, metal salt	13463-67-7	+	–	+
C706	Toluene	Benzene	108-88-3	+	–	+
C734	Trimethylphosphate	Alkyl ester of phosphoric or phosphonic acid	512-56-1	+	–*	+
C738	Tris(2-chloroethyl)phosphate	Alkylating agent	115-96-8	+	–	+
C744	Urethane	Alkyl carbamate	51-79-6	+	–*	+
NC8	dl-Amphetamine sulfate	Amine	60-13-9	–	–	+
NC13	l-Ascorbic acid	Carboxylic acid	50-81-7	–	–	+
NC49	Deltamethrin	Halogenated alkene	52918-63-5	–	–	+
NC59	1,1-Dichloroethane	Gem-dihalide	75-34-3	–	–	+
NC119	Methyl parathion	Alkyl ester of phosphoric or phosphonic acid	298-00-0	–	–	+
NC120	Monochloroacetic acid	Alkylating agent	79-11-8	–	–	+
NC133	Oxytetracycline HCl	Substituted vinyl ketone	2058-46-0	–	–	+
NC152	Resorcinol	Resorcinol or precursor	108-46-3	–	–*	+
NC173	Tolbutamide	Aryl sulphonamide	64-77-7	–	–	+
NC178	Triphenyltin hydroxide	Alkali, alkali earth, metal salt	76-87-9	–	–	+

*: positive response at both >10 mM and 2 mg/mL.
a: due to hypothermia.
b: mice, due to hypotheramia; negative in rat.

Table 15
Summary of performance for individual assays or their combinations.

Measure	Ames	*in vitro* CA	*in vivo* MN	TGR	Ames + *in vitro* CA	Ames + *in vivo* MN	Ames + TGR	Ames + *in vitro* CA + *in vivo* MN	Ames + *in vitro* CA + TGR	Ames + *in vivo* MN + TGR	Ames + *in vitro* CA + *in vivo* MN + TGR
Sensitivity (%)	59.0	62.8	41.0	72.4	74.3	68.7	83.8	80.8	89.1	87.5	89.3
Specificity (%)	73.9	48.5	60.5	NC	37.5	45.3	NC	21.3	NC	NC	NC
Concordance (%)	62.6	58.9	45.4	NC	NA	NA	NC	NA	NA	NA	NA

NC: Not calculated.
NA: Not applicable.

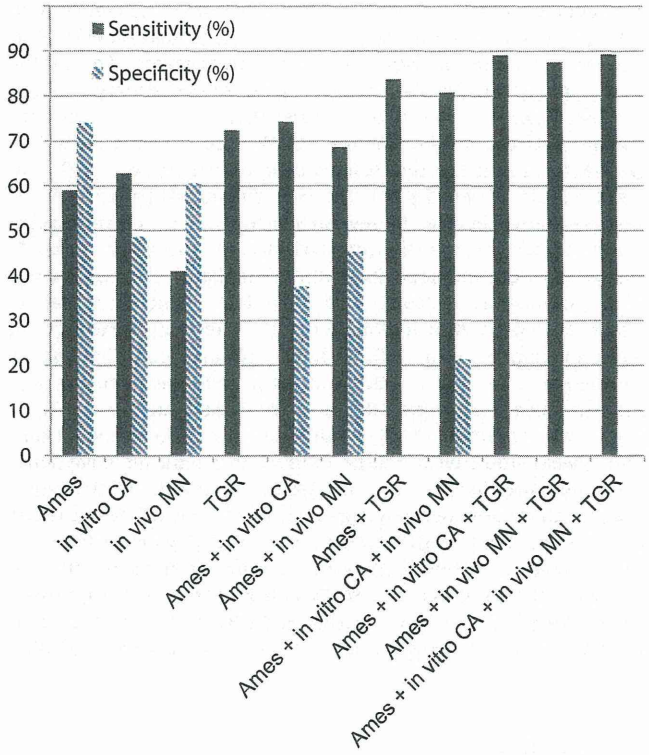

Fig. 1. Sensitivity and specificity for individual assays of Ames, *in vitro* CA, *in vivo* MN or TGR and their selected combinations.

aminobiphenyl and benzidine derivatives or heterocyclic amines, halogenated compounds or steroids and other hormones. As the CGX dataset includes many *N*-nitroso compounds, aromatic amines and halogenated compounds, it was to be expected that the *in vivo* MN test would have relatively low sensitivity when compared to previous investigations. Aromatic amines/halogenated or *N*-nitroso compounds would most likely produce effects in the liver or at a site of contact, respectively. Therefore, by knowledge of the chemical class it should be possible to determine whether a bone marrow MN test, a test for genotoxicity in liver or a site-of-contact assay would be most appropriate. Another important *in vivo* genotoxicity test, *i.e.*, TGR test, also showed good sensitivity (72.4%). However, its specificity, either alone or in combination with other test(s), could not be calculated due to the limited number of non-carcinogens in the CGX dataset that have been tested in the TGR assay. The sensitivity and specificity of the TGR test were previously reported to be 78% and 69% [15] or 76% and 78% [16], respectively. Similar sensitivity was obtained in our investigation.

Sensitivity and specificity of the combination of Ames + *in vivo* MN was 68.7% and 45.3%, respectively. These values are similar to those obtained with the combination of Ames + *in vitro* CA (sensitivity 74.3%, specificity 37.5%) (Table 15 and Fig. 1). Similar sensitivity (62%) was also seen for the combination of Ames + *in vivo* MN, but the specificity was much higher (67%) [12]. Higher sensitivity (83.8%) was seen for the combination of Ames + TGR; the specificity could not be calculated as discussed above. Similar sensitivity (88%) was also seen for the combination of Ames + TGR [15,16]. When one considers option 2 of ICH where two different *in vivo* tests are required in addition to the Ames test, then the combination of Ames + *in vivo* MN + TGR showed an even higher sensitivity (87.5%). This is similar to the sensitivity achieved by the combination of Ames + *in vitro* CA + *in vivo* MN (80.8%) as would often be used in option 1 of ICH. The sensitivity of the combination of Ames + *in vitro* CA + TGR was also high (89.1%). Apparently, option 1 and option 2 from ICH gave comparable sensitivities. These data indicate that, if the second *in vivo* test is selected adequately, option 2 of ICH can work equally as well as option 1 in terms of detecting carcinogens with a high sensitivity. In those cases where 4 tests will be performed (*e.g.*, Ames + *in vitro* CA + *in vivo* MN + TGR), usually where follow-up to an *in vitro* positive result is required, the sensitivity (89.3%) remained equally high. Good sensitivities were seen in the combinations of *in vitro* and *in vivo* tests, suggesting the usefulness of both *in vivo* MN and TGR tests as *in vivo* genotoxicity tests. The performances of Ames + *in vitro* CA and Ames + *in vivo* MN were also comparable, which is an important finding for the testing cosmetic ingredients in Europe [6,7].

Comparative analyses of *in vitro* and *in vivo* test results for the same or similar genotoxic endpoints were also investigated (*i.e.*, Ames and TGR test, or *in vitro* CA and *in vivo* MN test). Concordance for the Ames and TGR tests was good (79.5%, Table 11). Fifteen chemicals were identified as exhibiting inconsistent results (Table 12), but no clear explanations can be given for these inconsistencies. With respect to oxazepam (ID C605), chronic administration was required to detect a mutagenic response in the TGR test, which might be due to oxidative damage as a possible primary mechanism for this chemical [16]. For carbon tetrachloride (ID C137) and chloroform (ID C160), positive results in the Ames test were obtained from gaseous exposure [18]. On the other hand, carbon tetrachloride was administered by gavage and chloroform was administered by inhalation in the TGR test [16]. Some factors may differently affect chemical accessibility, metabolism and toxicity in bacteria and intact mammals. They include DNA structure, xenobiotic metabolism, antioxidant activity, and DNA damage repair. High levels of reductive enzymes of bacteria, compared to mammalian cells, can activate efficiently nitro and azo compounds to electrophilic metabolites [60]. Therefore, differences in metabolism or exposure levels between bacteria and mammalian tissues in whole animals might be explain the different responses. Seven out of the above 15 chemicals were halogenated alkenes or aromatic compounds (Table 12). For these chemicals, consideration of the route of exposure and the impact on metabolism may be important.

Concordance of the *in vitro* CA and *in vivo* MN tests was not so high (50.3%) based on a 300 chemical dataset (Table 13). Ninety one chemicals were positive in the *in vitro* CA, but negative in the *in vivo* MN test. Factors of the inconsistency will include detoxification/elimination, different metabolism or poor absorption in

in vivo, or extreme high concentration in *in vitro* [61,62]. Non-carcinogens within these chemicals are called misleading or false positives. Several recommendations including use of p53 competent cells or accurate cytotoxicity measurement have been made to avoid generation of false positives [61,63]. Twenty seven chemicals (17 carcinogens and 10 non-carcinogens) showed the opposite responses, *i.e.*, negative in the *in vitro* CA test, but positive in the *in vivo* MN test (Table 14). Benigni et al. reported that the concordance for these 2 tests was 60.2% (68/113) based on a 113 chemical dataset, and that the quite high number (n = 22) of *in vivo* MN positives that are negative *in vitro* CA may be due to aneugenic mechanisms of action that are not detected by the *in vitro* CA test [14]. However, this does not seem to be a logical explanation for the 27 inconsistent chemicals in Table 14, which do not seem to include many aneugens. The differences in concordance between the current analysis and Benigni et al. [14] may be due to the different chemical datasets used. The induction of MN *in vivo* by chlorpromazine (ID C179), reserpine (ID C660) and phenol (ID NC138) might be due to hypothermia [42,43,59]. For 3 of the chemicals, including trimethylphosphate (ID C734), urethane (ID C744) and resorcinol (ID NC152), the original CGX database expressed positive results in the *in vitro* CA test. However, as the positive responses were only seen at concentrations greater than both 10 mM and 2 mg/mL, they were assigned as negative in this analysis [28,29]. No specific chemical classes were identified amongst these inconsistent chemicals (Table 14), and, therefore, no clear explanations can be given to the other inconsistent *in vitro* CA and *in vivo* MN results. Some of the chemicals might induce MN via an indirect genotoxic mode of action (*i.e.*, secondary effect) such as body temperature change (hypothermia or hyperthermia) or erythropoiesis [52], or differences in metabolism including detoxification might be involved.

5. Conclusions

The combination of Ames and the *in vivo* MN test showed similar values to Ames + *in vitro* CA test in the performance of the different batteries (sensitivity and specificity to rodent carcinogenicity). This indicates that, as long as the second *in vivo* test is appropriately selected, option 2 in the ICH S2(R1) recommendations can work equally as well as option 1 in detection of carcinogens. Usefulness of the strategy of only *in vitro* test battery by the EU cosmetics is also indicated. Higher sensitivities with the combined use of the TGR test indicate the usefulness of this test in the genotoxicity testing strategies by COM, ECHA, EFSA, FSC, or ICH in case of *in vitro* positive results. An assay which can detect genotoxic effects in the liver or at a site of contact will be important as a second *in vivo* test. The standard test battery (Ames + *in vitro* CA + *in vivo* MN) is effective to detect rodent carcinogens, but it should be noted that this battery has low specificity.

Conflict of interest

There are no conflicts of interests.

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Appendix A. : Genotoxicity test results with rodent carcinogens.

ID	Chemical	CAS No.	Ames	*in vitro* CA	*in vivo* MN	*in vivo* MN Ref.	TGR	TGR Ref.
C1	Acetaldehyde	75-07-0	–	+	+	[11]		
C2	Acetaldehyde methylformylhydrazone	16568-02-8	–					
C3	Acetamide	60-35-5	–		–	[11]~		
C4	Acetaminophen	103-90-2	–	+	–	[64]	–	[16]~
C5	Acetone[4-(5-nitro-2-furyl)-2-thiazolyl]hydrazone	18523-69-8						
C6	Acetoxime	127-06-0	–					
C7	N-Acetoxy-2-acetylaminofluorene	6098-44-8	+	+				
C8	1′-Acetoxysafrole	34627-78-6	+					
C9	4-Acetylaminobiphenyl	4075-79-0						
C10	2-Acetylaminofluorene	53-96-3	+	+	+	[30]	+	[16]
C11	N′-Acetyl-4-(hydroxymethyl)phenylhydrazine	65734-38-5						
C12	1-Acetyl-2-isonicotinoylhydrazine	1078-38-2						
C13	1-Acetyl-2-phenylhydrazine	114-83-0	+					
C14	Acifluorfen	50594-66-6						
C15	Acronycine	7008-42-6						
C16	Acrylamide	79-06-1	E	+	+	[65,66]	+	[16,67]
C17	Acrylonitrile	107-13-1	+	+	–a	[11,41]	–^	[16]~
C18	Actinomycin D	50-76-0	–	+	+	[50]		
C19	Aflatoxicol	29611-03-8	+					
C20	Aflatoxin B1	1162-65-8	+	+	+	[30]	+	[16]
C21	Aflatoxin, crude	–						
C22	Aldrin	309-00-2	–	+	–	[68]		
C23	Allyl glycidyl ether	106-92-3	+	+	+	[69]		
C24	Allyl isothiocyanate	57-06-7	E	+	–	[34]		
C25	Allyl isovalerate	2835-39-4	–	+				
C26	1-Allyl-1-nitrosourea	760-56-5						
C27	Allylhydrazine HCl	52207-83-7						
C28	2-Aminoanthracene	613-13-8	+	E				
C29	2-Aminoanthraquinone	117-79-3	+					
C30	4-Aminoazobenzene	60-09-3	+		+	[11]		
C31	4-Aminobiphenyl	92-67-1	+	+	+	[11]	+	[16]
C32	4-Aminobiphenyl HCl	2113-61-3	+		+	[70]		
C33	1-Amino-2,4-dibromoanthraquinone	81-49-2	+	–				
C34	2-Amino-3,4-dimethylimidazo[4,5-f]quinoline (MeIQ)	77094-11-2	+	+			+	[16]
C35	2-Amino-3,8-dimethylimidazo[4,5-f]quinoxaline (MeIQx)	77500-04-0	+		+	[71]	+	[16]
C36	3-Amino-1,4-dimethyl-5H-pyrido[4,3-b]indole acetate (Trp-P-1 acetate)	68808-54-8	+	+				
C37	2-Aminodiphenylene oxide	3693-22-9						
C38	2-Aminodipyrido[1,2-a:3′,2′-d]imidazole (Glu-P-2)	67730-10-3	+	+				
C39	3-Amino-4-ethoxyacetanilide	17026-81-2	+	–				
C40	3-Amino-9-ethylcarbazole HCl	6109-97-3	+	–				
C41	3-Amino-9-ethylcarbazole mixture	Mixture	+					
C42	2-Aminofluorene	153-78-6	+					
C43	2-Amino-6-methyldipyridol[1,2-a:3′,2′-d]imidazole (Glu-P-1)	67730-11-4	+	+				
C44	2-Amino-3-methylimidazo[4,5-f]quinoline (IQ)	76180-96-6	+		–	[72]	+	[16]
C45	2-Amino-3-methylimidazo[4,5-f]quinoline HCl (IQ.HCl)	–	+				+	[16]
C46	2-Amino-1-methyl-6-phenylimidazo-[4,5-b]pyridine hydrochloride (PhiP.HCl)	105650-23-5	+	+	+	[73]	+	[16]
C47	3-Amino-1-methyl-5H-pyrido[4,3-b]indole acetate (Trp-P-2 acetate)	72254-58-1	+	+				
C48	2-Amino-5-(5-nitro-2-furyl)-1,3,4-oxadiazole	3775-55-1						
C49	2-Amino-5-(5-nitro-2-furyl)-1,3,4-thiadiazole	712-68-5						
C50	2-Amino-4-(5-nitro-2-furyl)thiazole	38514-71-5	+					
C51	trans-5-Amino-3[2-(5-nitro-2-furyl)vinyl]-1,2,4-oxadiazole	28754-68-9						
C52	2-Amino-4-nitrophenol	99-57-0	+	+	–	[50]		
C53	2-Amino-5-nitrophenol	121-88-0	+	+				
C54	4-Amino-2-nitrophenol	119-34-6	+	+				
C55	2-Amino-4-(p-nitrophenyl)thiazole	2104-09-8						
C56	2-Amino-5-nitrothiazole	121-66-4	+	+				
C57	2-Amino-9H-pyrido(2,3-b)indole (A-alpha-C)	26148-68-5	+	+			+	[16]