Mutation Research 653 (2008) 99–108

Contents lists available at ScienceDirect

Mutation Research/Genetic Toxicology and Environmental Mutagenesis

journal homepage: www.elsevier.com/locate/gentox
Community address: www.elsevier.com/locate/mutres

Recommended lists of genotoxic and non-genotoxic chemicals for assessment of the performance of new or improved genotoxicity tests: A follow-up to an ECVAM workshop☆

David Kirkland[a,*], Peter Kasper[b], Lutz Müller[c], Raffaella Corvi[d], Günter Speit[e]

[a] *Covance Laboratories Ltd., Otley Road, Harrogate HG3 1PY United Kingdom*
[b] *Federal Institute for Drugs and Medical Devices (BfArM), Kurt-Georg-Kiesinger Allee 3, D-53175 Bonn, Germany*
[c] *Non-clinical Drug Safety, F. Hoffmann-La Roche, PRBN-T, Building 73-311b, CH-4070 Basel, Switzerland*
[d] *European Centre for the Validation of Alternative Methods (ECVAM), Institute for Health and Consumer Protection (IHCP), European Commission – Joint Research Centre, TP 580, Via E. Fermi 1, I-21027 Ispra (Va), Italy*
[e] *Universitätsklinikum Ulm, Abteiling Humangenetik, D-89070 Ulm, Germany*

ARTICLE INFO

Article history:
Received 3 January 2008
Received in revised form 17 March 2008
Accepted 28 March 2008
Available online 11 April 2008

Keywords:
Genotoxicity *in vitro*
Reliability
Improved tests
New tests
Recommended chemicals

ABSTRACT

At a recent ECVAM workshop considering ways to reduce the frequency of irrelevant positive results in mammalian cell genotoxicity tests [D. Kirkland, S. Pfuhler, D. Tweats, M. Aardema, R. Corvi, F. Darroudi, A. Elhajouji, H.-R. Glatt, P. Hastwell, M. Hayashi, P. Kasper, S. Kirchner, A. Lynch, D. Marzin, D. Maurici, J.-R. Meunier, L. Müller, G. Nohynek, J. Parry, E. Parry, V. Thybaud, R. Tice, J. van Benthem, P. Vanparys, P. White, How to reduce false positive results when undertaking *in vitro* genotoxicity testing and thus avoid unnecessary followup animal tests: Report of an ECVAM Workshop, Mutat. Res. 628 (2007) 31–55], recommendations for improvements/modifications to existing tests, and suggestions for new assays were made. Following on from this, it was important to identify chemicals that could be used in the evaluation of modified or new assays. An expert panel was therefore convened and recommendations made for chemicals to fit three different sets of characteristics, namely:

Group 1: Chemicals that should be detected as positive in *in vitro* mammalian cell genotoxicity tests. Chemicals in this group are all *in vivo* genotoxins, either due to DNA-reactive or non DNA-reactive mechanisms (e.g., induction of aneuploidy, inhibition of topoisomerase). Most of them are also known carcinogens with a mutagenic mode of action.

Group 2: Chemicals that should give negative results in *in vitro* genotoxicity tests and routinely do give negative results in existing *in vitro* mammalian cell genotoxicity tests. Chemicals in this group are usually negative in *in vivo* genotoxicity tests (when tested) and non-DNA-reactive. They are either non-carcinogenic or rodent carcinogens with an assumed non-mutagenic mode of action.

Group 3: Chemicals that should give negative results in *in vitro* mammalian cell genotoxicity tests, but have been reported to induce chromosomal aberrations or *tk* mutations in mouse lymphoma cells, often at high concentrations or at high levels of cytotoxicity. Chemicals in this group are generally negative in *in vivo* genotoxicity studies (when tested) and negative in the Ames test. They are either non-carcinogenic or rodent carcinogens with an assumed non-mutagenic mode of action.

This paper therefore contains these three recommended lists of chemicals and describes how these should be used for any test-evaluation programme.

Abbreviations: CA, chromosomal aberrations; MLA, mouse lymphoma assay; MN, micronuclei; UDS, unscheduled DNA synthesis; IARC, International Agency for Research on Cancer; NTP, National Toxicology Program; RTG, relative total growth; E, equivocal; ip, intraperitoneal; HPRT, hypoxanthine-guanine phosphoribosyl transferase.

☆ *Disclaimer*: This document represents the consensus of the participants' views expressed as individual scientists and does not necessarily represent the policies and procedures of their respective institutions.

* Corresponding author. Tel.: +44 1423 848401; fax: +44 1423 848983.
E-mail address: david.kirkland@covance.com (D. Kirkland).

1383-5718/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.mrgentox.2008.03.008

1. Introduction

In 2007, Kirkland et al. [1] published the recommendations of a workshop, organised and funded by the European Centre for the Validation of Alternative Methods (ECVAM), in which ways to reduce the frequency of irrelevant positive results, particularly in mammalian cell tests (as highlighted in ref. [2]), were discussed. In order to judge the performance of *in vitro* genotoxicity tests the ECVAM workgroup report recommended that the following groups of chemicals be identified:

- chemicals that are *in vivo* genotoxins and DNA-reactive, mutagenic rodent carcinogens;
- chemicals that are not genotoxic in at least two *in vivo* tests, and induce tumours via a non-DNA-reactive, non-mutagenic mechanism;
- chemicals that are *in vivo* genotoxins but not carcinogenic, yet whose genotoxicity may be a relevant risk for human health;
- chemicals that are neither rodent carcinogens nor genotoxic in at least two *in vivo* tests.

Clearly we would expect genotoxicity tests to give positive results with those under bullets 1 and 3, but not with those under bullets 2 and 4. The workshop noted that in many cases chemicals that turn out not to be *in vivo* genotoxins, or not to induce tumours via a mutagenic or DNA-reactive mechanism, can give positive results in mammalian cell tests. These data have been called "false" or "irrelevant" positive results.

Several suggestions for possible improvements/modifications to existing tests, or new tests that showed potential, were identified. Such improvements or new assays need to show improved specificity (i.e. give fewer irrelevant positive results) without compromising sensitivity (i.e. still detecting *in vivo* genotoxins and DNA-reactive carcinogens). The four categories given above can be more conveniently listed in three groups. Thus, it was recommended that, in order to evaluate such improvements or new assays, an expert panel should be convened to identify and recommend the following three sets of chemicals:

Group 1: Chemicals that should be detected as positive in *in vitro* mammalian cell genotoxicity tests. Chemicals in this group are all *in vivo* genotoxins, either due to DNA-reactive or non DNA-reactive mechanisms (e.g. induction of aneuploidy, inhibition of topoisomerase). Most of them are also known carcinogens with a mutagenic mode of action.

Group 2: Chemicals that should give negative results in *in vitro* genotoxicity tests and routinely do give negative results in existing *in vitro* mammalian cell genotoxicity tests. Chemicals in this group are usually negative in *in vivo* genotoxicity tests (when tested) and non-DNA-reactive. They are either non-carcinogenic or rodent carcinogens with an assumed non-mutagenic mode of action.

Group 3: Chemicals that should give negative results in *in vitro* mammalian cell genotoxicity tests, but have been reported to induce chromosomal aberrations or *tk* mutations in mouse lymphoma cells, often at high concentrations or at high levels of cytotoxicity. Chemicals in this group are generally negative in *in vivo* genotoxicity studies (when tested) and negative in the Ames test. They are either non-carcinogenic or rodent carcinogens with an assumed non-mutagenic mode of action.

In order to fulfil this obligation, ECVAM kindly organised for the authors to meet to define these lists at a workshop at ECVAM, Ispra, Italy on 3–4 May 2007. Careful consideration was given to the published evidence that would support the inclusion of each chemical in each of the lists, and in the Tables presented here detailed justifications and supporting references are provided. However, complete and consistent data sets are not available for all chemicals. In many cases there are gaps and inconsistencies and our assessment is based on a weight-of-evidence approach. In order to develop weight-of-evidence decisions, data of various kinds were taken as being important. The information to build a weight of evidence that a chemical is a DNA-reactive carcinogen or an *in vivo* genotoxin, and should be detected with a new or modified genotoxicity test, will not necessarily be the same as that needed to decide that a chemical is not DNA-reactive and should not be detected.

Tables 1–3 summarise suggested reference substances for the three groups. Classification is mainly based on *in vivo* genotoxicity and DNA reactivity, while carcinogenicity data are used as a supplementary criterion. The definition of "DNA reactivity" is primarily based on results from bacterial mutagenicity tests ("Ames test"), i.e. Ames-test-positive indicates DNA reactivity while Ames-test-negative indicates non-DNA-reactivity. Exceptions from this rule are justified by the specific information given in the text. The Tables contain further information about the chemicals, including information on the requirement for metabolic activation, or the mode of action if available. Within the Tables, subgroups of chemicals are summarised based on chemical classes or a specific pattern of results in genotoxicity tests. It is recommended that examples from each subgroup are included for any test-evaluation program. Clearly some scientists may have their own "favourite" chemicals for inclusion in such an evaluation program, and the lists given here are not meant to be exhaustive.

Group 1 chemicals: *In vivo* genotoxins and DNA-reactive, mutagenic carcinogens that should be detected as positive in *in vitro* mammalian cell tests ("true positives").

A total of 20 chemicals have been identified for this group and they are detailed in Table 1, together with the reasons for their selection. We have chosen chemicals that represent different classes and exhibit different modes of action. The focus is on chemicals that are DNA-reactive carcinogens and *in vivo* genotoxins. However, we have included *in vivo* genotoxins such as aneugens and topoisomerase inhibitors that may not be carcinogenic, and are negative or equivocal in the Ames test, but which we would expect *in vitro* tests to detect as positive. The chemicals in Table 1 are arranged into two sections (Ames-positive and Ames-negative or equivocal) and several subgroups. They are not listed in any order of priority, but for any test-evaluation programme it is recommended that examples from each subgroup are included. The more examples from each subgroup that can be included, the more comprehensive will be the evaluation. It should be noted that the mode of action for tumour induction might not be the same as that leading to genotoxic responses.

Group 2 chemicals: Non-DNA-reactive chemicals (including non-genotoxic carcinogens) that should, and routinely do give negative results in *in vitro* mammalian cell genotoxicity tests ("true negatives").

Table 1
In vivo genotoxins which should be detected as positive in *in vitro* mammalian cell genotoxicity tests

Chemical (CAS number)	Genotoxicity profile			Carcinogenicity findings	Further information
	Ames test	*In vivo* genotoxicity tests	*In vitro* mammalian cell tests		
I. Ames-positive *in vivo* genotoxins					
(i) O^6 and N^7 alkylators					
Cyclophosphamide (6055-19-2)	+ve [3]	+ve for MN [8,17,29,30,35,75,84] and Comets [96]	+ve MLA [4], MN [5] and CA [6]	Tumours at multiple sites in rats and mice after oral and subcutaneous administration (IARC, Supplement 7) IARC Group 1 carcinogen	Requires metabolic activation (CYP2B6)
ENU (759-73-9)	+ve [3]	+ve for CA [7], MN [8] and transgenic mutations in many tissues [9]	+ve MN and CA tests in the range 10–100 μg/ml [5,6];	Nervous system, small intestine and thyroid tumours in rats. Skin tumours in mice after dermal application (IARC vol. 17). IARC Group 2A carcinogen	Strong gene mutagen (O^6 alkylation)
MMS (66-27-3)	+ve [3]	+ve for CA [97], MN,[17,98] and UDS [15] but more −ve than +ve results for gene mutations [9]	+ve MLA –S9 at <10 μg/ml [4]; +ve for MN [5]; +ve CA –S9 at <1 μg/ml [6]	Haematopoietic and lung tumours in male mice [10,11]. IARC Group 2A carcinogen	Strongt clastogen (N^7 alkylation)
(ii) Polycyclic aromatic hydrocarbons					
Benzo[a]pyrene (50-32-8)	+ve [3]	+ve for MN [8] and gene mutations [9]	+ve in all *in vitro* tests at <10 μg/ml [4–6] but needs metabolism	Stomach tumours in rats; oesophageal tumours in male mice [10]. Skin tumours in mice after dermal application (IARC vol. 3). IARC Group 1 carcinogen (vol 92)	Requires metabolic activation (CYP 1A1; 1B1, epoxide hydrolase); forms bulky adducts
7,12-Dimethylbenzanthracene (57-97-6)	+ve [3]	+ve for MN [8] and gene mutations [9]	+ve in MLA +S9 at <10 μg/ml [4]; +ve for MN [5]; variable CA responses from 1–200 μg/ml – and +S9 [6]	Vascular tumours in female mice (not tested systemically in rats). Skin tumours in mice, hamsters and gerbils following dermal application [12]. Not classified by IARC with regard to human carcinogenicity	Requires metabolic activation (CYP1B1); forms bulky adducts
(iii) Aromatic amines					
Dimethylnitrosamine (62-75-9)	+ve [3]	+ve for gene mutations [9] and UDS [15] in liver −ve for MN [14]	E for MLA in the range 9-250 μg/ml [4]; induces MN [5]; also +ve CA +S9 500–7500 μg/ml[6]	Liver tumours in rats and mice, but also lung, nervous system, kidney, testes and vascular tumours [10]. IARC Group 2A carcinogen	Alkylating agent after activation by CYP2E1(which is not highly expressed in rat liver S9): produces O^6- and N^7-methyl guanine adducts [13]
2-Acetylaminofluorene (53-96-3)	+ve [3]	+ve for many endpoints including MN [17], CA [18], UDS [15], Comet [19] and gene mutations in multiple tissues [9]	+ve MLA - & +S9 30-60 μg/ml [4]; +ve for MN [16]; variable CA responses 30–200 μg/ml [6]	Liver tumours rats and mice, bladder tumours in mice, mammary gland and skin tumours in rats [10]. Not classified by IARC with regard to human carcinogenicity	Hydroxylated by CYP1A2 and then acetylated. Forms C8 adduct on guanine [13]
2,4-diaminotoluene (95-80-7)	+ve [3]	+ve for UDS [15], transgenic mutations [9] and Comet [22] but –ve for MN [14]	+ve MLA [4] +ve CA [21]	Liver, kidney and mammary gland carcinogen after oral administration [10]. Induces mutations in liver/bladder after dermal application [20]. IARC Group 2B carcinogen	Aromatic amine, requires metabolic activation

Table 1 (*Continued*)

Chemical (CAS number)	Genotoxicity profile			Carcinogenicity findings	Further information
	Ames test	*In vivo* genotoxicity tests	*In vitro* mammalian cell tests		
IQ (2-amino-3methylimidazo[4,5-*f*]quinoline) (76180-96-6)	+ve [23]	+ve for transgenic mutations [9] CA in hepatocytes [18] and Comet [25] but −ve for MN [8] and for CA in bone marrow [26]	+ve MN [24]	Tumours in multiple organs, rats and mice (Gold database)	Heterocyclic amine with potent genotoxicity, requires metabolic activation [99]
PhIP.HCl (2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (no CAS no)	+ve [27]	+ve for UDS [100], transgenic mutations [9] and Comets in liver kidney and brain [25] but –ve for CA [101]	+ve MN and CA [5]; not tested in MLA	Mainly haematopoietic tumours; Also GI and prostate tumours in male rats [10]	Heterocyclic amine with potent genotoxicity, requires metabolic activation [99]
(iv) Others					
Aflatoxin B1 (1162-65-8)	+ve [10]	+ve *in vivo* for MN (see [32]), CA [33], UDS [15], and transgenic mutations in liver [9], but −ve for Comet [34]	+ve CA +S9 at 0.5 μg/ml [6]; +ve for MN [31]	Liver and large intestine tumours in rats; non-carcinogenic in mice [10]. IARC Group 1 carcinogen	Activated by CYP3A4, which is not highly expressed in rats compared with humans. Forms various adducts
Cadmium chloride (10108-64-2)	+ve [40]	+ve for CA and MN *in vivo* [39]	+ve MN [5] +iv CA [6]	Haematopoietic, lung, prostate and testicular tumours in rats [10]	Inorganic carcinogen
Cisplatin (15663-27-1)	+ve [41]	+ve for CA (IARC Suppl 7)	+ve CA at low concentrations [42]	Induced lung adenomas in mice and leukaemia in rats (IARC Suppl. 7), IARC Group 2A carcinogen	Cross-linking agent
p-chloroaniline (106-47-8)	+ve [3]	+ve for comets [22] but equivocal for MN [51,52]	Variable MLA [53] and CA [6] results	Non-carcinogenic [10]	No adducts
II. *In vivo* genotoxins negative or equivocal in Ames					
Etoposide (33419-42-0)	E [IARC V-76]	+ve for MN and CA [36] *in vivo* but –ve for transgenic mutations *in vivo* [9]	+ve in CA [37], MLA, Comet and MN [38] *in vitro*	Carcinogenicity not established	Topoisomerase inhibitor
Hydroquinone (123-31-9)	−ve [3]	+ve for CA (IARC vol 71) and MN [43]	+ve MLA [4], +ve MN and CA [5] at <10 μg/ml	Kidney, liver and haematopoietic tumours in rats and mice [10]	MOA: aneugen
Azidothymidine (30516-87-1)	−ve [45]	+ve for MN [44]	+ve for CA [45]	Vaginal squamous cell carcinomas in mice (IARC, vol 76);	MOA: nucleoside analogue
Sodium arsenite (7784-46-5)	−ve [47]	+ve for MN [46]	+ve for CA at only 13 μg/ml [48]	Weak inducer of lung, kidney and bladder tumours in rats, but arsenous acid (arsenite); IARC Group 1 carcinogen (IARC vol 84);	Inorganic carcinogen MOA: oxidant? repair inhibitor?
Taxol (33069-62-4)	−ve	Strong +ve for *in vivo* MN [49]	+ve for MN *in vitro* [50]	Carcinogenicity not established	MOA: aneugen
Chloramphenicol (56-75-7)	−ve [56]	+ve for CA [54] and MN [55]	+ve for CA (-S9) [56,57]	Rodent carcinogenicity data is inadequate	MOA: clastogen that binds to DNA

IARC classification: Group 1, human carcinogen; Group 2A, probable human carcinogen; Group 2B, possible human carcinogen; MOA, mode of action.

Table 2
Non-DNA-reactive chemicals (including non-genotoxic carcinogens) that should give negative results in *in vitro* mammalian cell genotoxicity tests

Chemical (CAS number)	Genotoxicity profile			Carcinogenicity findings
	Ames test	*In vivo* genotoxicity tests	*In vitro* mammalian cell tests	
(i) Non-carcinogens with negative *in vivo* genotoxicity data				
Ampicillin trihydrate (7177-48-2)	−ve [3]	−ve for MN [83]	−ve for MLA up to 5000 μg/ml [4]; −ve for CA up to 1500 μg/ml [28]	−ve in rats and mice [10]
D-mannitol (69-65-8)	−ve [3]	−ve for MN and CA [84]	−ve for CA [28] and MLA [4] up to 5000 μg/ml	−ve in rats and mice [10]
(ii) Non-carcinogens with no *in vivo* genotoxicity data				
Phenformin HCl (834-28-6)	−ve [3]	No data	−ve for MLA and CA [28] −ve CA at 50% toxicity [62]	−ve in rats and mice [10]
n-butyl chloride (109-69-3)	−ve [3]	No data	−ve for 6/7 MLA trials [4]; −ve for CA up to 5000 μg/ml [28]	−ve in rats and mice [10]
(2-chloroethyl)trimethyl-ammonium chloride (999-81-5)	−ve [3]	No data	−ve for MLA and CA up to 5000 μg/ml [28]	−ve in rats and mice [10]
Cyclohexanone (108-94-1)	−ve [3]	No data	−ve for MLA up to 5000 μg/ml [4]; −ve for CA (detailed data not available) [85]	−ve in rats and mice [10]
N,N-dicyclohexyl thiourea (1212-29-9)	−ve [3]	No data	−ve for MLA up to 90% toxicity [4]; −ve for CA to 1600 μg/ml [28]	−ve in rats and mice [10]
Trisodium EDTA trihydrate (150-38-9)	−ve [3]	No data	−ve at high concentrations (non-toxic) namely 5000 μg/ml in MLA [28]; only tested to 100 μg/ml in CA [28]	−ve in rats and mice [10]
Ephidrine sulphate (134-72-5)	−ve [3]	No data	−ve for MLA up to 90% toxicity [28]; −ve for CA up to 30% toxicity [28]	−ve in rats and mice [10]
Erythromycin stearate (643-22-1)	−ve [3]	No data	−ve for MLA up to 90% toxicity but +ve at >90% toxicity [4]; −ve for CA up to 500 μg/ml [28]	−ve in rats and mice [10]
Fluometron (2164-17-2)		No data	−ve for MLA up to 90% toxicity but +ve at >90% toxicity [4]; −ve for CA up to 2380 μg/ml [21]	−ve in rats and mice [10]
Phenanthrene (85-01-8)	−ve (IARC, vol 32)	No data	−ve for CA (details not available, IARC, vol. 32)	−ve in mice after dermal, ip and subcutaneous administration (IARC, vol. 32)
(iii) Non-genotoxic carcinogens				
D-limonene (5989-27-5)	−ve [3]	No data	−ve for CA [28] but technically compromised MLA [4]	Male rat kidney tumours [10] tumours due to α2 μ-globulin nephropathy
Di-(2-ethylhexyl)phthalate (117-81-7)	−ve [28]	−ve for CA [28], MN [28], UDS [15] and transgenic mutations [9]	−ve for MLA and CA [28]	Liver carcinogen in rats and mice [28] due to peroxisome proliferation
Amitrole (61-82-5)	−ve [28]	−ve for MN and CA [86]	−ve for MLA and CA [28]	Thyroid and liver tumours (IARC, vol 79) due to hormonal effects and prolactin secretion

Table 2 (*Continued*)

Chemical (CAS number)	Genotoxicity profile			Carcinogenicity findings
	Ames test	*In vivo* genotoxicity tests	*In vitro* mammalian cell tests	
Tert-butyl alcohol (75-65-0)	−ve [28]	−ve for MN in blood after 90 days [28]	−ve for MLA and CA [28]	Kidney and bladder tumours [28] due to mineralisation in kidney and bladder
Diethanolamine (111-42-2)	−ve [3]	−ve for MN [28]	−ve for MLA [28] and CA [87]	Tumours of mouse liver and renal tubules [28] due to choline deficiency
Melamine (108-78-1)	−ve [3]	−ve for MN [88]	−ve for MLA [4] and CA [28]	Bladder and ureteral carcinomas [28] due to calculus formation
Methyl carbamate (598-55-0)	−ve [3]	−ve for MN [89]	−ve for MLA [4] and CA [28]	Liver tumours in rats [28] due to inflammation and hyperplasia resulting from bioaccumulation (poor clearance)
Progesterone (57-83-0)	−ve [3]	No conventional studies *in vivo*, weak inducer of MN in liver [90] but not an initiating liver carcinogen in rats and −ve for MN in monkeys after 12 weeks [91]	−ve for MLA [4] and CA [6]	Ovarian, uterine and mammary tumours (IARC, vol. 6) due to hormonal effects; no adducts in liver of female rats
Pyridine (110-86-1)	−ve [3]	−ve for MN (IARC, vol. 77) and UDS [92]	−ve for MLA [4] and CA [28]	Renal tubule carcinogen of F344 rats [28]; Strain specific effects for tumourigenicity
Tris(2-ethylhexyl)phosphate (78-42-2)	−ve [3]	−ve for MN and CA [84]	−ve for MLA [4] and CA [28]	Liver tumours in female mice [28]; Tumours to peroxisome proliferation
Hexachloroethane (67-72-1)	−ve [3]	−ve for MN [95]	−ve for MN [94] and CA [28]	Tumours due to α-2-μglubulin in rat kidney: promotion in mouse liver; however, does induce DNA adducts but not strand breaks in mouse liver [93]

Table 3
Non-DNA-reactive chemicals (including non-genotoxic carcinogens), metabolic poisons and others that should give negative results in *in vitro* mammalian cell genotoxicity tests, but have been reported to induce chromosomal aberrations or *tk* mutations in mouse lymphoma cells, often at high concentrations or at high levels of cytotoxicity

Chemical (CAS number)	Genotoxicity profile			Carcinogenicity findings
	Ames test	*In vivo* genotoxicity Tests	*In vitro* mammalian cell tests	
(i) Non-carcinogens that are negative or equivocal for genotoxicity *in vivo*				
D,L-menthol (15356-70-4)	−ve [60]	−ve for MN [61]	−ve/inconclusive MLA [4] +ve CA, 3 h −S9 (+17 hr recovery), 1.6–1.9 mM with toxicity [62]	−ve in rats and mice [10]
Phthalic anhydride (85-44-9)	−ve [28]	−ve for gene mutations [63]	+ve MLA at >90% toxicity [28]; +ve CA at 10 mM −S9 with toxicity [62]	−ve in rats and mice [28]
Tertiary-butylhydroquinone (1948-33-0)	−ve or E [28]	−ve for MN and CA [28]	+ve for CA but only where few cells could be scored (toxicity?) [28]	−ve in rats and mice [28]
o-Anthranilic acid (118-92-3)	−ve [3]	−ve for MN and CA [64]	+ve MLA [4] and CA [65] at toxic concentrations; +ve MN *in vitro* above 4000 μg/ml [66]	−ve in rats and mice [10]
1,3-Dihydroxybenzene (resorcinol) (108-46-3)	−ve [77]	−ve for MN [102]	+ve in MLA at 250–300 μg/ml; +ve CA at 20–80 μg/ml [28]	−ve in mice [78]; not tested in rats
2-Ethyl-1,3-hexanediol (94-96-2)	−ve [79]	−ve for MN and CA [79]	+ve CA +S9 at 4000 μg/ml; −ve HPRT [79]	−ve in mice [78]; not tested in rats
Sulfisoxazole (127-69-5)	−ve [3]	−ve MN and CA *in vivo* [28]	−ve CA [6,28] but inconclusive [4] or weakly +ve at <20% RTG [82] MLA	−ve in rats and mice [10]:
(ii) Non-carcinogens with no *in vivo* genotoxicity data				
Ethionamide (536-33-4)	−ve [28]	No *in vivo* genotoxicity data	Weak +ve MLA at 70–90% toxicity [28]; Weak +ve CA 5–8 mM with precipitate [62]	−ve in rats; possible thyroid tumours in mice [28]
Curcumin (458-37-7)	−ve [28]	No *in vivo* genotoxicity data for curcumin alone	+ve for MN only with apoptosis [73]	Anti-carcinogen [72]
Benzyl alcohol (100-51-6)	−ve [3]	No *in vivo* genotoxicity data	Weak +ve MLA at 4500 μg/ml and weak +ve CA at 4000 μg/ml i.e. 30–40 mM [28]	−ve in rats and mice [10]
Urea (57-13-6)	−ve [3]	No *in vivo* genotoxicity data	+ve for CA at >10 mM [6]	−ve in rats and mice [10]
(iii) Non-genotoxic carcinogens or carcinogenic by irrelevant (for humans) mechanism				
Sodium saccharin (128-44-9)	−ve [3]	Mainly −ve for CA [76]	−ve MLA [4] but +ve CA at 8000 μg/ml [6]	Rat and mouse bladder tumours [10]; Tumours due to ionic imbalance and microcrystalline deposits
(iv) Supplementary list (prediction of *in vitro* genotoxicity results less clear)				
Propyl gallate (121-79-9)	−ve [28]	−ve for MN but questionable for CA [28]	+ve in MLA but mainly tested <20% RTG; +ve for CA −S9 but strongly reduced +S9 [28]	−ve in rats and mice [28]
p-Nitrophenol (100-02-7)	−ve [28]	No *in vivo* genotoxicity data	+ve CA +S9 at 1500 μg/ml [28] −ve HPRT [80], but inconclusive MLA [4]	−ve in mice [28]; not tested in rats
Sodium xylene sulfonate (1300-72-7)	−ve [28]	No *in vivo* genotoxicity data	E for MLA at 4000 μg/ml −ve CA [28]	−ve in mice [28]; not tested in rats
Ethyl acrylate (140-88-5)	−ve [3]	Weak +ve for MN and CA in splenocytes at near-toxic doses [81]; −ve for CA in bone marrow [28]	+ve MLA at 20 μg/ml [4] and +ve CA at 300 μg/ml +S9, probably associated with high levels of cytotoxicity [21]	Forestomach tumours rats and mice [10]; tumours due to cytotoxicity/chronic irritation
Eugenol (97-53-0)	−ve [3]	Equivocal for MN (+ve at LD50 dosed ip, but weak [67] or −ve [68,69] if dosed orally)	+ve MLA [4] and CA [70,71] under conditions probably due to high cytotoxicity	−ve in rats and mice [10]
Isobutyraldehyde (78-84-2)	−ve or E [28]	−ve for MN, +ve for CA at an inter-mediate dose only by ip dosing [28]	+ve MLA −S9 (not tested +S9) and +ve CA −S9 but −ve +S9 [28]; may be oxidative mechanism	−ve in rats and mice by inhalation [28]
2,4-Dichlorophenol (120-83-2)	−ve with rat S9 E with hamster S9 [3,28]	Weak +ve for CA [74]	+ve MLA at >90% toxicity [4,28]; +ve CA in some conditions [62]	−ve in rats and mice [28]

A total of 23 non-DNA-reactive (Ames test-negative) chemicals have been identified for this group and they are detailed in Table 2. The chemicals in Table 2 are arranged into three subgroups. The two chemicals in subgroup (i) are the only ones that had clearly negative *in vivo* genotoxicity data as well as being negative *in vitro* and non-carcinogenic. There are a large number of non-carcinogens that are non-genotoxic *in vitro*, but for which no published *in vivo* genotoxicity data could be found. However, these are included in this group because the existing data suggest they should be negative in any modified or new *in vitro* genotoxicity test systems.

Group 3 chemicals: Non-DNA-reactive chemicals (including non-genotoxic carcinogens), metabolic poisons and others that should give negative results, but have been reported to induce chromosomal aberrations or *tk* mutations in mouse lymphoma cells, often at high concentrations or at high levels of cytotoxicity ("false positives").

A total of 19 non-DNA-reactive (Ames test-negative) chemicals have been identified for this group and they are detailed in Table 3, together with the reasons for their selection. The chemicals in this group have been selected primarily because most are negative for *in vivo* genotoxicity. A small subgroup (iv) that gave some inconsistent or equivocal results *in vivo* are included because they are either non-carcinogenic or carcinogenic via an accepted non-genotoxic mechanism, as well as giving negative results in the Ames test. Also in Table 3 are compounds such as benzyl alcohol, 2,4-dichlorophenol, urea and sodium saccharin where the only reported positive results occurred at concentrations or levels of cytotoxicity that exceed those mentioned in current guidelines. It is suggested to include such chemicals into a validation study for a new or modified *in vitro* mammalian cell genotoxicity test because they may have given positive results at lower concentrations/levels of toxicity, but these chemicals should give negative results in new or improved *in vitro* genotoxicity tests. The chemicals in Table 3 are arranged into four subgroups. Those in subgroup (i) are negative *in vivo* and should be given priority. However, for the chemicals in subgroup (ii) no published *in vivo* genotoxicity data could be found. Only one chemical (sodium saccharin) is included in subgroup (iii) being mainly negative for genotoxicity *in vivo* but with tumours induced via a non-genotoxic mechanism. Also the majority of chemicals in subgroup (iv) have carcinogenicity or genotoxicity findings that are uncertain or controversial, including 2,4-dichlorophenol, which is reported positive for chromosomal aberrations *in vivo*, although the response is quite weak. The chemicals in subgroup (iv) are also expected to be negative *in vitro* but may be considered a lower priority for testing.

It should be noted that "concordance" relies on having approximately equal numbers of carcinogens/genotoxins and non-carcinogens/non-genotoxins. Many previous collaborative or validation trials have contained large numbers of carcinogens but few non-carcinogens. In Tables 1 and 2 there are similar numbers of carcinogens/genotoxins and non-carcinogens/non-genotoxins, providing a good balance for concordance calculations. It should also be noted that whilst we believe all of the chemicals listed in these Tables are commercially available, laboratories should be aware of quality and try to obtain the purest samples available for test.

2. Conclusions

After careful consideration of the published literature the authors have compiled lists of chemicals that can be used in the evaluation of modified or new mammalian cell genotoxicity assays. These lists basically arrange the chemicals according to whether positive results should be expected *in vitro* or whether negative results should be expected, and the latter includes chemicals currently suspected of giving irrelevant positive results in existing assays. It is hoped these lists may provide useful reference points for those scientists seeking to reduce irrelevant positive results by modification of existing assays or introduction of new assays.

Acknowledgement

The authors would like to thank ECVAM for organising and funding the workshop that enabled this project to be completed.

References

[1] D. Kirkland, S. Pfuhler, D. Tweats, M. Aardema, R. Corvi, F. Darroudi, A. Elhajouji, H.-R. Glatt, P. Hastwell, M. Hayashi, P. Kasper, S. Kirchner, A. Lynch, D. Marzin, D. Maurici, J.-R. Meunier, L. Müller, G. Nohynek, J. Parry, E. Parry, V. Thybaud, R. Tice, J. van Benthem, P. Vanparys, P. White, How to reduce false positive results when undertaking *in vitro* genotoxicity testing and thus avoid unnecessary follow-up animal tests: report of an ECVAM Workshop, Mutat. Res. 628 (2007) 31–55.

[2] D. Kirkland, M. Aardema, L. Henderson, L. Müller, Evaluation of the ability of a battery of 3 *in vitro* genotoxicity tests to discriminate rodent carcinogens and non-carcinogens. I. Sensitivity, specificity and relative predictivity, Mutat. Res. 584 (2005) 1–256.

[3] E. Zeiger, Genotoxicity database, in: L/S. Gold, E. Zeiger (Eds.), Handbook of Carcinogenic Potency and Genotoxicity Databases, CRC Press Inc., Boca Raton, 1997, pp. 687–729.

[4] A.D. Mitchell, A.E. Auletta, D. Clive, P.E. Kirby, M.M. Moore, B. Myhr, The L5187/tk$^{+/-}$ mouse lymphoma specific gene and chromosomal mutation assay. A phase III report of the U.S. Environmental Protection Agency Gene-Tox Program, Mutat. Res. 394 (1997) 177–303.

[5] T. Matsushima, M. Hayashi, A. Matsuoka, M. Ishidate Jr., K.F. Miura, H. Shimizu, Y. Suzuki, K. Morimoto, H. Ogura, K. Mure, K. Koshi, T. Sofuni, Validation of the *in vitro* micronucleus test in a Chinese hamster lung cell line (CHL/IU), Mutagenesis 14 (1999) 569–580.

[6] M. Ishidate Jr., M. Harnois, T. Sofuni, A comparative analysis of data on the clastogenicity of 951 chemical substances tested in mammalian cell cultures, Mutat. Res. 195 (1988) 151–213.

[7] S.W. Soukup, W. Au, The effect of ethylnitrosourea on chromosome aberrations *in vitro* and *in vivo*, Humangenetik 29 (1975) 319–328.

[8] K.H. Mavournin, D.H. Blakey, M.C. Cimino, M.F. Salamone, J.A. Heddle, The *in vivo* micronucleus assay in mammalian bone marrow and peripheral blood. A report of the U. S. Environmental Protection Agency Gene-Tox Program, Mutat. Res. 239 (1990) 29–80.

[9] I.B. Lambert, T.M. Singer, S.E. Boucher, G.R. Douglas, Detailed review of transgenic rodent mutation assays, Mutat. Res. 590 (2005) (2005) 1–280.

[10] L.S. Gold, The carcinogenic potency project, <http://www.potency.berkeley.edu/cpdb.html>, 2004.

[11] N.K. Clapp, A.W. Craig, R.E. Toya Sr., Oncogenicity by methyl methanesulfonate in male RF mice, Science 161 (1968) 913–914.

[12] S. Nesnow, M. Argus, H. Bergman, K. Chu, C. Frith, T. Helmes, R. McGaughy, V. Ray, T.J. Slaga, R. Tennant, E. Weisburger, Chemical carcinogens. A review and analysis of the literature of selected chemicals and the establishment of the Gene-Tox Carcinogen Data Base. A report of the U. S. Environmental Protection Agency Gene-Tox Program, Mutat. Res. 185 (1986) 1–195.

[13] M. Otteneder, W.K. Lutz, Correlation of DNA adduct levels with tumor incidence: carcinogenic potency of DNA adducts, Mutat. Res. 424 (1999) 237–247.
[14] T. Morita, N. Asano, T. Awogi, Y.F. Sasaki, S. Sato, H. Shimada, S. Sutou, T. Suzuki, A. Wakata, T. Sofuni, M. Hayashi, Evaluation of the rodent micronucleus assay in the screening of IARC carcinogens (Groups 1, 2A and 2B). The summary report of the 6th collaborative study by CSGMT/JEMS.MMS, Mutat. Res. 389 (1997) 3–122.
[15] S. Madle, S.W. Dean, U. Andrae, G. Brambilla, B. Burlinson, D.J. Doolittle, C. Furihata, T. Hertner, C.A. McQueen, H. Mori, Recommendations for the performance of UDS tests *in vitro* and *in vivo*, Mutat. Res. 312 (1984) 263–285.
[16] F. Nesslany, D. Marzin, A micromethod for the *in vitro* micronucleus assay, Mutagenesis 14 (1999) 403–410.
[17] J.A. Heddle, M. Hite, B. Kirkhart, K. Mavournin, J.T. MacGregor, G.W. Newell, M.F. Salamone, The induction of micronuclei as a measure of genotoxicity. A report of the U. S. Environmental Protection Agency Gene-Tox Program, Mutat. Res. 123 (1983) 61–118.
[18] S. Sawada, T. Yamanaka, K. Yamatsu, C. Furihata, T. Matsushima, Chromosome aberrations, micronuclei and sister-chromatid exchanges (SCEs) in rat liver induced *in vivo* by hepatocarcinogens including heterocyclic amines, Mutat. Res. 251 (1991) 59–69.
[19] Y.F. Sasaki, E. Nishidate, F. Izumiyama, N. Matsusaka, S. Tsuda, Simple detection of chemical mutagens by the alkaline single-cell gel electrophoresis (Comet) assay in multiple mouse organs (liver, lung, spleen, kidney and bone marrow), Mutat. Res. 391 (1997) 215–231.
[20] D. Kirkland, C. Beevers, Induction of *LacZ* mutations in Muta™ Mouse can distinguish carcinogenic from non-carcinogenic analogues of diaminotoluenes and nitronaphthalenes, Mutat. Res. 608 (2006) 88–96.
[21] K.S. Loveday, B.E. Anderson, M.A. Resnick, E. Zeiger, Chromosome aberration and sister chromatid exchange tests in Chinese hamster ovary cells *in vitro*. V: Results with 46 chemicals, Environ. Mol. Mutagen. 16 (1990) 272–303.
[22] Y.F. Sasaki, K. Fujikawa, K. Ishida, N. Kawamura, Y. Nishikawa, S. Ohta, M. Satoh, H. Madarame, S. Ueno, N. Susa, N. Matsusaka, S. Tsuda, The alkaline single cell gel electrophoresis assay with mouse multiple organs: results with 30 aromatic amines evaluated by the IARC and U.S. NTP, Mutat. Res. 440 (1999) 1–18.
[23] A. Brams, C. de Meester, Mutagenic potency of heterocyclic amines towards *Salmonella typhimurium*; possible causes of variability in the results observed, Mutat. Res. 280 (1992) 103–107.
[24] S. Knasmüller, C.E. Schwab, S.J. Land, C.Y. Wang, R. Sanyal, M. Kundi, P. Parzefall, F. Darroudi, Genotoxic effects of heterocyclic amines in human derived hepatoma (HepG2) cells, Mutagenesis 14 (1999) 533–539.
[25] Y.F. Sasaki, A. Saga, M. Akasaka, E. Nishidate, M. Watanabe-Akanuma, T. Ohta, N. Matsusaka, S. Tsuda, *In vivo* genotoxicity of heterocyclic amines detected by a modified alkaline single cell gel electrophoresis assay in a multiple organ study in the mouse, Mutat. Res. 395 (1997) 57–73.
[26] J.L. Minkler, A.V. Carrano, *In vivo* cytogenetic effects of the cooked-food-related mutagens Trp-P-2 and IQ in mouse bone marrow, Mutat. Res. 140 (1984) 49–53.
[27] M.A. Malfatti, M.H. Buonarati, K.W. Turteltaub, N.H. Shen, J.S. Felton, The role of sulfation and/or acetylation in the metabolism of the cooked-food mutagen 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine in *Salmonella typhimurium* and isolated rat hepatocytes, Chem. Res. Toxicol. 7 (1994) 139–147.
[28] NTP, NTP website at http://www.ntp-server.niehs.nih.gov.
[29] L. Machemer, D. Lorke, Method for testing mutagenic effects of chemicals on spermatogonia of the Chinese hamster: results obtained with cyclophosphamide, saccharin, and cyclamate, Arzneimittelforschung 25 (1975) 1889–1896.
[30] I. Emerit, A. Levy, J. Feingold, Chromosome effect of cyclophosphamide in various strains of mice, Ann. Genet. 19 (1976) 203–206.
[31] C. Crofton-Sleigh, A. Doherty, S. Ellard, E.M. Parry, S. Venitt, Micronucleus assays using cytochalasin-blocked MCL-5 cells, a proprietary human cell line expressing five human cytochromes P-450 and microsomal epoxide hydrolase, Mutagenesis 8 (1993) 363–372.
[32] S. Madle, A. Korte, B. Beek, Species differences in mutagenicity testing: I. Micronucleus and SCE tests in rats, mice and Chinese hamsters with Aflatoxin B1, Teratogen. Carcinogen. Mutagen. 6 (1986) 1013.
[33] L. Fabry, M. Roberfroid, Mutagenicity of aflatoxin B1: observations *in vivo* and their relation to *in vitro* activation, Toxicol. Lett. 7 (1981) 245–250.
[34] B. Watzl, C. Neudecker, G.M. Hänsch, G. Rechkemmer, B.L. Pool-Zobel, Short-term moderate aflatoxin B1 exposure has only minor effects on the gut-associated lymphoid tissue of Brown Norway rats, Toxicology 5 (1999) 93–102.
[35] A. Leonard, G. Deknudt, Comparison *in vivo* of the clastogenic properties of busulfan and cyclophosphamide, C R Seances Soc. Biol. Fil. 177 (1983) 239–242.
[36] R.C. Choudhury, A.K. Palo, P. Sahu, Cytogenetic risk assessment of etoposide from mouse bone marrow, J. Appl. Toxicol. 24 (2004) 115–122.
[37] S.M. Galloway, J.E. Miller, M.J. Armstrong, C.L. Bean, T.R. Skopek, W.W. Nichols, DNA synthesis inhibition as an indirect mechanism of chromosome aberrations: comparison of DNA-reactive and non-DNA-reactive clastogens, Mutat. Res. 400 (1998) 169–186.
[38] G. Boos, H. Stopper, Genotoxicity of several clinically used topoisomerase II inhibitors, Toxicol. Lett. 116 (2000) 7–16.
[39] A. Mukherjee, A.K. Giri, A. Sharma, G. Talukder, Relative efficacy of short-term tests in detecting genotoxic effects of cadmium chloride in mice *in vivo*, Mutat. Res. 206 (1988) 285–295.
[40] R. Mandel, H. Ryser, Mutagenicity of cadmium in *Salmonella typhimurium* and its synergism with two nitrosamines, Mutat. Res. 138 (1984) 9–16.
[41] M.A. Hannan, A.A. al-Dakan, S.S. Hussain, M.H. Amer, Mutagenicity of cisplatin and carboplatin used alone and in combination with four other anticancer drugs, Toxicology 55 (1989) 183–191.
[42] G. Krishnaswamy, W.C. Dewey, Cisplatin induced cell killing and chromosomal aberrations in CHO cells: treated during G1 or S phase, Mutat. Res. 293 (1993) 161–172.
[43] I.-D. Adler, U. Kliesch, Comparison of single and multiple treatment regimens in the mouse bone marrow micronucleus assay for hydroquinone (HQ) and cyclophosphamide (CP), Mutat. Res. 234 (1990) 115–123.
[44] M.D. Phillips, B. Nascimbeni, R.R. Tice, M.D. Shelby, Induction of micronuclei in mouse bone marrow cells: an evaluation of nucleoside analogues used in the treatment of AIDS, Environ. Mol. Mutagen. 18 (1991) 168–183.
[45] K.M. Ayers, D. Clive, W.E. Tucker Jr., G. Hajian, P. de Miranda, Nonclinical toxicology studies with zidovudine: genetic toxicity tests and carcinogenicity bioassays in mice and rats, Fund. Appl. Toxicol. 32 (1996) 148–158.
[46] H. Tinwell, S.C. Stephens, J. Ashby, Arsenite as the probable active species in the human carcinogenicity of arsenic: mouse micronucleus assays on Na and K arsenite, orpiment, and Fowler's solution, Environ. Health Perspect. 95 (1991) 205–210.
[47] S. De Flora, A. Camoirano, P. Zanacchi, C. Bennicelli, Mutagenicity testing with TA97 and TA102 of 30 DNA-damaging compounds, negative with other *Salmonella* strains, Mutat. Res. 134 (1984) 159–165.
[48] B. Wan, R.T. Christian, S.W. Soukup, Studies of cytogenetic effects of sodium arsenicals on mammalian cells *in vitro*, Environ. Mutagen. 4 (1982) 493–498.
[49] H. Tinwell, J. Ashby, Genetic toxicity and potential carcinogenicity of taxol, Carcinogenesis 15 (1994) 1499–1501.
[50] L. Digue, T. Orsière, M. De Méo, M.G. Mattéi, D. Depetris, F. Duffaud, R. Favre, A. Botta, Evaluation of the genotoxic activity of paclitaxel by the in vitro micronucleus test in combination with fluorescent in situ hybridization of a DNA centromeric probe and the alkaline single cell gel electrophoresis technique (comet assay) in human T-lymphocytes, Environ. Mol. Mutagen. 34 (1999) 269–278.
[51] NTP, Comparative toxicity studies of *o*-, *m*-, and *p*-choroaniline and B6C3F1 mice, NTP Toxicity Report Series, vol. 43, 1998, 79 pp.
[52] Beratergremium für umweltrelevante Alstoffe (BUA), vol. 153, *p*-Chloroaniline, December 1993, 164 pp.
[53] W.J. Caspary, D. Spencer Daston, B.C. Myhr, A.D. Mitchell, C.J. Rudd, P.S. Lee, Evaluation of the L5178Y mouse lymphoma cell mutagenesis assay: interlaboratory reproducibility and assessment, Environ. Mol. Mutagen. 12 (suppl. 13) (1988) 195–229.
[54] N. Teodoreanu, I. Voiculescu, M. Manolache, Chromosome breakage by triethylenemelamine and chloramphenicol in golden hamster *in vivo*, Proc. Int. Congr. Genet. 12 (1968) 210.
[55] W.R. Bruce, J.A. Heddle, The mutagenic activity of 61 agents as determined by the micronucleus, *Salmonella*, and sperm abnormality assays, Can. J. Genet. Cytol. 21 (1979) 319–334.
[56] J. Ashby, C.R. Richardson, P.A. Lefevre, R.D. Callander, J.A. Styles, Chloracetamide-*N*-metholol: an example of an *in vitro* and *in vivo* clastogen which is non-mutagenic to *Salmonella*, Mutat. Res. 156 (1985) 19–32.
[57] W.J. Mitus, N. Coleman, *In vitro* effect of chloramphenicol on chromosomes, Blood 35 (1970) 689–694.
[60] E. Zeiger, B. Anderson, S. Haworth, T. Lawlor, K. Mortelmans, *Salmonella* mutagenicity tests. 4: Results from the testing of 300 chemicals, Environ. Mol. Mutagen. 11 (suppl. 12) (1988) 1–158.
[61] Screening Information Data Set (SIDS) for High Production Volume: Chemicals, 2004, 296 pp.
[62] C.A. Hilliard, M.J. Armstrong, C.I. Bradt, R.B. Hill, S.K. Greenwood, S.M. Galloway, Chromosome aberrations *in vitro* related to cytotoxicity of nonmutagenic chemicals and metabolic poisons, Environ. Mol. Mutagen. 31 (1998) 316–326.
[63] J.A. Heddle, M.A. Khan, C. Urlando, M.E. Pagura, Measuring gene mutation *in vivo*, Prog. Clin. Biol. Res. 372 (1991) 281–289.
[64] A.F. McFee, P.P. Jauhar, K.W. Lowe, J.T. MacGregor, C.M. Wehr, Assays of three carcinogen/non-carcinogen chemical pairs for *in vivo* induction of chromosome aberrations, sister chromatid exchanges and micronuclei, Environ. Mol. Mutagen. 14 (1989) 207–220.
[65] M.D. Shelby, S. Stasiewicz, Chemicals showing no evidence of carcinogenicity in long-term, two-species rodent studies: the need for short-term test data, Environ. Mutagen. 6 (1984) 871–878.
[66] Y. Oshiro, C.E. Piper, P.S. Balwierz, S.G. Soelter, Chinese hamster ovary cell assays for mutation and chromosome damage: data from non-carcinogens, J. Appl. Toxicol. 11 (1991) 167–177.
[67] C.J. Woolverton, P.G. Fotos, M.J. Mokas, M.E. Mermigas, Evaluation of eugenol for mutagenicity by the mouse micronucleus test, J. Oral Pathol. 15 (1986) 450–453.
[68] A. Allavena, A. Martelli, L. Robbiano, G. Brambilla, Evaluation in a battery of *in vivo* assays of four *in vitro* genotoxins proved to be noncarcinogens in rodents, Teratogen. Carcinogen. Mutagen. 12 (1992) 31–41.

[69] A. Maura, A. Pino, R. Ricci, Negative evidence *in vivo* of DNA-damaging, mutagenic and chromosomal effects of eugenol, Mutat. Res. 227 (1989) 125–129.
[70] S.M. Galloway, M.J. Armstrong, C. Reuben, S. Colman, B. Brown, C. Cannon, A.D. Bloom, F. Nakamura, M. Ahmed, S. Duk, J. Rimpo, B.H. Margolin, M.A. Resnick, B. Anderson, E. Zeiger, Chromosome aberrations and sister chromatid exchanges in Chinese hamster ovary cells: evaluations of 108 chemicals, Environ. Mol. Mutagen. 10 (suppl. 10) (1987) 1–175.
[71] M. Ishidate Jr., T. Sofuni, K. Yoshikawa, M. Hayashi, T. Nohmi, M. Sawada, A. Matsuoka, Primary mutagenicity screening of food additives currently used in Japan, Food Chem. Toxicol. 22 (1984) 623–636.
[72] A. Duvoix, R. Blasius, S. Delhalle, M. Schnekenburger, F. Morceau, E. Henry, M. Dicato, M. Diederich, Chemopreventive and therapeutic effects of curcumin, Cancer Lett. 223 (2005) 181–190.
[73] S. Mientières, A. Biola, M. Pallardy, D. Marzin, Using CTLL-2 and CTLL-2 *bcl2* cells to avoid interference by apoptosis in the *in vitro* micronucleus test, Environ. Mol. Mutagen. 41 (2003) 14–27.
[74] S.M. Amer, F.A. Aly, Genotoxic effect of 2,4-dichlorophenoxy acetic acid and its metabolite 2,4-dichlorophenol in mouse, Mutat. Res. 494 (2001) 1–12.
[75] C. Barbarasa, D. Luca, F. Postica, M. Covic, *In vivo* cytogenetic screening for determination of the mutagenic potential of cyclophosphamide, Morphol. Embryol. (Bucur) 25 (1979) 369–372.
[76] J. Ashby, The genotoxicity of sodium saccharin and sodium chloride in relation to their cancer-promoting properties, Food Chem. Toxicol. 23 (1985) 507–519.
[77] A. Hakura, Y. Tsutsui, H. Mochida, Y. Sugihara, T. Mikami, F. Sagami, Mutagenicity of dihydroxybenzenes and dihydroxynaphthalenes for Ames *Salmonella* tester strains, Mutat. Res. 371 (1996) 293–299.
[78] F. Stenback, P. Shubik, Lack of toxicity and carcinogenicity of some commonly used cutaneous agents, Toxicol. Appl. Pharmacol. 30 (1974) 7–13.
[79] R.S. Slesinski, P.J. Guzzie, D.L. Putman, B. Ballantyne, *In vitro* and *in vivo* evaluation of the genotoxic potential of 2-ethyl-1,3-hexanediol, Toxicology 53 (1988) 179–198.
[80] T.J. Oberly, M.A. Rexroat, B.J. Bewsey, K.K. Richardson, K.C. Michaelis, An evaluation of the CHO/HGPRT mutation assay involving suspension cultures and soft agar cloning: results for 33 chemicals, Environ. Mol. Mutagen. 16 (1990) 260–271.
[81] A.D. Kligerman, A.L. Atwater, M.F. Bryant, G.L. Erexson, P. Kwanyuen, K.L. Dearfield, Cytogenetic studies of ethyl acrylate using C57BL/6 mice, Mutagenesis 6 (1991) 137–141.
[82] B.C. Myhr, W.J. Caspary, Chemical mutagenesis at the thymidine kinase locus in L5178Y mouse lymphoma cells: results for 31 coded compounds in the National Toxicology Program, Environ. Mol. Mutagen. 18 (1991) 51–83.
[83] G. Stemp, S. Pascoe, D. Gatehouse, *In vitro* and *in vivo* cytogenetic studies of three ß-lactam antibiotics (penicillin VK, ampicillin and carbenicillin), Mutagenesis 4 (1989) 439–445.
[84] M.D. Shelby, K.L. Witt, Comparison of results from mouse bone marrow chromosome aberration and micronucleus tests, Environ. Mol. Mutagen. 25 (1995) 302–313.
[85] US Environmental Protection Agency database on Toxic Substances Control Act Test Submissions (TSCATS), <http://www.rtknet.org/datadoc/tscats.html>.
[86] J.H. Carver, J. Bootman, M.C. Cimino, H.J. Esber, P. Kirby, B. Kirkhart, Z.A. Wong, J.A. MacGregor, Genotoxic potential of acephate technical: *in vitro* and *in vivo* effects, Toxicology 35 (1985) 125–142.
[87] K.S. Loveday, M.H. Lugo, M.A. Resnick, B.E. Anderson, E. Zeiger, Chromosome aberration and sister chromatid exchange tests in Chinese hamster ovary cells *in vitro*: II. Results with 20 chemicals, Environ. Mol. Mutagen. 13 (1989) 60–94.
[88] R.W. Mast, R.W. Naismith, M.A. Friedman, Mouse micronucleus assay of melamine, Environ. Mutagen. 4 (1982) 340–341 (abstract).
[89] M.D. Shelby, R.R. Tice, Methyl carbamate: negative results in mouse bone-marrow micronucleus test, Mutat. Res. 260 (1991) 311.
[90] A. Martelli, E. Mereto, M. Ghia, P. Orsi, A. Allavena, C.R. De Pascalis, G. Brambilla, Induction of micronuclei and of enzyme-altered foci in the liver of female rats exposed to progesterone and three synthetic progestins, Mutat. Res. 419 (1998) 33–41.
[91] J. Schuppler, J. Dammé, R. Schulte-Hermann, Assay of some endogenous and synthetic sex steroids for tumor-initiating activity in rat liver using the Solt-Farber system, Carcinogenesis 4 (1983) 239–241.
[92] J.A. MacGregor, C.M. Hamilton, J.E. Kubicek, J.C. Mirsalis, Pyridine does not induce unscheduled DNA synthesis (UDS) in hepatocytes of male B6C3F1 mice treated *in vivo*, J. Appl. Toxicol. 20 (2000) 389–393.
[93] M. Taningher, S. Parodi, S. Grilli, A. Colacci, M. Mazzullo, R. Bordone, L. Santi, Lack of correlation between alkaline DNA fragmentation and DNA covalent binding induced by polychloroethanes after *in vivo* administration. Problems related to the assessment of a carcinogenic hazard, Cancer Detect. Prevent. 15 (1991) 35–39.
[94] J.M. Parry, E.M. Parry, R. Bourner, A. Doherty, S. Ellard, J. O'Donovan, B. Hoebee, J.M. de Stoppelaar, G.R. Mohn, A. Önfelt, A. Renglin, N. Schultz, C. Söderpalm-Berndes, K.G. Jensen, M. Kirsch-Volders, A. Elhajouji, P. Van Hummelen, F. Degrassi, A. Antoccia, D. Cimini, M. Izzo, C. Tanzarella, I.-D. Adler, U. Kliesch, G. Schriever-Schwemmer, P. Gasser, R. Crebelli, A. Carere, C. Andreoli, R. Benigni, P. Leopardi, F. Marcon, Z. Zinjo, A.T. Natarajan, J.J.W.A. Boei, A. Kappas, G. Voutsinas, F.E. Zarani, A. Patrinelli, F. Pachierotti, C. Tiveron, P. Hess, The detection and evaluation of aneugenic chemicals, Mutat. Res. 353 (1996) 11–46.
[95] R. Crebelli, A. Carere, P. Leopardi, L. Conti, F. Fassio, F. Raiteri, D. Barone, P. Ciliutti, S. Cinelli, J.A. Vericat, Evaluation of 10 aliphatic halogenated hydrocarbons in the mouse bone marrow micronucleus test, Mutagenesis 14 (1999) 207–215.
[96] D. Anderson, A. Dhawan, T.-W. Yu, M.J. Plewa, An investigation of bone marrow and testicular cells *in vivo* using the comet assay, Mutat. Res. 370 (1996) 159–174.
[97] J.V. Frei, S. Venitt, Chromosome damage in the bone marrow of mice treated with the methylating agents methyl methanesulphonate and *N*-methyl-*N*-nitrosourea in the presence or absence of caffeine, and its relationship with thymoma induction, Mutat. Res. 30 (1975) 89–96.
[98] D. Jenssen, C. Ramel, Dose response at low doses of X-irradiation and MMS on the induction of micronuclei in mouse erythroblasts, Mutat. Res. 41 (1976) 311–320.
[99] E.G. Snyderwine, H.A.J. Schut, R.H. Adamson, U.P. Thorgeirsson, S.S. Thorgeirsson, Metabolic activation and genotoxicity of heterocyclic arylamines, Cancer Res. 52 (suppl.) (1992) 2099s–2102s.
[100] K.R. Kaderlik, G.J. Mulder, J.G. Shaddock, D.A. Casciano, C.H. Teitel, F.F. Kadlubar, Effect of glutathione depletion and inhibition of glucuronidation and sulfation on 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) metabolism, PhIP-DNA adduct formation and unscheduled DNA synthesis in primary rat hepatocytes, Carcinogenesis 15 (1994) 1711–1716.
[101] J.D. Tucker, A.V. Carrano, N.A. Allen, M.L. Christenesen, M.G. Knize, C.L. Strout, J.S. Felton, *In vivo* cytogenetic effects of cooked food mutagens, Mutat. Res. 224 (1989) 105–113.
[102] A.T. Natarajan, G. Obe, How do *in vivo* mammalian assays compare to *in vitro* assays in their ability to detect mutagens? Mutat Res. 167 (1986) 189–201.