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DETAILED REVIEW PAPER ON CELL TRANSFORMATION ASSAYS FOR DETECTION OF CHEMICAL CARCINOGENS

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Environment Directorate

Organisation for Economic Co-operation and Development

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LIST OF ABBREVIATIONS

BALB/c 3T3	Cell line derived from embryos of BALB/c mice
BaP	Benzo(a)pyrene
BrdU	Bromodeoxyuridine
CA	Chromosomal aberration
CCRIS	Chemical Carcinogenesis Research Information System
CHO	Chinese hamster ovary cells
C3H10T1/2	Cell line derived from embryos of C3H mice
CPDB	Carcinogenic potency database
CTA	Cell transformation assay
DDT	Dichlorodiphenyl trichloroethane
DEHP	di (2-Ethylhexyl) phthalate
DES	Diethylstilbestrol
DRP	Detailed review paper
ECM	Expert Consultation Meeting
ECVAM	The European Centre for the Validation of Alternative Methods.
EPA	EPA/GENETOX Program database
FCS	Fetal calf serum
HPRT	Hypoxanthine Phosphoribosyl Transferase
IARC	International Agency for Research on Cancer
IPCS	International Program on Chemical Safety
ITES	Insuline, transferrin, ethanolamine and sodium selenite supplemented medium
MCA	3-Methylcholanthrene
MEHP	Mono(2-ethylhexyl)phthalate
ML	Mouse lymphoma
MN	Micronucleus
MNNG	N-Methyl-N'-nitro-N-nitrosoguanidine
NCI	National Cancer Institute
NTP	National Toxicology Program
SHE	Syrian hamster embryo
TEHP	Tris(2-ethylhexyl)phosphate

FOREWORD

1. An introductory document pointing on the urgency to develop *in vitro* assays to detect non-genotoxic carcinogens was presented by the Nordic Council of Ministers in 1997.

2. A draft Detailed Review Paper (DRP) entitled "Non-Genotoxic Carcinogens Detection: the Performance of *In Vitro* Cell Transformation Assays" was prepared thereafter for the OECD, by Dr. Claude Lasne (Ministère de l'Ecologie et du Developpement Durable) and Pr. Paule Vasseur (CNRS UFR Sciences-Metz), in France. The draft DRP focused on the three *in vitro* cell transformation assays (CTA), including the Syrian Hamster embryo (SHE) cell assay, and the BALB/c 3T3 and the C3H10T1/2 established cell line assays. The first draft DRP was circulated to the Working Group of National Coordinators of the Test Guidelines Programme (WNT) for comments in 2001, and the Secretariat revised the draft DRP in accordance with the comments received from member countries. However, due to the extensive commenting, the revised DRP was not considered ready for finalization at that time.

3. A 2nd revised version was circulated to the WNT on 9 December 2002 together with a compilation of comments from the 1st circulation round and the Secretariat's responses to these. Since a considerable amount of comments were also received after the 2nd circulation, the Secretariat arranged for the establishment of an expert revision group in January 2003 to assist the French authors with the revision of the DRP. Several experts offered their help in assisting in the revision, some as reviewers, and some more actively as experts within the different technical areas that were to be covered. The Canadian expert assisted with data on the C3H10T1/2 assay, the Japanese experts for the BALB/c 3T3 assay, and the French authors for the SHE assay.

4. An extensive revision has been continuously performed since January 2003 by the expert revision group. Special attention has been paid to the comments received from member countries after the 2nd circulation round in 2002/03. The draft DRP has been restructured and completely revised with considerable addition of data for the three assays. In order to also address the comments received on the 1st draft DRP, the 3rd version included updated CTA results that are presented in parallel with results of current *in vitro* genotoxicity tests, using mammalian and non-mammalian cell systems, and *in vivo* short term genotoxicity tests.

5. The 3rd revised version was circulated in 3rd quarter of 2006. Despite the thorough revision and massive adding of data, a rather extensive number of comments were received after the last circulation. In order to cope with these comments and finalize the draft DRP, an expert consultation meeting (ECM) was held in Washington DC, US on the 3rd to 5th October, 2006. The ECM was hosted by US Environmental Protection Agency; the meeting successfully addressed all the comments received from member countries in the last commenting round and produced a revised 4th version. The revised 4th version was approved by the WNT19 in April 2007 with some minor changes.

6. Many experts have participated in different OECD meetings including the above ECM, and the Secretariat would especially like to mention the experts that have been actively involved in the development and drafting of this document, including:

Abigail Jacobs, US-FDA, US
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The OECD Secretariat gratefully acknowledges these experts and others who have contributed to the project for their professional assistance and their indispensable contributions to the finalisation of this important DRP.

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I. INTRODUCTION

Objectives of the DRP

7. *In vivo* rodent models remain the only reliable way for experimental investigation of the carcinogenic risk of chemicals to humans, however, the rodent carcinogenicity assay is expensive and time-consuming. Several *in vitro* alternatives to animal-based methods have been developed. Among these methods, cell transformation assays (CTA), which mimic some stages of *in vivo* multistep carcinogenesis, have been proposed for predicting carcinogenic potential of chemicals.

8. Compared to *in vivo* based-assays, *in vitro* CTA tests are fast, cost efficient and provide a means for initial screening of carcinogenic potential. The objective of this DRP is to provide an overview of the three main CTAs; (the Syrian hamster embryo cell (SHE), the BALB/c 3T3 and the C3H10T1/2 assays) and to correlate them with *in vivo* rodent assays and assess their performances in predicting chemical carcinogenicity. A large number of chemicals have been tested using the three CTAs and the data from these tests have been used for evaluation of the performances of the three CTAs in order to judge whether any of these assays are ready to be suggested for further development into OECD Test Guidelines.

Definitions

9. Cell transformation has been defined as the induction of certain phenotypic alterations in cultured cells that are characteristic of tumorigenic cells (Barrett and Ts'o, 1978). These phenotypic alterations can be induced by exposing mammalian cells to carcinogens. Transformed cells that have acquired the characteristics of malignant cells have the ability to induce tumours in susceptible animals (Berwald and Sachs, 1963, 1965).

10. *In vitro* transformed cells exhibit morphological changes related to neoplasia. The phenomenon of morphological cell transformation involves changes in behaviour and growth control of cultured cells, such as alteration of cell morphology, disorganized pattern of colony growth, and acquisition of anchorage-independent growth (Combes *et al.,* 1999). Later on, transformed cells become able to grow in semi-solid agar (anchorage-independent growth), produce autocrine growth factors and can evolve to tumorigenicity when injected into appropriate hosts. They acquire the ability to divide indefinitely (immortalized) which is associated with other alterations like aneuploid karyotype and altered genetic stability. Accumulated evidence strongly supports the assumption that cellular and molecular processes involved in cell transformation *in vitro* are similar to those of *in vivo* carcinogenesis. (For review: Combes *et al.,* 1999; LeBoeuf *et al.,* 1999).

11. Chemical carcinogens can be classified into two categories according to their ability to interact directly or indirectly with DNA.

- *genotoxic carcinogens* (or their metabolites) are defined as compounds able to initiate cells to carcinogenesis through direct interaction with DNA. These interactions result in DNA damages and/or structural/numerical chromosomal aberrations which can be detected by one or multiple genotoxicity tests. Generally, an evaluation of genotoxic potential focuses on the assessment of gene mutations and structural/numerical chromosomal aberrations. Recommended test batteries for genotoxicity evaluation have been issued by regulatory agencies, and reports have been published by the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH 1997) and the OECD.

- *non-genotoxic carcinogens* are carcinogenic agents that are, at least initially, devoid of direct interaction with DNA. The indirect modifications to DNA structures, amount or function may induce altered gene expression and/or signal transduction. Non-genotoxic carcinogens in this DRP refer to carcinogens negative in genotoxicity assays performed to measure biological endpoints including gene mutations and chromosomal damages (chromosomal aberrations, micronuclei formation).

Presentation of the DRP

12. This document focuses on the three main *in vitro* CTAs, the SHE, the BALB/c 3T3 and the C3H10T1/2 assays. The SHE assay uses karyotypically normal cells and is believed to detect early steps of carcinogenesis. The other two assays are based on immortalized aneuploid cell lines which measure later stages of carcinogenesis.

13. The DRP weighs together the data of the three CTAs for testing of chemicals and the data is reported alongside genotoxicity test data using mammalian and non-mammalian cell systems. The performances of the CTAs in predicting carcinogenic potential are analyzed in terms of classification as rodent and/or human carcinogens.

14. The DRP discusses CTAs as means for *in vitro* carcinogenicity detection in general, without considering the genotoxic or non-genotoxic character of the carcinogens. This particular aspect will be considered in a second step, when the ability of the CTAs to identify non-genotoxic carcinogens is analysed as well as their advantages over mammalian genotoxicity tests in this respect.

15. The second chapter where the results are presented is divided into three parts:

- (1) SHE
- (2) BALB/c 3T3
- (3) C3H10T1/2

Each CTA is discussed as an entire sub-chapter, comprising: (i) state of the art, (ii) principle of the test (iii) results, (iv) rodent carcinogens, (v) rodent non-carcinogens, and (vi) chemicals not evaluated for carcinogenicity and not included in the data set.

16. The CTA results are reported in parallel to current genotoxicity test results in tables with separate rodent carcinogens and non-carcinogens. The lists of chemicals in tables 2-10 are restricted to chemicals with CTA and genotoxicity results available. Tables 11-13 lists all chemicals with CTA results that will be used to evaluate performances in predicting carcinogenicity. *In vivo* carcinogenicity was based on the evaluation by IARC (International Agency for Research on Cancer), Gold and Zeiger (1997) or CCRIS (Chemical Carcinogenesis Research Information System). The genotoxicity tests considered are as follows:

- *in vitro* mutagenicity tests measuring point mutations, using:

  bacteria: Salmonella mutation test TG 471 (Ames test)

  mammalian cells: measuring gene mutations at thymidine kinase (TK), the L5178Y mouse lymphoma (ML) cell mutation test and at hypoxanthine-guanine phosphoribosyl transferase (HPRT) locus.

- mammalian chromosome aberration (CA) tests carried out;

  in vitro: mammalian chromosome aberration test (TG 473)

  in vivo: bone marrow chromosome aberration test (TG 475) and micronucleus test (TG 474)

17. The last part of the DRP is devoted to a discussion of the performances of the three CTAs in predicting carcinogenic potential and recommendations for specific CTAs to be developed into OECD Test Guidelines. CTA data are analyzed regarding classification of rodent and human carcinogens according to IARC (International Agency for Research on Cancer) and NTP (National Toxicology Program).

18. A brief description of the three CTA protocols can be found in Annexes I-III.

Methods

19. The genotoxicity data presented in the document were obtained from:

- NTP;
- GENETOX (US EPA GENetic TOXicology Program);
- CCRIS;
- IARC;
- CPDB (The Carcinogenic Potency Database) (Gold and Zeiger, 1997); and;
- Genetic Activity Profiles in a few cases.

The scientific literature is also cited to confirm results when necessary.

20. The results from the CTAs were extracted from published articles and peer review articles, especially those by Heidelberger *et al.,* (1983) on SHE, BALB/c 3T3 and C3H/10T1/2 assays, and by Matthews *et al.,* (1993a, b) on BALB/c 3T3.

21. A synthesis of CTA results is presented in tables 11-13, which distinguish organic and inorganic chemicals according to their carcinogenicity to mammals based on:

- the IARC classification;
- human carcinogens class 1, 2A (probably) and 2B (possibly);
- animal carcinogens with sufficient (SE), limited (LE), inadequate (IE) and equivocal (EE) evidence;
- the CPDB, CCRIS and NTP databases were consulted for rodent carcinogenicity when chemicals were not documented by IARC or were referred to as human carcinogen class 3 (not classifiable as to carcinogenicity to humans).

22. The purpose of this DRP is to evaluate the performance of three CTAs in predicting chemical carcinogenicity to mammals. Chemicals are generally identified by their CAS-RN and therefore mixtures of chemicals and chemical formulations were not taken into consideration in the evaluation.

23. The performance of each CTA to predict mammal carcinogenicity is evaluated using a typical 2 x 2 Cooper ratio table, providing a binary outcome (positive or negative). Such tables provide measures of a test's sensitivity, specificity, positive and negative predictivity (see example below with definitions according to Zeiger, (1998)). The proportion of false negatives and positives and of chemicals not included in the calculation (inconclusives, equivocals, etc.) is also presented.

		In vivo Carcinogenicity	
		Carcinogen	Non-carcinogen
Cell Transformation Assay	+	a	b
	-	c	d

Concordance: % agreement with *in vivo* experiment (a + d) / (a + b + c + d) * 100
Sensitivity: % carcinogens that are positive a / (a + c) * 100
Specificity: % non-carcinogens that are negative d / (b + d) * 100
Positive predictivity: % positives that are carcinogens a / (a + b) * 100
Negative predictivity: % negatives that are non-carcinogens d / (c + d) * 100
False negatives: c / (a + c) * 100
False positives: b / (b + d) * 100

24. The criteria for determining a chemical's activity (*i.e.,* positive (+), negative (–), inconclusive (?), etc.) in the CTAs were defined by the reporting authors. Although many of the chemicals had only one result for an assay, there were a number of chemicals that had results from more than one test in a single laboratory or in multiple laboratories. Although the majority of such chemical test results were replicated among laboratories, a number of the chemicals had divergent results within a laboratory or among several laboratories. In such situations, it was necessary to combine the results into a single value that could be used for evaluating the assay's performance.

25. Because of differences in protocols among some laboratories, and the evolution and improvement of the protocols over time, many of the apparent intra- or inter-lab differences may be the result of protocol differences or the use of different test concentration ranges. In lieu of a detailed reevaluation of the specific publications to identify potential protocol or treatment factors that led to the different results, it was recommended that, where there are different results for the same chemical in a particular assay, the results be summarized as "D" to indicate that there was no reproducibility. Similarly, a number of chemicals did not yield a clear positive or negative response in the test, and were reported as inconclusive ("?", or "+?").

26. The results were summarized by the individual CTA. The SHE assay results were summarized according to the protocol used (*i.e.,* pH 6.7 and pH $\geq$ 7.0). The rule adopted for summarizing the test results by chemical for inclusion in Tables 11-13 was that chemicals that were consistently positive or negative among laboratories in an assay were summarized as positive or negative. The only exception to this rule would be when there are multiple tests of a single chemical (*i.e.,* $\geq$ 4) and all the results, except one, agree. Non-reproduced results, recorded as "D" or "?" or other than + or -, were included in the summary tables but not in the calculations of test performance (Table 14).

27. The genetic toxicity and carcinogenicity results were defined by the publications or databases from which they were obtained. Where there were multiple results for a chemical in a test, a weight of evidence approach was used to determine whether the summary call would be positive, negative, or equivocal.

II. INTERLABORATORY REPRODUCIBILITY

28. Although cell transformation methods have been the focus of extensive studies worldwide (Kuroki and Sasaki, 1985, Dunkel *et al.,*1988; Swierenga and Yamasaki, 1992; LeBoeuf *et al.,* 1996; Isfort *et al.,* 1996; LeBoeuf *et al.,* 1999), and modifications to the protocols have been described for all three CTAs, studies on the intra- and inter-laboratory reproducibility have been limited. The results are summarised below.

The SHE cell transformation assay

29. A collaboration initiated by the NTP involved three different laboratories testing model and coded chemicals using the same basic 'traditional' protocol using medium at pH 7.35 (Tu *et al.,*1986; Jones *et al.,* 1988). Two or three assays from each laboratory were carried out to determine the cytotoxicity and transforming potential of each chemical. In order to eliminate the influence of some variables, one trial utilised common cell pools and the same lot of fetal calf serum. The results of this trial are summarised in Table 1-1. A positive response (+) was defined as a level of transformation (T) at two or more concentrations that exceeded three times the level of spontaneous transformation in each assay.

30. The second trial evaluated 18 coded chemicals using the same protocol except that different cell and serum sources were used (Table 1-2). Seven chemicals were tested in all 3 laboratories while eleven were tested in two laboratories. The results showed that, of the seven chemicals tested by all three laboratories, 5 produced concordant results and 2 produced discrepant results. Of the 11 chemicals evaluated by only 2 laboratories, 5 produced concordant data, while 6 produced discrepant results. The response for 12 chemicals was in agreement with the rodent carcinogenicity data. The major factors influencing intra- and inter-laboratory reproducibility were the source of cells and serum, and low cloning efficiency.

31. At the same time, LeBoeuf and Kerckaert (1987) demonstrated that the morphological transformation of the SHE cells induced by benzo(a)pyrene was increased under conditions of reduced bicarbonate concentration and pH. They conducted an inter-laboratory evaluation to determine whether the low pH-enhanced morphological transformation frequency observed with benzo(a)pyrene occurs with other carcinogens and whether enhanced low pH transformation of SHE cells could respond qualitatively in a similar manner in independent laboratories with different lots of serum and cells (LeBoeuf *et al.,* 1989). Two laboratories tested four carcinogens under code using media at pH 6.7 and pH 7.35. Positive responses were obtained in both laboratories with all four carcinogens and a negative response with the non-carcinogen when the assay was performed in medium at low pH. In contrast, when medium at pH 7.35 was used, only benzo(a)pyrene produced a positive response in both laboratories The other three carcinogens were negative. Qualitative agreement between the two laboratories was good, despite the use of different pools of cells and sera.

32. Additional studies have also indicated that the 7 day pH 6.7 assay is reproducible between laboratories. 18 chemicals previously evaluated were re-tested and the results were highly concordant (90%) with the previous results; only 2 chemical evaluations were discordant (Engelhardt *et al.,* 2004).

Table 1-1 : Summary results of an interlaboratory comparison of transformation responses in Syrian hamster embryo cells with model and coded chemicals (Tu *et al.,* 1986)

Chemical	SHE cell transformation in : Lab. A	Lab. B	Lab. C	Overall evaluation	In vivo Carcinogenesis
Benzo(a)pyrene	+	+	+	+	+
7,12-dimethylbenzanthracene	+	+	+	+	+
N-methyl-N'-nitro-N-Nitrosoguanidine	+	+	+	+	+
4-Nitroquinoline-N-oxide	-	+	+	+?	+
Lead chromate	+	+	+	+	+
Pyrene	-	-	-	-	-
N-2 fluorenylacetamide	-	-	-	-	+
Anthracene	-	-	-	-	-
2,6-dichloro-p-phenylene diamine	+	+	E	+	+
4,4'-oxydianiline	+	+	E	+	+
Cinnamyl anthranilate	-	E	+	Inc.	+
Dichlorvos	+	+	+	+	+
Reserpine	+	+	-	+	+

In all cases common cell pools and the same lot of fetal calf serum were used in order to eliminate the influence of some variables. +: positive result; -: negative result; E: equivocal result; Inc.: inconclusive overall evaluation of transformation;

Table 1-2 : Results of an interlaboratory comparison of transformation responses in Syrian hamster embryo cells with eighteen coded chemicals (Jones *et al.,* 1988)

Chemical	SHE cell transformation in : Lab. A	Lab. B	Lab. C	Overall evaluation	In vivo Carcinogenesis
Diethylhexyl phthalate	+	+	+	+	+
Diphenyl hydantoin	+	ND	+	+	+
Sodium fluoride	+	+	ND	+	E
O-Toluidine-hydrochloride	+	+	+	+	+
L- ascorbic acid	-	ND	-	-	-
Benzoin	-	E	E	-	-
Bisphenol A	E	ND	-	-	-
Caprolactam	+	+	+	+	-
Chloramphenicol sodium succinate	-	+	ND	Inc.	+
FD andYellow n°6	-	ND	E	-	-
Geranyl acetate	+	+	+	+	-
5-Azacytidine	+	-	ND	Inc.	+
Benzidine dihydrochloride	ND	-	+	Inc.	+
Ethylene thiourea	-	+	ND	Inc.	+
Methapyriline hydrochloride	ND	+	+	+	+
HC blue 1	E	+	+	+	+
HC blue 2	-	-	-	-	-
HC red 3	-	ND	-	-	-

NB: In contrast to Table 1-1, individual serum sources were used in each experiment.
+ : positive result ; - : negative result ; E : equivocal result ; ND : not determined ; Inc. : inconclusive overall evaluation of transformation;

The BALB/c 3T3 cell transformation assay

33. Inter-laboratory studies sought to determine whether the ITES-medium-improved BALB/c 3T3 cell transformation model (Tsuchiya and Umeda, 1995) was reproducible and if the modified two-stage protocol was appropriate to detect non-genotoxic carcinogens. 19 laboratories reproduced the same type of experiments, using the typical rodent carcinogen MCA selected at an initiating concentration in association with the promoter TPA (Tsuchiya *et al*., 1999). Cells from the A 31-1-1 clone and chemicals were from the same source and the same batch of serum was employed. The data were reproducible among laboratories since the initiating activity of MCA and the promoting activity of TPA were detected in 100 % in the 2-stage protocol *in vitro*. When MCA was tested alone, a significant positive response was obtained in 89 %

of laboratories (17/19) and with TPA tested alone, a significant positive response was obtained in 47 % of laboratories (8/17). Some variation in the dose response relationship was noticed between the different laboratories that may be explained by individual cell culture conditions.

34. A recent study involving 4 to 5 laboratories tested 4 chemicals for initiation, 3 of which gave consistent results (Umeda 2004). 3 to 5 laboratories obtained highly consistent results with 7 compounds (comprising 1 non-carcinogen, and 6 carcinogens among which 4 displayed promoting activity).

The C3H 10T1/2 cell transformation assay

35. Two laboratories tested a total of 46 chemicals, the majority under code. Seven chemicals were active in both laboratories and 14 were inactive. 34 chemicals were evaluated for reproducibility (the results of 12 chemicals were not taken into account because 25% toxicity was not reached). Reproducibility was concordant across laboratories for 21/34 chemicals or 62% of the chemicals tested, though no effort was made to rigorously standardize the testing procedure. There were differences in the number of target cells, the medium used, the batches of serum selected and dose selection protocol (Dunkel *et al.,* 1988).

Conclusions

36. The data contained in this DRP enables an assessment of some measure of reproducibility beyond that suggested by reports in the scientific literature referenced above. Excluding chemicals with only one result, consistency between laboratories is 87.7% for the SHE assay (57/65 chemicals), 68.4% for the BALB/c 3T3 assay (39/57 chemicals), and 54.3% for the C3H 10T1/2 assay (38/70 chemicals). The lower apparent reproducibility for the latter 2 assays may be attributable to substantial differences in protocols. When a European research team compared 121 replicate rodent carcinogenicity studies from the US NTP database with those in the published scientific literature, it found that the studies produced concordant results for only 57 % of the time indicating that rodent carcinogenicity assays are not inferior in reproducibility than CTAs (Gottman *et al.,* 2001).

37. The European Centre for the Validation of Alternative Methods (ECVAM) at the EU DG JRC, is presently engaged in validation studiesof the SHE and BALB/c 3T3 assays.

III. RESULTS

Mechanisms of cell transformation

38. *In vitro* cell-transformation systems have been developed to simulate a part of the carcinogenesis process. They are the only *in vitro* systems in which tumor production can be utilized as an end-point: most transformed BALB/c 3T3 and C3H/10T1/2 cells are tumorigenic in nude mice (Kakunaga and Yamazaki, 1985) and morphologically transformed SHE cells can acquire tumorigenicity after subsequent passages (Berwald and Sachs, 1963; Barrett and Ts'o, 1978). Because of their direct biological link to cancer, cell-transformation systems have been extensively used to understand the molecular and cellular mechanisms of carcinogenesis, especially the role of oncogenes and tumor-supressor genes (Yamasaki *et al.,* 1996). In detailed studies of SHE cell transformation, Carl Barrett, Paul Ts'o and their colleagues in the late 1970s, and Newbold (1985) demonstrated multiple cellular changes required for malignant transformation and the capacity for anchorage-independent (AI) growth as an essential event which occurs at a relatively late stage in the process for malignancy. An infinite cellular lifespan was an early characteristic of transformed cultures, and morphological changes and enhanced fibrinolytic activity were considered to be important early markers.

39. Like the multistep process of carcinogenesis, cell transformation is a staged process. LeBoeuf *et al.,* (1999) identified at least four phenotypic steps in cell transformation. They included (i) a block in cellular differentiation visualized as morphological transformation in the SHE cell transformation assay, (ii) acquisition of immortality expressed by unlimited lifespan, an aneuploid karyotype and genetic instability, (iii) acquisition of tumorigenicity closely associated with the in-vitro phenotypes of foci formation, anchorage independent growth in semi solid agar and autocrine factor production, and (iv) full malignancy when cells are injected into a suitable host.

40. Cell transformation has been related to structural alterations and changes in the expression of genes involved in cell cycle control, proliferation and differentiation. Genomic changes may result from direct interaction as well as indirect and non-genotoxic mechanisms. Indirect mechanisms include oxidative stress, alteration of DNA repair, changes in DNA methylation, allelic loss (*e.g.,* aneuploidy). Non-genotoxic mechanisms involve cell cycle deregulation, alteration of signal transduction pathways, inhibition of intercellular communication, resulting in altered cell proliferation, differentiation and finally neoplastic transformation. These views are supported by studies that have shown the morphological transformed colonies that arise in SHE cell transformation assays harbour activated versions of proto-oncogenes (Notario *et al.,* 1990; Albor *et al.,* 1994), and frequently display methylation-associated suppression of gene expression known to be associated with embryonic differentiation and development (Isfort *et al.,* 1997). Further, the introduction of an activated oncogene, by transfection, will morphologically transform normal SHE cells (Thomassen *et al.,* 1985). Also notable is that the morphologically transformed SHE cell phenotype has been linked to a stable loss of cell cycle G_2 checkpoint control (Ashra and Rao, 2006). Oxidative stress was shown to be involved in acrylonitrile-induced morphological transformation in SHE cells (Zhang *et al.,* 2000a, 2000b). Imbalance of cell proliferation via an inhibition of apoptosis has been related to cell transforming effects of some HPP (Maire *et al.,* 2005) and some transforming agents in SHE cells (Alexandre *et al.,* 2003).

41. The BALB/c 3T3 cell line displays an aneuploid karyotype and ability to undergo tumorigenic conversion either spontaneously or after treatment with a carcinogen. The conversion to tumorigenic phenotype is associated with either activation of oncogenes or inactivation of tumor suppressor genes (Nakazawa *et al.,* 1990; Zerrahn *et al.,* 1992; Olson *et al.,* 1993; Whong, 1999; Lin *et al.,* 2000; Spruill *et al.,* 2002). Activated forms of several proto- and viral oncogenes have been shown to morphologically

transform (Silingardi *et al.,* 1994; Vaziri and Faller, 1995) and alter cell-cycle functioning (Sinibaldi *et al.,* 2000) of BALB/c 3T3 cells when introduced by transfection techniques. Conversely, antisense RNA down-regulation experiments can reverse the oncogene-mediated transformed phenotype (Joseph *et al.,* 2002). C3H/10T1/2 cells are also morphologically transformed by transfection of activated proto-oncogenes (Parada and Weinberg, 1983; Hsiao *et al.,* 1984; Denhardt *et al.,* 1987; Manoharan *et al.,* 1985; Trimble *et al.,* 1987; van der Hoorn and Miller, 1985). The frequency of transformation can be enhanced by co-operative interactions among the activated proto-oncogenes introduced in this way (Taparowsky *et al.,* 1987; Taylor *et al.,* 1992). Transformed foci that develop during C3H/10T1/2 cell transformation assays frequently exhibit mutated oncogenes (Chen and Herschman, 1989; Krolewski and Little, 1993; Syljuasen, 1999), increased proto-oncogene expression (Shuin *et al.,* 1986; Billings *et al.,* 1987; Landolph, 1994; Coleman *et al.,* 1994a, 1994b), hyper- and hypo-methylation of oncogene DNA sequences (Hsiao *et al.,* 1986; Thomas and Guernsey, 1991) as well as gene amplifications (Krolewski and Little, 1995). Such changes are likely to underlie the loss of G_1-checkpoint control, which appears to be a consistent feature of transformed C3H/10T1/2 cells (Syljuasen, 1999). Many of these genes are also altered during the conversion from immortality to tumorigenicity in the SHE cell systems (Isfort and LeBoeuf, 1995; Notario and DiPaolo, 1998). The expression of genes - such as p53, p27, cyclins - which could explain cell cycle disturbances were studied to understand the molecular and cellular mechanisms contributing to *in vitro* two-stage transformation of BALB/c 3T3 cells by carcinogens (Fang *et al.,* 2001a; 2001b; 2002). The same approach has been developed to identify mechanisms of tumour promoter–induced responses in C3H/10T1/2 (Parfett *et al.,* 1993; 1996; 2000; Hanlon *et al.,* 2005). The mechanisms by which tumour promoters alter DNA stability is also a current approach to elucidate multi-stage carcinogenesis (Parfett and Healy, 2006). Our understanding of mechanisms of chemical carcinogenesis requires detailed knowledge of cellular behaviour, signal transduction, cell cycle control and genomic instability, and will support the proposal that cell transformation represents carcinogenesis *in vivo* relatively accurately and that cell transformation assays may be used to predict carcinogenic potential.

The SHE cell transformation assay

State of the art

42. Syrian hamster embryo cells have been used to study transformation *in vitro* since Berwald and Sachs (1963) confirmed the key observation by Earle (1943) that morphological changes in cell cultures were associated with the oncogenicity of these cells *in vivo*. These authors demonstrated oncogenicity of SHE cells which presented a transformed phenotype after exposure to chemical carcinogens *in vitro*. Thereafter, DiPaolo *et al.,* (1969, 1971, 1972a), Pienta *et al*. (1977), Pienta (1979, 1980), Barrett *et al*., (1978, 1979, 1984) established the close correlation between the ability of compounds from different chemical classes to induce tumours *in vivo* and morphological transformation *in vitro*. The SHE cell transformation system has also been used extensively to elucidate the mechanisms of carcinogenesis (Barrett and Ts'o, 1978; Pienta *et al.*, 1977).

43. In parallel to Earle's studies (1943), Berenblum developed the "initiation-promotion" concept from his experiments on mouse skin cancer (1941). He showed that tumour promoters, when applied after an initiator, allow cells to progress from an initiated to a transformed state, gaining immortality and malignancy. Later on, Chouroulinkov and Lasne, (1976, 1978) showed that substances with promoting activity could be detected *in vitro* using cells previously treated with an initiator. Their results were at the origin of the use of sequential treatment applied to SHE cells and to established cell lines, in order to detect initiator- or promoter-like activities.

44. SHE cells are primary and normal diploid cells, which derive from mid-gestation embryos. The cells are metabolically competent. Exposure to carcinogenic agents results in an increase in the percentage of morphologically transformed (MT) colonies compared to controls. The time necessary for

morphological transformation is short (8-10 days). The level of spontaneously morphologically transformed colonies is very low. MT colonies are characterized by a random growth pattern of spindle shaped cells and a piling up of cells in a criss-cross pattern, which express a loss of growth inhibition and of cell-cell orientation at confluency.

45. Subsequent passages of such transformed cells would result in the acquisition of other characteristics related to a malignant state, including the ability to grow in a semi-solid medium, to acquire immortality and the capacity to produce tumours in syngeneic or immunosuppressed animals (Barrett *et al*.,1979; Leboeuf *et al*., 1990). Therefore, *in vitro* transformation in SHE cells is a multistage phenomenon requiring multiple mutational events for complete transformation to occur (Berwald and Sachs, 1963; Leboeuf *et al.*, 1999, Tsuda, 2003).

Principle of the test

46. SHE cells are obtained from primary cell cultures of individual Syrian hamster embryos at 13 days of gestation. After enzyme tissue digestion, cells are collected and stored in liquid nitrogen. One part will be used as feeder cells, the other part as target cells.

47. The test consists in seeding target cells at clonal density onto a feeder layer of 5000 rad X-irradiated SHE cells. The target cells are treated 24 hours later. After 7 days necessary for clonal expansion, cells are washed, fixed and stained with Giemsa. Dishes are coded and colonies are scored for their morphological phenotype under stereomicroscope (or image analyzer).

48. Cytotoxicity is evaluated by inhibition of cloning efficiency. The number of MT colonies reported to the total number of colonies is calculated for each concentration tested. A statistically higher percentage of MT colonies at two concentration levels compared to control vehicle will yield a conclusion of a positive response.

49. The test medium is the Dulbecco's modified Eagle's medium (DMEM) without phenol red, supplemented with fetal calf serum. Usually, the exposure-time to the test agent is 7 days, and pH of the test medium is in the range 7.0-7.35.

50. Several variations to the classical protocol have been proposed:

- the use of a reduced pH 6.7 by Leboeuf and Kerckaert (1986, 1987). The acidic pH modifies cell metabolism and reduces cell-cell communication (Ruch *et al.,* 1990), resulting in an increased sensitivity of SHE cells to morphological transformation (Leboeuf *et al.,* 1989; 1990; Isfort *et al.,* 1996).

- the use of a 24-hr exposure in addition to a 7 day treatment (Leboeuf *et al.,* 1996).

- an initiation-promotion protocol (sequential treatment) (see paragraph 43) to mimic a two-stage in vivo malignant transformation and to detect substances with promoting activity. Chouroulinkov and Lasne (1976; 1978) proposed to use cells previously treated with an initiator (BaP, MCA or MNNG) applied at a non-transforming concentration during a short period of time (24h). Thereafter, the medium is replaced and the chemical is applied and tested for its tumour-promoting-like activity. After a seven-day exposure, cells are fixed, stained and colonies scored as usual.

- limiting the incubation time of the target cells to < 5 h after thawing (Zhang *et al.,* 2004)

- Only results obtained after 7 days of exposure have been considered in the evaluation of performances. Studies with addition of S9 or the SA7/SHE assay employing the simian adenovirus SA7 and SHE cells have been excluded from the analysis.

51. Sequential treatment can also be used to detect an initiator-like chemical: then the test chemical is applied first during a short period of time, thereafter cells are treated with a typical tumour-promoter such as TPA.

Results

52. The data set of SHE results (264 chemicals in total) involves:

- 203 organic chemicals

  138 classified as rodent carcinogens (table 2 lists chemicals with SHE and genotoxicity results)
  65 as rodent non-carcinogens (table 3)

- 61 inorganic chemicals

  53 rodent carcinogens (table 4)
  8 non-carcinogens (table 4)

Tables 2-4 are restricted to the chemicals with SHE and genotoxicity results available. All the chemicals used in the analysis of the performances of the SHE assay to predict carcinogenicity are presented in table 11 (rodent organic carcinogens), table 12 (non-carcinogenic and inconclusive organic chemicals) and table 13 (inorganic chemicals, carcinogens and non-carcinogens).

Rodent carcinogens

53. 78% of the rodent organic carcinogens and 94% of the inorganic carcinogens are positive in SHE assay. The latter include asbestos, glass and ceramic fibres, beryllium salts, chromates, nickel compounds, arsenites and arsenates. Examples of the organic carcinogens with positive responses are: aromatic amines, chlorinated hydrocarbons (*e.g.,* aroclor 1260), benzene, estradiol, diethylstilbestrol, the carcinogenic polycyclic aromatic hydrocarbons, most *N*-nitroso-compounds, alkylating agents, etc.

54. The positive calls include tumour promoters which induced morphological transformation of pre-initiated cells, when tested using a sequential (also called “two-stage” or initiation-promotion) treatment as recommended by Chouroulinkov (1988): benzoyl peroxide, butylated hydroxytoluene (BHT), chlordane and the phorbol esters TPA and PDD.

55. The cell system failed to detect 9% of the carcinogens. The false negatives included: aniline, anthraquinone, arochlor 1254 (while arochlor 1260 is positive; Rivedal *et al.,* 2000), decabromodiphenyl oxide, DDT, ethinylestradiol, ethyl alcohol, furfuryl alcohol, d-limonene, metaproterenol hemisulfate, methyl carbamate, nitrilotriacetic acid, 5-nitro-o-toluidine, phenacetine, pyridine, tetrahydrofuran, TEHP and titanium dioxide.

56. Some compounds were classified as inconclusive: for example hydrazine sulfate, nitrosodimethylamine, 1,3-propane sultone.

57. A specific pH value of the test medium cannot be correlated with negative results:

- ethyl alcohol, d-limonene, methylcarbamate are negative at both physiological and acidic pH;

- furfuryl alcohol, phenacetine, pyridine, tetrahydrofurane, TEHP are negative at acidic pH, but were not tested at physiological pH;

- cinnamyl anthranilate is negative at pH 6.7, while positive at physiological pH;

- conversely, 2,4-dinitrotoluene is positive at acidic pH and negative at physiological pH.

58. The pH conditions are known to influence the lipophilicity of ionisable organic chemicals such as phenolic and aromatic amines, as well as the ionic character of inorganic compounds. Both forms play a role for the bioavailability of these chemicals. However, it does not appear from the present results that one pH condition is better than the other (see performances section IV).

Rodent non-carcinogens

59. The following compounds induce SHE cell transformation, as an example :

- 2,4-dichlorophenol and resorcinol, two phenolic compounds weakly positive (pH 7.0);

- phenol, not carcinogenic in the standard rodent assay, but classified as tumor promoter by IARC;

- the aneuploidogens colcemid and vincristin.

Chemicals not evaluated for carcinogenicity and not included in the data set

60. Several chemicals tested on SHE cells are not included in the analysis because no information is available on their rodent carcinogenicity. The results are mentioned below for information. The response was negative for the following chemicals (with their CAS numbers in parentheses):

- dimethyl sulfoxide (67-68-5) (Heidelberger *et al.,* 1983; Leboeuf *et al.,* 1996);

- ethylene glycol methyl ether (109-86-4) (Elias *et al.,* 1996a; Dhalluin *et al.,* 1999);

- 5-fluorodeoxy-uridine (50-91-9) (Heidelberger *et al.,* 1983),

- 4-O-methyl TPA (57716-89-9) (Rivedal and Sanner, 1982; Elias *et al.,* 1996b) and 4-α-phorbol 12,13-didecanoate (257536-56-7) (Rivedal and Sanner, 1982; Engelhardt *et al.,* 2004), two non-active analogs of the tumor promoter TPA.

- titanium (7420-32-6) (Coombs et al. 1989), goethite (1310-14-1) (Elias *et al.,* 1995) and xonotlite (12141-77-4) (Elias *et al.,* 1996b)

The response of the SHE cell transformation assay was positive for :

- econazole nitrate (24169-02-6), an imidazole-derivative fungicide (Leboeuf *et al.,* 1996)

- MEHP (4376-20-9), the active metabolite of DEHP (Mikalsen *et al.,* 1990; Tsutsui and Barrett, 1991; Cruciani *et al.,* 1999)

- trenbolone (10161-33-8), an anabolic steroid (Lasne *et al.,* 1990; Schiffmann *et al.,* 1985, 1988).

Table 2: Rodent carcinogenic organic chemicals tested with the SHE cell transformation assay

Carcinogen CAS Number	Point Mutations			Cytogenetics (rodent and human cells)			SHE cell transformation			References SHE cell transformation assays
	Ames	Mammalian cells		In vitro	In vivo					
		Mouse Lymphoma TK	HPRT		Chrom. Ab.	Micro-nucleus	6.7	7.0	7.1-7.35	
Acetamide 60-35-5	– (1) – (2)					+u (2)			+ 	Heidelberger et al., 1983
2-Acetylaminofluorene 53-96-3	+ (2)	+ (1) + (2*) + (3)	+ (2^9) + (3)	+ (1)	+ (1)	+ (2)	+ –		+ + + –	Heidelberger et al., 1983 Poiley et al., 1983 Tennant et al., 1985 Tu et al., 1986 LeBoeuf et al., 1996 Engelhardt et al., 2004
Acrylamide 79-06-1	– (1) – (3) – (5)	+ (2*) + (3) + (5)		+ (1) + (5)	+ (1) +/– (2) +/– (5)	+ (5)	+		+	Pienta, 1980 Park et al., 2002
Acrylonitrile 107-13-1	+ (1) + (2) +/– (5)	+ (1) + (2*) – (4) + (5)	+ (5)	+ (1) +/– (5)	– (5)	– (1) – (2) – (5)	+ 	+	+ +	Heidelberger et al., 1983 Sanner and Rivedal, 1985 Barrett and Lamb, 1985 Zhang et al., 2000
Actinomycin D 50-76-0	– (3)	+ (2*) + (3)	+ (2^9)	+ (2)		+ (1) ? (2)			+	Hirakawa et al., 1979
Aflatoxin B_1 1162-65-8	+ (1) + (3) + (5)	+ (3)	+ (2^9) + (3) + (5)	+ (5)	+ (2) +/– (5)	+ (2)			+	Heidelberger et al., 1983
2-Aminoanthracene 613-13-8	+ (1) + (2)	+ (2*)		? (1)					+	Heidelberger et al., 1983
p-Aminoazobenzene 60-09-3	+(1) + (2) +(3)								+	Heidelberger et al., 1983
o-Aminoazotoluene 97-56-3	+ (2) +(3)	+(3)							+	Heidelberger et al., 1983
4-Aminobiphenyl 92-67-1	+ (1) +(2) +/– (3) +/– (5)	+ (2*) + (5)	+ (3) + (5)	+ (1)		+ (1) ? (2)			+	Heidelberger et al., 1983

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	Ames	Mammalian cells		In vitro	In vivo					
		Mouse Lymphoma TK	HPRT		Chrom. Ab.	Micro-nucleus	6.7	7.0	7.1-7.35	
Amitrole (3-Aminotriazole) 61-82-5	– (1) – (2) – (3)	– (1) – (3) – (4)		– (1) – (4)		? (2) – (4)		+	+ + + +	Inoue et al., 1981 Tsutsui et al., 1984a Dunkel et al, 1981 Heidelberger et al., 1983 Mikalsen et al., 1990
Aniline 62-53-3	– (1) – (2) – (3) – (5)	+ (1) + (2*) + (3) + (5)	+/– (3)	+w (1) +/– (5)		+ (1) ? (2) – (5)			–	Heidelberger et al., 1983
o-Anisidine 90-04-0	+ (1) ? (2)	+ (2*) + (3) + (4)		+ (1)				+w		Rivedal et al., 2000
o-Anisidine HCl 134-29-2	+/– (1) +/– (3)					– (1)	+*/– +			LeBoeuf at al., 1996 Engelhardt et al., 2004
Anthraquinone 84-65-1	+/– (1) +/– (3)	– (3)				– (1)	–			Kerckaert et al., 1996a
Aroclor 1254 11097-69-1	– (1) – (3)			– (1)	– (1)	– (1) ? (2)			–	Heidelberger et al., 1983
Auramine, C.I. Basic Yellow 2; 2465-27-2	+ (1) – (3)	# (2*)	+ (3)			? (2)			?	Heidelberger et al., 1983
5-Azacytidine 320-67-2	+ (1) +/– (5)	+ (1) + (2*) + (3) + (5)	+ (3) +/+w (5)	+ (1) +/– (5)		+ (1)			+ +/– + +	Barrett et al., 1984 Jones et al.,1988 Klein et al., 1990 Stopper et al., 1992
Benomyl 17804-35-2	– (1) – (3)	+ (2*)					+*/–			Gibson et al., 1996
Benz(a)anthracene 56-55-3	+ (1) + (2) + (5)	+ (1) + (2*) +(3) +/– (5)	+ (2^{10}) + (3) – (5)	+/– (5)	+/– (5)	+ (5)			+	Heidelberger et al., 1983

Table 2: Rodent carcinogenic organic chemicals tested with the SHE cell transformation assay

Carcinogen CAS Number	Point Mutations			Cytogenetics (rodent and human cells)			SHE cell transformation			References SHE cell transformation assays
	Ames	Mammalian cells		In vitro	In vivo					
		Mouse Lymphoma TK	HPRT		Chrom. Ab.	Micro-nucleus	6.7	7.0	7.1-7.35	
Benzene 71-43-2	– (1)	– (1) + (2*) +/– (3) +/– (4)	– (3)	– (1) + (2) + (4)	+ (2) + (4)	+ (1) + (2)			+ + + +	Sanner and Rivedal, 1985 Amacher and Zelljadt, 1983 Barrett and Lamb, 1985 Tsutsui et al., 1997a
Benzidine 92-87-5	+ (1) + (2) +/– (3)	+ (2*) + (3)	+ (2^9) +/– (3)	+ (1)		+ (2)			+	Heidelberger et al., 1983
Benzidine dihydrochloride 531-85-1	+ (1) + (2)	+ (1) + (2*) + (3)		+ (1)	+ (1)	+ (1)			+ ?	Pienta, 1980 Jones et al., 1988
Benzo(a)pyrene 50-32-8	+ (1) + (2)	+ (1) + (2*) + (3)	+ (2^9) + (3)	+ (1)	+ (1)	+ (2)	+ +	+ +(ST)	+ + +(ST) +	Heidelberger et al., 1983 Rivedal and Sanner, 1980 Sala et al., 1987 LeBoeuf et al., 1989 Bessi et al., 1994 Elias et al., 1996b Lasne et al., 1990 Slamenova et al., 1992a Dusinska and Slamenova, 1994 Engelhardt et al., 2004
Benzoyl peroxide 94-36-0	– (1) – (3)							– +(ST)	 +/–	Rivedal et al., 2000 Elias et al.,1996b Yamasaki et al.,1996
Benzyl chloride 100-44-7	+ (1) +/– (5)	+ (2*) + (5)	? (2^9) + (5)	+/– (5)		– (2) – (5)			+	Heidelberger et al., 1983
HC Blue 1 2784-94-3	+ (1)	+ (1) ? (2*) +/– (3)		+ (1)	+ (1)	? (2)			+	Jones et al., 1988
5-Bromo-2'-deoxyuridine 59-14-3	– (3)		+ (2^9) + (3)	+ (2)		+ (2)			+ +?	Tsutsui et al., 1979 Heidelberger et al., 1983

Table 2: Rodent carcinogenic organic chemicals tested with the SHE cell transformation assay

Carcinogen CAS Number	Point Mutations			Cytogenetics (rodent and human cells)			SHE cell transformation			References SHE cell transformation assays
	Ames	Mammalian cells		In vitro	In vivo					
		Mouse Lymphoma TK	HPRT		Chrom. Ab.	Micro-nucleus	6.7	7.0	7.1-7.35	
Butylated hydroxytoluene 128-37-0	– (1) – (2) – (3) – (8)	+ (1) + (2*)	+ (8)	– (1) +/– (4)	– (4)	– (1) ? (2) – (4) – (8)			–,+	Potenberg et al., 1986
Butylbenzylphthalate 85-68-7	– (1)	– (1) # (2*) + (3) – (4)		– (1) – (4)	+ (1)	– (1)	+			LeBoeuf et al., 1996
Catechol 120-80-9	– (1)	+ (2*) + (3) + (4)	+ (3)	+ (4)		– (1) ? (2) ? (3) – (4)		+	+ +	Tsutsui et al., 1997a Elias et al., 1998; 1999a
Chloral hydrate 302-17-0	+ (1) + (2)	+ (3)		+ (1)		+ (1) ? (2)	+			LeBoeuf et al, 1996
Chlordane Analytical 57-74-9	– (1)			– (1)				+(ST)	+/– +(ST)	Yamasaki et al., 1996 Bessi et al., 1995 Elias et al., 1996b
p-Chloroaniline 106-47-8	+/– (1) +/– (5)	+ (1) + (2*) + (3) + (5)		+ (1) + (5)					+ +	Pienta and Kawalek, 1981 Heidelberger et al., 1983
Chlorothalonil 1897-45-6	– (1)	+ (1) + (2*)		+ (1)		– (4)			+	Bessi et al., 1994
Chlorpromazine 69-09-0	– (1)		+ (3)	– (1)			+*			Mauthe et al., 2001
Chrysene 218-01-9	+ (2) +/– (3)		+ (3)						+ +	Heidelberger et al., 1983 Sala et al., 1987
Cinnamyl anthranilate 87-29-6	– (1) – (3)	+ (1) + (2*) + (7)		– (1) – (7)	– (1)	– (1) – (7)	–		+ ?	LeBoeuf et al., 1996 Tennant et al., 1985 Tu et al., 1986

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Carcinogen CAS Number	Point Mutations			Cytogenetics (rodent and human cells)			SHE cell transformation			References SHE cell transformation assays
	Ames	Mammalian cells		In vitro	In vivo					
		Mouse Lymphoma TK	HPRT		Chrom. Ab.	Micro-nucleus	6.7	7.0	7.1-7.35	
Clofibrate 637-07-0	– (3) – (5)		– (3) – (5)	– (5)			+ + + + +		–,+ST + –	LeBoeuf et al., 1996 Cruciani et al., 1999 Cruciani et al., 1999 Mikalsen et al., 1990 Elias et al., 1996 b Cruciani et al.,1997 Tsutsui et al.,1993 Rivedal et al., 2000 Engelhardt et al., 2004
Cyclophosphamide 50-18-0	+ (2) +/– (3) +/– (5)	+ (1) + (2*) + (3) + (5)	+ (2^{9}) + (5)	+ (2) + (5)	+ (2) +/– (5)	+ (1) + (2) + (5)			+	Hirakawa et al., 1979
Cyclophosphamide, H_2O 6055-19-2	+ (1)			+ (1)		+ (1)	+*		+	Pienta, 1980 Custer et al., 2000
Cyclosporin A 59865-13-3	– (1)		– (3)			– (2)	+*			Custer et al., 2000
Decabromo diphenyloxide 1163-19-5	– (1) – (3)	– (1) – (4)	– (3)	– (1) – (4)		+ (1)	–			LeBoeuf et al., 1996
2,4-Diaminotoluene 95-80-7	+ (1) + (2)	+ (2*)	– (3)	+ (1)		– (1)	+ + +	+	+	Heidelberger et al., 1983 Holen et al., 1990 Kerckaert et al., 1998 LeBoeuf et al., 1996 Engelhardt et al., 2004
Dibenz(a,h)anthracene 53-70-3	+ (2) +/– (3) + (5)	+ (2*) + (5)	+ (2^{10}) + (3) + (5)	+ (5)					+	Heidelberger et al., 1983
Dichlorodiphenyl trichloroethane (DDT) 50-29-3	– (1) – (2) – (3)	– (1)	+ (2^{10})	– (1) + (2) – (4)		? (2)		– –		Roseng et al., 1994 Rivedal et al., 2000
2,6-Dichloro-p-phenylenediamine 609-20-1	+ (1)	+ (1) + (2*)		+ (1)	+ (1)	– (1)			+ +	Tennant et al., 1985 Tu et al., 1986

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Carcinogen CAS Number	Point Mutations			Cytogenetics (rodent and human cells)			SHE cell transformation			References SHE cell transformation assays
	Ames	Mammalian cells		In vitro	In vivo					
		Mouse Lymphoma TK	HPRT		Chrom. Ab.	Micro-nucleus	6.7	7.0	7.1-7.35	
Dichlorvos 62-73-7	+ (1) + (2)	+ (1) + (2*) + (3)	+ (3)	+ (1)	– (1) – (2)	– (1) ? (2)			+ +	Tennant et al., 1985 Tu et al., 1986
Dieldrin 60-57-1	– (1) – (2) – (3)	+ (1) eq (2*) + (3)	+ (2^{10})	– (1)			+*			Mauthe et al., 2001
Diepoxybutane, 1,2 :3,4 1464-53-5	+ (2) + (3) +/– (5)	+ (2*) + (5)	+ (5)	+ (5)	+ (5)	+ (5)			+	Heidelberger et al., 1983
Diethanolamine 111-42-2	– (1) – (3)	– (1)		– (1)		– (1)	+			Kerckaert et al., 1996a
Di(2-ethylhexyl)phthalate (DEHP) 117-81-7	– (1) – (3) – (5)	– (1) # (2*) +/– (3) +/– (4) +/– (5)	– (5)	– (1) – (4) – (5)	– (1) – (4) – (5)	– (1) + (4) – (5)	+	+ + + +	– + + +	LeBoeuf et al.,1996 Cruciani et al., 1999 Elias et al., 1996b Sanner and Rivedal, 1985 Rivedal et al., 2000 Mikalsen et al., 1990 Barrett and Lamb., 1985 Tsutsui et al., 1993 Jones et al., 1988
Diethylstilbestrol (DES) 56-53-1	– (1) – (2) – (3) – (5)	+ (1) + (2*) +/– (3) +/– (5)	– (5)	+ (1) +/– (5)	+ (5)	+/– (1) + (2) – (5)	+*/– +*	– +w ST	+ +? + + + + +	Barrett et al., 1981 Heidelberger et al, 1983 Sanner and Rivedal, 1985 Barrett andLamb,1985 Tu et al., 1986 Klein et al., 1990 Hayashi et al., 1996 Elias et al., 1996b Tsutsui and Barrett, 1997 Gibson et al., 1995 Mauthe et al., 2001
N,N'Diethyl-2-thiourea 105-55-5	– (1)	+ (2*) + (1)		– (1)			+			LeBoeuf et al., 1996

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	Ames	Mammalian cells		In vitro	In vivo					
		Mouse Lymphoma TK	HPRT		Chrom. Ab.	Micro-nucleus	6.7	7.0	7.1-7.35	
N,N-Dimethyl-4-aminoazobenzene 60-11-7	+ (1) + (2) +/– (3) +/– (5)	– (2*) +/– (3) + (5)	– (3) – (5)	+/– (5)		+ (2) – (5)			+ ?	Pienta, 1980 Heidelberger et al., 1983
7,12-Dimethyl benzanthracene 57-97-6	+ (1) + (2) + (3)	+ (1) + (2*) + (3)	+ (2^{9}) + (3)	+ (2)	+ (2)	+ (1) + (2)			+	Tu et al., 1986
1,2-Dimethylhydrazine 540-73-8	– (2)					? (2)			+	Pienta, 1980
2,4-Dinitrotoluene 121-14-2	+ (1)	+ (1)	? (2^{9})	– (1)		– (2)	–			Holen et al., 1990 Kerckaert et al., 1998 Engelhardt et al., 2004
							+*			
							+			
2,6-Dinitrotoluene 606-20-2	+ (1) +/– (2)		? (2^{9})				+			Engelhardt et al., 2004
Diphenylhydantoin 57-41-0	– (1)	– (1) ? (2*) – (3)	– (3)	– (1)	– (1)	– (1) ? (2)			+	Jones et al., 1988
Epichlorhydrin 106-89-8	+ (5) + (2) +/– (3)	+ (2*) + (5)	– (3) +/– (5)	+ (2) +/– (5)	+/? (2) +/– (5)	– (5) ? (2^{12})	+			Kolman et al., 1994 Kolman and Dusinska, 1995
							+			
1,2-Epoxybutane 106-88-7	+/– (1) + (2) + (3)	+ (1) + (2*) + (3)		+ (1)		– (1)			+	Heidelberger et al., 1983
Estradiol 50-28-2	– (1) – (3) – (5)	? (2*)	+/– (3) – (5)		– (7)	– (1)			+ + +	Tsutsui et al., 1987 Hayashi et al., 1996, Tsutsui and Barrett, 1997
							+*			Mauthe et al., 2001
Ethinylestradiol 57-63-6	– (1) – (3)					– (1)			–	Heidelberger et al, 1983
l-Ethionine 13073-35-3	– (2)					+ (2)			+ + +	Brown et al., 1983a Heidelberger et al., 1983 Amacher and Zelljadt, 1983

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Carcinogen CAS Number	Point Mutations			Cytogenetics (rodent and human cells)			SHE cell transformation			References SHE cell transformation assays
	Ames	Mammalian cells		In vitro	In vivo					
		Mouse Lymphoma TK	HPRT		Chrom. Ab.	Micro-nucleus	6.7	7.0	7.1-7.35	
Ethyl Alcohol Ethanol 64-17-5	– (1) – (2) – (3) – (5)	– (2*) – (3) + (4) – (5)		– (2) – (4) +/– (5)	? (4) – (5)	– (2) – (4) +/– (5)	–		–	Heidelberger et al., 1983 LeBoeuf et al., 1996
Ethyl benzene 100-41-4	– (1) – (3)	+ (1) + (2*)		– (1)		– (1) ? (2^{12})	+ +			Kerckaert et al., 1996a Engelhardt et al., 2004
Ethyl methanesulfonate (EMS) 62-50-0	+/? (1) + (2) +/– (3) +/– (5)	+ (2*) + (3)	+ (2^{9}) + ($2^{10,11}$) + (3)	+ (1) + (5)	+ (1) +/? (2) + (5)	+ (2) + (5)			+	Heidelberger et al., 1983
Ethylene glycol butyl ether 2-Butoxyethanol 111-76-2	– (1) – (3)		+ (13)	– (1)		– (1)	+*			Kerckaert et al., 1996a
Ethylene thiourea 96-45-7	+w/– (1) + (2)	+ (1) # (2*) + (3)		– (1)		? (2)			?	Jones et al., 1988
Formaldehyde 50-00-0	+(1) +/– (3) +/– (5)	+ (2*)	+ (5) ? (2^{9}) + (6)	+ (1) + (5)	+/– (5)	? (2) +/– (5)	+			LeBoeuf et al, 1996
Furfuryl alcohol 98-00-0	– (1) – (3)			? (1)			–			Kerckaert et al., 1996a
Furylfuramide (AF-2) 3688-53-7	+ (1) + (2) + (3)	+ (2*) + (3)	+ (2^{10})	+ (1)		? (2^{12})			+	Heidelberger et al., 1983
Genistein 446-72-0	– (14)	+ (14)		+ (14)		– (14)	–			Harvey et al, 2005
Glycidaldehyde 765-34-4	+ (1) + (2) + (3)	+ (2*)							+	Heidelberger et al., 1983
Griseofulvin 126-07-8	– (1) ? (2) – (3)	+ (3)				? (2)	+*/–			Gibson et al., 1995
Hexachloro-1,3-butadiene 87-68-3	– (1)			– (1)					+	Schiffmann et al., 1984

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Carcinogen CAS Number	Point Mutations			Cytogenetics (rodent and human cells)			SHE cell transformation			References SHE cell transformation assays
	Ames	Mammalian cells		In vitro	In vivo					
		Mouse Lymphoma TK	HPRT		Chrom. Ab.	Micro-nucleus	6.7	7.0	7.1-7.35	
Hexamethyl phosphoramide (HMPA) 680-31-9	– (1) – (5)	+ (1) + (2*) +/– (3) + (5)	+/– (3)	– (1) – (5)	+ (1) +/– (5)	+ (2) + (5)		+ 	 + +	Sanner and Rivedal, 1985 Barrett and Lamb, 1985 Zdzienicka et al., 1985
Hydrazine sulfate 10034-93-2	+ (2) + (3)	+ (2*)		? (2)		? (2[12])			+?	Heidelberger et al., 1983.
Hydroquinone 123-31-9	– (1)	+ (1) + (2*) + (4)		+ (1)		+ (1) +(2) + (3)		+	 +	Elias et al., 1998, 1999a Tsutsui et al , 1997a
N-Hydroxy-2-acetylaminofluorene 53-95-2	+ (2) + (3)		+ (2[9])						+	Heidelberger et al., 1983
d-Limonene 5989-27-5	– (1) – (3)	– (1) ? (2*) + (3)		– (1)			–	+/–	 –	Rivedal et al., 2000 Pienta, 1980 Engelhardt et al., 2004
Melphalan 148-82-3	+ (1) + (2) + (3) +/– (5)	+ (5)		+ (1) + (5)	+ (5)	+ (1)	+*		+	Klein et al., 1990 Mauthe et al., 2001
Metaproterenol hemisulfate 5874-97-5	– (14)		– (14)	– (14)		– (14)	–			Harvey et al., 2005
Methapyrilene HCl 135-23-9	– (1)	– (1) + (2*) +/– (3)		+ (1)			+		+	Jones et al., 1988 LeBoeuf et al., 1996
3-Methoxy-4-aminoazobenzene 3544-23-8	+ (2) + (3)								+	Heidelberger et al., 1983
3-Methyl-4'-(dimethyl amino)azobenzene 55-80-1	+ (3)								+?	Heidelberger et al., 1983
Methyl eugenol 93-15-2	– (1) – (3)			– (1)		– (1)	+ +*			Engelhardt et al., 2004 Kerckaert et al., 1996b

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Carcinogen CAS Number	Point Mutations			Cytogenetics (rodent and human cells)			SHE cell transformation			References SHE cell transformation assays
	Ames	Mammalian cells		In vitro	In vivo					
		Mouse Lymphoma TK	HPRT		Chrom. Ab.	Micro-nucleus	6.7	7.0	7.1-7.35	
Methyl iodide 74-88-4	+ (3) +/– (5)	? (2*) + (3)							+ +	Pienta, 1980 Heidelberger et al., 1983
Methyl methane sulfonate (MMS) 66-27-3	+ (1) + (2) +/– (3) +/– (5)	+ (2*) + (3) + (5)	+ (2^{9}) +/– (5)	+ (2)	+ (2) +/– (5)	+ (2) + (5)	–*		+ + +	Popescu et al., 1981 Heidelberger et al., 1983 Popescu et al., 1984 Dusinska and Slamenova, 1994
Methylazoxymethanol acetate 592-62-1	+ (1) + (3)	+ (2*) + (3)							+	Heidelberger et al., 1983
Methylcarbamate 598-55-0	– (1) – (2) – (3)	– (1) # (2*) – (3)		– (1)		– (1)	–		–	Heidelberger et al., 1983 Engelhardt et al., 2004
3-Methylcholanthrene (MCA) 56-49-5	+ (1) + (2)	+ (2*)	+ (2^{9}) + (3)	– (1) – (2)		? (2)	+ + +		+	Heidelberger et al., 1983 LeBoeuf et al, 1989 Dusinska et al., 1993 Dusinska and Slamenova, 1994
Methylclofenapate 21340-68-1	– (3)							+(ST) +	+	Yamasaki et al., 1996 Cruciani et al., 1997 Cruciani et al., 1999
4,4'-Methylene bis-(N,N'-dimethylaniline) 101-61-1	+/– (1) + (2) + (9)	+ (1) + (2*) + (9)		– (1)					+	Heidelberger et al., 1983
Mezerein 34807-41-5	– (1) – (3)								+	Tu et al., 1992
Mitomycin C 50-07-7	+/– (3) +/– (5)	+ (2*) + (3)	+ (2^{9}) + (3)	+ (2) + (5)	+ (1) + (2) + (5)	+ (1) + (2) + (5)			+ +	Popescu et al., 1984 Klein et al., 1990
Monuron 150-68-5	– (1) – (3)	– (1) + (2*)		+ (1)	– (1)	+ (1)			+	Swierenga and Yamasaki, 1992
2-Naphthylamine 91-59-8	+ (1) + (2) +/– (3) + (6) +/– (5)	+ (1) + (2*) + (5)	+ (2^{9}) +/– (5)	+ (1) + (5)	? (1)	– (1) + (2) – (5) + (2^{12})	+		+	Heidelberger et al., 1983 Engelhardt et al. 2004

Table 2: Rodent carcinogenic organic chemicals tested with the SHE cell transformation assay

Carcinogen CAS Number	Point Mutations			Cytogenetics (rodent and human cells)			SHE cell transformation			References SHE cell transformation assays
	Ames	Mammalian cells		In vitro	In vivo					
		Mouse Lymphoma TK	HPRT		Chrom. Ab.	Micro-nucleus	6.7	7.0	7.1-7.35	
Nitrilotriacetic acid 139-13-9	– (1) – (3)	– (3)		– (1)			+*/– –		–	Lanfranchi et al.,1988 LeBoeuf et al., 1996 Engelhardt et al., 2004
N-(4-(5-Nitro-furanyl)-2-thiazolyl)formamide 24554-26-5	+ (1) + (2) + (3)								+	Heidelberger et al., 1983
2-Nitro-1,4-phenylenediamine 5307-14-2	+ (1) + (2) +/– (5)	+ (1) + (2*) + (3) + (5)	+ (3)	+ (1) + (2) + (5)	– (5)	? (2) – (5)			+	Heidelberger et al., 1983
5-Nitro-*o*-toluidine 2-Amino-4-nitrotoluene 99-55-8	+ (1) +/– (3) +/– (5)			+ (1)			+*	–		Holen et al., 1990 Kerckaert et al., 1998
2-Nitropropane 79-46-9	+ (1)		+/– (3)	– (1)		? (2)		+(ST)		Elias et al., 1996b
4-Nitroquinoline-N-oxide 56-57-5	+ (2) + (3)	+ (1) + (2*) + (3)	+ (2^9) + (3)	+ (1) + (2)	+ (1) + (2)	+ (2)			+ + +?	Heidelberger et al., 1983 Tennant et al., 1985 Tu et al., 1986
N-Nitroso-*N*-ethylaniline 612-64-6	– (3)								+	Heidelberger et al., 1983
N-Nitroso-*N*-ethylurea (ENU) 759-73-9	+ (1) + (2) + (3) + (5)		+ (3) + (9)	+ (1) + (2) + (5)	+ (2) + (5)	+ (1) + (2) + (5)			? + + +	Heidelberger et al., 1983 De Kok et al., 1985a De Kok et al., 1985 b De Kok et al., 1988
N-Nitroso-*N*-methylnitroguanidine (MNNG) 70-25-7	+ (2) + (3)	+ (2*) + (3)	+ (2^9) + (3)	+/– (2)	? (2)	+ (2)	+ + +		+ + +	Dunkel et al., 1981; Popescu et al., 1981 Heidelberger et al., 1983 LeBoeuf et al., 1996 Dusinska and Slamenova, 1994 Slamenova et al., 1994
N-Nitroso-*N*-methylurea (MNU) 684-93-5	+ (2) + (3) + (5)	+ (2*)	+ (2^9) + (5)	+ (2) + (5)	+ (2) + (5)	+ (1) ? (2)	+* +*			Slamenova et al., 1992b Dusinska and Slamenova, 1994

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Carcinogen CAS Number	Point Mutations			Cytogenetics (rodent and human cells)			SHE cell transformation			References SHE cell transformation assays
	Ames	Mammalian cells		In vitro	In vivo					
		Mouse Lymphoma TK	HPRT		Chrom. Ab.	Micro-nucleus	6.7	7.0	7.1-7.35	
N-Nitrosodiethylamine (NDEA) 55-18-5	+/– (1) + (2) +/– (3)	+ (2*) + (3)	+ (2^{9}) +/– (3)	+ (2)		? (2)			+ 	Heidelberger et al., 1983
N-Nitrosodimethylamine (NDMA) 62-75-9	+/– (1) + (2) +/– (3) + (5)	+ (2*) +(3) + (5)	+ (2^{9}) + (3) + (5)	+/– (1) + (2) + (5)	– (2) + (6)	+ (2)			? 	Heidelberger et al., 1983
N-Nitrosodiphenylamine 86-30-6	– (1) – (2) – (3)	+ (1) – (2*)	– (2^{10})	– (1)		? (2)			+ 	Heidelberger et al., 1983
Okadaic acid 78111-17-8	– (3)	+ (3)						–	+ 	Rivedal et al., 1990a Afshari et al., 1993
4,4'-Oxydianiline 101-80-4	+ (1)	+ (1) + (2*)		+ (1)	+ (1)	+ (1)			+ +	Pienta, 1980 Tu et al., 1986
Oxymetholone 434-07-1	– (1) – (3)	– (3)		– (1)		– (1)	+ +			Kerckaert et al., 1996a Holden et al., 1999
Phenacetin 62-44-2	– (1) +/– (3) +/– (5)	– (1) # (2*)		+/– (5)	+ (5)	+ (2) +/– (5)	–			Mauthe et al., 2001
Phenobarbital 50-06-6	+w (1) – (5)	+ (1) ? (2*) +/– (3) – (5)	+ (3) + (5)	+ (1)		+ (2)		+ +	– – + +*	Pienta, 1980 Barrett and Lamb, 1985 Sanner et Rivedal., 1985 Rivedal et al., 2000 LeBoeuf et al., 1996 Mauthe et al., 2001
Phenobarbital sodium 57-30-7	– (2) +/– (3)							+	 +	Rivedal et al., 2000 Engelhardt et al., 2004
Phenolphtalein 77-09-8	+/– (1) – (3)			+ (1)	– (1)	+ (1)			+ +*	Tsutsui et al., 1997b Kerckaert et al., 1996a
Polybrominated biphenyls Firemaster FF1 67774-32-7		– (1)			– (1)	– (1)	+			LeBoeuf et al., 1996

Table 2: Rodent carcinogenic organic chemicals tested with the SHE cell transformation assay

Carcinogen CAS Number	Point Mutations			Cytogenetics (rodent and human cells)			SHE cell transformation			References SHE cell transformation assays
	Ames	Mammalian cells		In vitro	In vivo					
		Mouse Lymphoma TK	HPRT		Chrom. Ab.	Micro-nucleus	6.7	7.0	7.1-7.35	
Procarbazine HCl Natulan 366-70-1	– (1) – (2) – (3)	+ (2*) + (3)	+ (3)			+ (2)			+ 	Heidelberger et al., 1983
Progesterone 57-83-0	– (1) – (3)	– (1) eq (2*)							– +	Heidelberger et al., 1983 Tsutsui et al., 1995
1,3-Propane sultone 1120-71-4	+ (2) + (3) + (5)			+ (5)					?	Heidelberger et al., 1983
β-Propionolactone 57-57-8	+ (1) + (3) +/– (5)	+ (2*) + (3) + (5)	+ (2^9) + (5)	+ (1) + (5)	+ (5)	? (2) +/– (5)			+	Heidelberger et al., 1983
Propylene oxide 75-56-9	+ (1) + (3) +/– (5)	+ (1) + (2*) + (3) + (5)	+ (2^9) + (5)	+ (1) + (5)	+ (1) +/– (5)	– (1) +/– (5)	+ +			Kolman et al., 1994 Kolman and Dusinska, 1995
1,2-Propyleneimine 75-55-8	+ (1) + (2) + (3) +/– (5)								+	Heidelberger et al., 1983
Pyridine 110-86-1	– (1) – (3)	– (1) – (2*) – (3)		– (1)	– (1) ? (2)	? (2)	–			Kerckaert et al., 1996a
Reserpine 50-55-5	– (1) – (3)	– (1) # (2*)		– (1)	+ (1)	– (1)	 +		+ + 	Tu et al.,1986 Tsutsui et al., 1994 LeBoeuf et al., 1996
p-Rosaniline (Magenta) CI Basic Red 9 569-61-9	+? (1) – (2) +/– (3) +/– (5)	+ (2*) + (5)	– (1) – (6)	– (1) – (5)		– (1)			+ ?	Pienta, 1979 Heidelberger et al., 1983
Safrole 94-59-7	– (1) – (2)	+ (1) + (2*) +/– (3)	+/– (3)	– (1)		? (2)			+ + +	Pienta, 1980 Heidelberger et al., 1983 Barrett and Lamb, 1985
Sulfamethoxazole 723-46-6	– (1)						+*			Mauthe et al.,2001

Table 2: Rodent carcinogenic organic chemicals tested with the SHE cell transformation assay

Carcinogen CAS Number	Point Mutations			Cytogenetics (rodent and human cells)			SHE cell transformation			References SHE cell transformation assays
	Ames	Mammalian cells		In vitro	In vivo					
		Mouse Lymphoma TK	HPRT		Chrom. Ab.	Micro-nucleus	6.7	7.0	7.1-7.35	
Testosterone 58-22-0		? (2*)							– +(ST) +	Schiffmann et al., 1985 Lasne et al., 1990 Tsutsui et al., 1995
12-*O*-Tetradecanoyl phobol 13-acetate (TPA) 16561-29-8	– (2)		– (2[10])				+ + +(ST) + + +		– +(ST)	Rivedal and Sanner, 1980 Heidelberger et al., 1983 Mikalsen et al., 1990 Lasne et al.,1990 Bessi et al., 1995 Elias et al., 1996b Rivedal et al., 2000 LeBoeuf et al., 1996 Engelhardt et al., 2004
Tetrahydrofuran 109-99-9	– (1) – (3)	? (2*)		– (1)		– (1)	–			Kerckaert et al., 1996a
Thioacetamide 62-55-5	– (1) – (2) – (3)	+ (1) ? (2*) +/– (3)				+ (2)			+	Heidelberger et al., 1983
Thiourea 62-56-6	– (1) – (2) – (3)	+/– (1) eq (2*) +/– (3) + (4)				– (4)			+	Heidelberger et al., 1983
o-Toluidine 95-53-4	+ (1) ? (2) – (5)	+ (1) ? (2*) – (3) +/– (4) +/– (5)	+/– (3) +/– (5)	+ (1) +/– (5)		+/– (1) – (5)		+	+ +	Sanner and Rivedal,1985 Barrett and Lamb,1985
o-Toluidine HCl 636-21-5	+/– (1)			? (1)	? (1)	? (2)	+* +		+	Jones et al., 1988 Kerckaert et al., 1998 Engelhardt et al., 2004
2,4,6-Trichlorophenol 88-06-2	– (1) – (2)	+ (1) + (2*) +/– (3) + (4)		– (1)				+		Elias et al., 1998, 1999a

Table 2: Rodent carcinogenic organic chemicals tested with the SHE cell transformation assay

Carcinogen CAS Number	Point Mutations			Cytogenetics (rodent and human cells)			SHE cell transformation			References SHE cell transformation assays
	Ames	Mammalian cells		In vitro	In vivo					
		Mouse Lymphoma TK	HPRT		Chrom. Ab.	Micro-nucleus	6.7	7.0	7.1-7.35	
Tris(2-ethylhexyl)phosphate (TEHP) 78-42-2	– (1) – (3)	– (1) # (2*)		– (1)		– (1)	–			LeBoeuf et al., 1996
Urethane (ethyl carbamate) 51-79-6	+/– (1) – (2) – (3)	– (3) ? (2*)		– (1) + (3)		+ (1) + (2)	+		+	Heidelberger et al., 1983 Engelhardt et al., 2004
Wyeth-14,643 50892-23-4	– (3)						+ +		+	Tsutsui and al, 1993 LeBoeuf et al., 1996 Mauthe et al., 2001

(1) NTP database
(2) GENETOX database
(3) CCRIS database
(4) IUCLID database
(5) Genetic Activity Profile
(6) CICADS (IPCS)
(7) IARC Monographs
(8) Great Lakes Water Quality Agreement Genetic Activity Profiles
(2*) GENETOX Phase III: review (Mitchell *et al.,* 1997)
(2^{9}) GENETOX Phase III: HPRT/CHO (Li *et al.,* 1988)
(2^{10}) in GENETOX : HPRT/V79 (Bradley *et al.,* 1981)
(2^{11}) in GENETOX : HPRT/CHO (Hsie *et al.,* 1981)
(2^{12}) in GENETOX : (Mavournin *et al.,* 1990)
(13) Elias *et al.,* 1996
(14) Harvey *et al.,* 2005

(ST) Sequential treatments
\+ Positive result
+u Positive unconfirmed
– Negative result
+w Weakly positive result
? Inconclusive result
+? Positive at one concentration or at two non-consecutive concentrations
+/– Diverging results inside a database
Not testable
eq equivocal
* Short term treatment 24 hours or 30 minutes

Table 3: Non-carcinogenic and inconclusive for carcinogenicity organic chemicals tested with the SHE cell transformation assay

Chemical CAS Number	Point Mutations			Cytogenetics (rodent and human cells)			SHE cell transformation			References SHE cell transformation assays
	Ames	Mammalian cells		In vitro	In vivo					
		Mouse Lymphoma TK	HPRT		Chrom. Ab.	Micro-nucleus	6.7	7.0	7.1-7.35	
Acetone 67-64-1	– (1) – (2) – (3)	? (2*) – (4)		– (1) – (2)	– (2)	– (1) – (2) – (3)			–	Heidelberger et al., 1983
4-Acetylaminofluorene 28322-02-3	+ (2)	+/– (1) + (2*) +/– (3)		– (1)	– (1)	? (2)	– –		– –	Heidelberger et al.,1983 Tsutsui and Barrett, 1991 LeBoeuf et al., 1996 Engelhardt et al., 2004
Acid red 14 (IC) 3567-69-9	– (1) – (2)	– (1) – (2*)		– (1)		– (1)	+*/–			LeBoeuf et al., 1996
Acrylic Acid 79-10-7	– (1) – (3)	+ (2*) + (3) + (4)	– (3)						–	Wiegand et al., 1989
Ampicillin 7177-48-2	– (1) – (3)	– (2*) – (3)		– (1)			+*/–			Mauthe et al., 2001
Anilazine 101-05-3	– (1) – (3)	+ (1) # (2*) + (3)	– (3)	– (1)			–			LeBoeuf et al., 1996
p-Anisidine 104-94-9	+ (14)	+ (14)		+ (14)		– (14)	+			Harvey et al., 2004
Anthracene 120-12-7	+w (1) – (2) +/– (3)	+ (1) + (2*) + (3) +/– (4)		– (2)		? (2)	–		– – – –	Heidelberger et al., 1983 Tennant et al., 1985 Tu et al., 1986 Tsutsui and Barrett, 1991 LeBoeuf et al., 1996
l-Ascorbic acid 50-81-7	+w/– (1) ? (2) – (3)	? (1) # (2*)		– (1)	+ (1)	+ (1) ? (2)	–		–	Jones et al., 1988 LeBoeuf et al., 1996
Benzo(e)pyrene 192-97-2	+ (1) + (2) +/– (3)	+ (1) + (2*) +/– (3)	– (2^{10})						–	Heidelberger et al., 1983
Benzoin 119-53-9	+w/– (1) +/– (3)	+ (1) – (2*) +/– (3)	+/– (3)	– (1)	– (1)	– (1)		–	– –	Sanner and Rivedal, 1985 Barrett and Lamb, 1985 Jones et al., 1988

Table 3: Non-carcinogenic and inconclusive for carcinogenicity organic chemicals tested with the SHE cell transformation assay

Chemical CAS Number	Point Mutations			Cytogenetics (rodent and human cells)			SHE cell transformation			References SHE cell transformation assays
	Ames	Mammalian cells		In vitro	In vivo					
		Mouse Lymphoma TK	HPRT		Chrom. Ab.	Micro-nucleus	6.7	7.0	7.1-7.35	
Bisphenol A 80-05-7	– (1) – (3)	– (1) – (2*) – (3) – (4)		– (1)		– (1)	 –		– +	Jones et al, 1988 Tsutsui et al., 1998 LeBoeuf et al., 1996
HC Blue 2 33229-34-4	+ (1)	+ (1) ? (2*) + (3)		– (1)	– (1)		 –		–	Jones et al., 1988 Kerckaert et al., 1998
n-Butyl acrylate 141-32-2	– (1)	+ (3)		+ (1)				–		Wiegand et al., 1989
2-tert-Butyl-1,4-hydroquinone 1948-33-0	– (1) – (3)			+ (1)	– (1)	– (1)	–			Kerckaert et al., 1996a
Caffeine 58-08-2	– (1) – (2) – (3)	? (2*)	– (2^{10})	+ (2)	? (2)	+ (2)			– –	Doniger and DiPaolo,1981 Heidelberger et al., 1983
Caprolactam 105-60-2	– (1) – (3)	– (1) – (2*) – (3) – (4)	– (2^{9}) – (3)	– (1)	– (1)	– (1) – (4)	 –	?	 +w +	Sanner and Rivedal, 1985 Barrett and Lamb, 1985 Jones et al., 1988 LeBoeuf et al., 1996
Chloramphenicol 56-75-7	– (2)								? – –	Jones et al., 1988 Tsutsui and Barrett, 1991 Suzuki, 1987
o-Chloroaniline 95-51-2	– (1) – (3)	+ (1) + (2*) + (3) + (4)				+/– (1)			–	Heidelberger et al., 1983
Colcemid (IC) 477-30-5	– (3)					+ (2)	 +		+	Tsutsui et al., 1984b Gibson et al., 1995
Cytosine arabinoside 147-94-4	– (3)	+ (2*) + (3)	+ (2^{10}) +/– (3)	+ (2)		+ (2)			– +	Hirakawa et al., 1979 Heidelberger et al., 1983
2,6-Diaminotoluene 823-40-5	+ (3)						– – –			LeBoeuf et al., 1996 Kerckaert et al., 1998 Engelhardt et al., 2004

Table 3: Non-carcinogenic and inconclusive for carcinogenicity organic chemicals tested with the SHE cell transformation assay

Chemical CAS Number	Point Mutations			Cytogenetics (rodent and human cells)			SHE cell transformation			References SHE cell transformation assays
	Ames	Mammalian cells		In vitro	In vivo					
		Mouse Lymphoma TK	HPRT		Chrom. Ab.	Micro-nucleus	6.7	7.0	7.1-7.35	
Diazepam 439-14-5	– (1) ? (2) – (3)			– (1)		? (2)	+*/–			Gibson et al., 1995
2,4-Dichlorophenol 120-83-2	? (1) ? (2) – (3)	+ (1) ? (2*) + (3) + (4)	+ (3)	– (1)				+w		Elias et al., 1998, 1999a
2,4-Dichlorophenoxyacetic acid 94-75-7	– (1) – (2) – (3)		+ (2^{10}) + (3)	+ (1) + (2)	+ (2)	? (2)		–		Mikalsen et al., 1990
2,4-Dimethoxyaniline HCl 54150-69-5	+ (1)	+ (1) ? (2*)		+ (1)		– (1)	–			Kerckaert et al., 1998
Dimethylformamide 68-12-2	– (1) – (3)	+/– (1) – (2*) – (3) +/– (4)		– (1)		? (2)			–	Heidelberger et al., 1983
Ethylenediaminetetracetic acid (EDTA) 60-00-4		+ (3) ? (2*)					–			Engelhardt et al., 2004
Ethylenediaminetetracetic acid, trisodium salt 150-38-9	– (1) – (3)	– (1) – (2*)		– (1)			–		– –	LeBoeuf et al.,1996 Fukuda, 1987 Tsutsui and Barrett., 1991
Eugenol 97-53-0	– (1)	+ (1) + (2*) + (3)		+ (1)	? (1)	– (1) + (2)	 –		+ +	Fukuda, 1987 Tsutsui and Barrett, 1991 Engelhardt et al., 2004
Geranyl acetate 105-87-3	– (1)	+ (1) # (2*)		– (1)	– (1)	– (1)			+	Jones et al., 1988
8-Hydroxyquinoline 148-24-3	+ (1) + (2)	+ (1) + (2*)		+w (1)	– (1)	– (1) ? (2)		–		Elias et al., 1998, 1999a
Isobutyraldehyde 78-84-2	–/ ? (1) – (3)	+ (1) + (3)		+ (1)		– (1)	–			Kerckaert et al., 1996a

Table 3: Non-carcinogenic and inconclusive for carcinogenicity organic chemicals tested with the SHE cell transformation assay

Chemical CAS Number	Point Mutations			Cytogenetics (rodent and human cells)			SHE cell transformation			References SHE cell transformation assays
	Ames	Mammalian cells		In vitro	In vivo					
		Mouse Lymphoma TK	HPRT		Chrom. Ab.	Micro-nucleus	6.7	7.0	7.1-7.35	
Lithocholic acid 434-13-9	– (1) – (3)	+/– (1) ? (2*) +/– (3)		– (1)					+ ?	Kelsey and Pienta, 1981 Heidelberger et al., 1983
d-Mannitol 69-65-8	– (1) – (3)	– (1) – (2*)		– (1)	– (1)	– (1)	– –			LeBoeuf et al., 1996 Mauthe et al., 2001
6-Mercaptopurine 50-44-2	+/– (3)	+ (2*)	+ (3)	+ (1)	+ (2)	+ (2)			–	Klein et al., 1990
Methotrexate 59-05-2	? (2) – (3)	+ (2*) + (3)	– (3)	+ (2)	+ (2)	+ (2)			– –	Heidelberger et al., 1983 Klein et al., 1990
Methoxychlor 72-43-5	– (1) – (2)	+ (1) # (2*) +/– (3)	– (3)	– (1)			+ +		–	Heidelberger et al., 1983 LeBoeuf et al., 1996 Engelhardt et al., 2004
1-Naphthylamine 134-32-7	+ (1) + (2) +/– (3)	? (2*)		+ (1) +/– (3)		+ (2) + (2^{12})	–		–	Heidelberger et al., 1983 Engelhardt et al., 2004
Naphthylisothiocyanate 551-06-4	+ (1)								–	Pienta, 1980
4-Nitro-*o*-phenylenediamine 99-56-9	+ (1) + (2) +/– (3)	+ (1) eq (2*) + (3)	– (3)	– (1) + (2)	– (1)	eq (1) ? (2)	– –		+ +	Pienta and Kawalek, 1981 Heidelberger et al., 1983 LeBoeuf et al., 1996 Engelhardt et al., 2004
1-Nitropropane 108-03-2	– (1)		+ (3)			? (2)		+(ST)		Elias et al., 1996b
3-Nitropropionic acid 504-88-1	+ (1) +/– (3) +/– (5)	+ (1) # (2*) + (3) + (5)	– (3) – (5)	+w (1)			–			LeBoeuf et al., 1996
Pentoxifylline 6493-05-6	– (3)	– (3)	+ (3)				–			Slamenova et al., 1994
Phenanthrene 85-01-8	+w (1) – (2) +/– (3)			– (2)		? (2)			–	Heidelberger et al., 1983
Phenol	– (1)	+ (1)	– (3)	+ (1)	+ (1)	+ (1)			+	Tsutsui et al., 1997a

Table 3: Non-carcinogenic and inconclusive for carcinogenicity organic chemicals tested with the SHE cell transformation assay

Chemical CAS Number	Point Mutations			Cytogenetics (rodent and human cells)			SHE cell transformation			References SHE cell transformation assays
	Ames	Mammalian cells		In vitro	In vivo					
		Mouse Lymphoma TK	HPRT		Chrom. Ab.	Micro-nucleus	6.7	7.0	7.1-7.35	
108-95-2		# (2*) + (3) ? (4)		+ (4)		? (2) ? (4)		+w (ST)		Elias et al.,1998, 1999a
p-Phenylenediamine dihydrochloride 624-18-0	+ (1)	+ (1) eq (2*) + (3)	– (3)	+ (1)			–			Kerckaert et al., 1998
Phtalic anhydride 85-44-9	– (1) – (3)	+ (1)		– (1)			–	 – –		LeBoeuf et al., 1996 Elias et al,1996b Dhalluin et al.,1998
Pyremethamine (IC) 58-14-0	– (1) ? (2)			+ (1)			+*			LeBoeuf et al., 1996
Pyrene 129-00-0	+/– (1) – (2) +/– (3)	+ (1) + (2*) + (3)	– (2^{10}) – (3)	– (1) – (2)	– (1)	? (2)			– – –	Heidelberger et al., 1983 Tu et al., 1986 Tennant et al., 1985
HC Red 3 2871-01-4	+ (1)	+ (1)		+ (1)					–	Jones et al., 1988
Resorcinol 108-46-3	– (1) – (2)	+ (1) + (2*) + (3) + (4)		+ (1)		+ (1) ? (2)	–	+w		Elias et al., 1998, 1999a Harvey et al., 2005
Rotenone 83-79-4	– (14)	+ (14)		+ (14)		+ (14)	+			Harvey et al., 2005
Saccharin 81-07-2	– (1)	? (2)							– –	Pienta, 1980 Heidelberger et al., 1983
Sodium xylenesulfonate 1300-72-7	– (1) – (3)	? (1) – (3)		– (1)			–			Kerckaert et al., 1996a
Sorbic acid 110-44-1	– (1)		– (3)						–	Schiffmann and Schlatter, 1992
Succinic anhydride 108-30-5	– (1) – (2) – (3)	? (2*)		– (1)					+	Heidelberger et al., 1983
Sulfisoxazole 127-69-5	– (1) – (3)	+ (1) ? (2*)		– (1)		– (1)	–			Custer et al., 2000

Table 3: Non-carcinogenic and inconclusive for carcinogenicity organic chemicals tested with the SHE cell transformation assay

Chemical CAS Number	Point Mutations			Cytogenetics (rodent and human cells)			SHE cell transformation			References SHE cell transformation assays
	Ames	Mammalian cells		In vitro	In vivo					
		Mouse Lymphoma TK	HPRT		Chrom. Ab.	Micro-nucleus	6.7	7.0	7.1-7.35	
Tetracycline HCl 64-75-5	– (1) – (3)	? (1) – (2*) +/– (3)		– (1)					– –	Suzuki, 1987 Tsutsui and Barrett, 1991
Tetraethylthiuramdisulfide 97-77-8	– (1)	+ (1) eq (2*) + (3)		+ (1)		? (2)	+			LeBoeuf et al., 1996
Thiabendazole 148-79-8	+ (1)					+ (2)	–			Gibson et al., 1995
2,4,5-Trichlorophenoxyacetic acid 93-76-5	– (1) – (3)			+ (1)	+ (2)	? (2)		–		Mikalsen et al 1990
Triphenyltin Hydroxide 76-87-9	– (1) – (3)	+ (1)	– (3)	– (1)			–			LeBoeuf et al., 1996
Vincristine sulfate (IC) 2068-78-2	– (3)								+ + +	Hirakawa et al., 1979 Tsutsui et al., 1986 Klein et al., 1990
FD and C Yellow n°6 2783-94-0	– (1)	+ (1) – (2*)		– (1)	– (1)	– (1)			–	Jones et al.,1988

NTP database
GENETOX database
CCRIS database
IUCLID database
Genetic Activity Profile
(2*) GENETOX Phase III: review (Mitchell *et al.,* 1997)
(2^{9}) GENETOX Phase III: HPRT/CHO (Li *et al.,* 1988)
(2^{10}) in GENETOX : HPRT/V79 (Bradley *et al.,* 1981)
(2^{12}) in GENETOX : (Mavournin *et al.,* 1990)
(14) Harvey *et al*., 2005

(ST) Sequential treatments
– Negative result
+w Weakly positive result
? Inconclusive result
+/– Diverging results inside a database
IC Inconclusive for carcinogenicity
Not testable
eq equivocal
+ Positive result
* Short term treatment 24 hours or 30 minutes

Table 4: Inorganic compounds tested with the SHE cell transformation assay and classification for carcinogenicity

Inorganic compound CAS number	Point mutations			Cytogenetics (rodent and human cells)			Carcinog. Class.	SHE cell transformation			References SHE cell transformation assay
	Ames	Mammalian cells		*in vitro*	*in vivo*						
		Mouse Lymphoma TK	HPRT		Chrom. Ab.	Micro nucleus		6.7	7.0	7.1-7.35	
Amosite 12172-73-5	– (6)		+w (6)	+ (6)			+ (8)		+	 +	Mikalsen et al., 1988 DiPaolo et al., 1983
Barium chromate 10294-40-3	– (5)						IE (8)			+w	Elias et al., 1989
Beryllium sulfate 13510-49-1	– (1) – (9)			+/– (9)		– (9)	+ (8)			+	Heidelberger et al., 1983
Beryllium sulfate tetrahydrate 7787-56-6	– (1) – (3)						+ (8)			+ +	Heidelberger et al., 1983 Klein et al., 1990
Cadmium acetate 543-90-8			+ (3)				+ (8)		+	 +	Rivedal and Sanner, 1981 Heidelberger et al., 1983
Cadmium chloride 10108-64-2	– (1) ? (2) – (3)	+ (1) ? (2*)	+ (3)	+ (1) – (2)		– (2)	+ (8)	 +		+ +	Pienta, 1980 Swierenga and Yamasaki, 1992 Gibson et al., 1995
Calcium chromate 13765-19-0	+ (1) + (2) + (5)	+ (1) + (2*) + (3)	+ (3) + (5)	– (1) + (5)		– (5)	+ (8)			+ + +	Heidelberger et al., 1983 Lanfranchi et al., 1988 Elias et al.,1991
Chromium [III] chloride 10025-73-7	? (2) – (5)		+ (3)	+ (5)	– (2)		IE (8)		–		Rivedal and Sanner, 1981
Chromium [III] oxide 1308-38-9	– (5)		+ (3) + (5)				IE (8)			+	Elias et al., 1982
Chromium (metallic) 7440-47-3					+ (5)		IE (8)			+	Coombs et al., 1989
Chromium trioxide 1333-82-0	+ (2) + (3) + (5)			+ (2) + (5)	+ (5)		LE (8)			+	Elias et al., 1982
Chrysotile 12001-29-5	– (6)		+w (6)	+ (6)			+ (8)	 +	+ +	 + + +	Mikalsen et al., 1988 Elias et al.,1996 b DiPaolo et al., 1983 Hersterberg and Barrett, 1984 Tsutsui and Barrett, 1991 Pang et al., 1991 Gibson et al., 1995
cis-Platinium diammine	+ (1)	+ (2*)	+ (2[9])	+ (1)		+ (2)	+ (8)			+	Heidelberger et al., 1983

Table 4: Inorganic compounds tested with the SHE cell transformation assay and classification for carcinogenicity

Inorganic compound CAS number	Point mutations			Cytogenetics (rodent and human cells)			Carcinog. Class.	SHE cell transformation			References SHE cell transformation assay
	Ames	Mammalian cells		*in vitro*	*in vivo*						
		Mouse Lymphoma TK	HPRT		Chrom. Ab.	Micro nucleus		6.7	7.0	7.1-7.35	
dichloride Cisplatin 15663-27-1	+ (2) +/– (3)		+ (3)							+ +	Popescu et al., 1985 Klein et al., 1990
Cobalt sulfate heptahydrate 10026-24-1	+w						+ (8)	+*			Kerckaert et al.,1996 (a, b)
Cristobalite 14464-46-1	– (3)						+ (8)		+		Elias et al., 1996c; 2000
Crocidolite 12001-28-4	– (3)		+/– (3)	+ (6)			+ (8)		+	+ +	Mikalsen et al., 1988 Hersterberg and Barrett, 1984 DiPaolo et al., 1983
Diatomaceous earths 68855-54-9	– (3)						IE (8)		+ +		Elias et al., 1996c; 2000; Elias et al., 2002a
Ferric oxide 1309-37-1	– (3)						IE (8)			–	DiPaolo and Casto, 1979
Gallium arsenide 1303-00-0	– (1) – (3)					– (1)	ND	+*			Kerckaert et al., 1996(a,b)
Glass fibre 100 No CAS	– (6)			+ (6)			LE (8)		+	+	Mikalsen et al., 1988 Hesterberg and Barrett, 1984
Glass fibre 110 No CAS	– (6)			+/– (6)			LE (8)		–	+	Mikalsen et al.,1988 Hersterberg and Barrett, 1984
Hydrogen peroxide 7722-84-1	? (2) +/– (3) + (9)	+ (2*) + (3) + (9)	+ (3)	+ (9)	+ (9)		+ (10)	+ 	+		Mikalsen et al., 1990 Leboeuf et al., 1996
Lead acetate 301-04-2	– (1)	– (1) # (2*) – (3)	+ (3)	– (2)	– (2)	– (1) + (2)	+ (8)	+ 		+	Heidelberger et al., 1983 LeBoeuf et al., 1996
Lead chromate 7758-97-6	+/– (if solubilized) (5)		– (3)	+ (5)		+ (5)	+ (8)			+ + + +	Heidelberger et al., 1983 Tu et al , 1986 Tennant et al ., 1985 Elias et al., 1991
Lead molybdenum chromates [a] 12709-98-7	+/– (if solubilized) (5)			+ (5)			+ (5)			+	Elias et al., 1989
Lead sulphate chromates [b]	+/– (if			+ (5)			+ (5)			+	Elias et al., 1989

Table 4: Inorganic compounds tested with the SHE cell transformation assay and classification for carcinogenicity

Inorganic compound CAS number	Point mutations			Cytogenetics (rodent and human cells)			Carcinog. Class.	SHE cell transformation			References SHE cell transformation assay
	Ames	Mammalian cells		*in vitro*	*in vivo*						
		Mouse Lymphoma TK	HPRT		Chrom. Ab.	Micro nucleus		6.7	7.0	7.1-7.35	
7752-97-6	solubilized) (5)										
Lead silicochromate basic[c] 1344-38-3			+ (5)				+ (8)			+	Elias et al., 1989
Molybdenum trioxide 1313-27-5	– (1) – (3)	+ (3)		– (1)			+	+*			Kerckaert et al., 1996(a,b)
Nickel (metallic) 7440-02-0	– (3)						+ (8)			+	Ping et al., 1988
Nickel chloride 7718-54-9	– (3)	+ (2*)	+ (3)	+ (5)	+ (5)	– (5)	+ (8)			+ + +	Conway et al., 1987 Zhang and Barrett, 1988 Ping et al., 1988
Nickel monoxide 1313-99-1	– (1)		+ (3)			– (1)	+ (8)			+	Sunderman et al.,1987
Nickel subsulfide 12035-72-2	? (1) – (3)		+ (3)			– (1)	+ (8)			+ + + +	DiPaolo and Casto, 1979 Costa and Mollenhauer, 1980 Costa et al., 1981 Sunderman et al., 1987
Nickel sulfate 7786-81-4	– (5)		+ (5)	+ (5)	– (5)		+ (8)		+ +	 + +	Rivedal and Sanner, 1981 Mikalsen et al., 1990 Pienta, 1980 Zhang and Barrett, 1988
Nickel sulfate heptahydrate 10101-98-1		+ (2*)					+ (8)	+			Kerckaert et al., 1996b
Nickel sulfate hexahydrate 10101-97-0	– (1)	+ (1) + (3)	+ (3)				+ (8)			+ +	DiPaolo and Casto, 1979 Heidelberger et al., 1983
Nickel sulfide(crystalline) 16812-54-7			+ (5)	+ (5)			+ (8)			+ + +	Costa et al., 1982 Ping et al., 1988 Sunderman et al., 1987
Potassium chromate 7789-00-6	+ (2)	+ (2*) + (5)	+ (3)	+ (5)		+ (2) + (5)	+ (8)		+	 + +	Rivedal and Sanner, 1981 Elias et al., 1982 Conway et al., 1987
Potassium dichromate 7778-50-9	+ (1)	+ (2*) + (5)	+ (2^{9}) + (5)	+ (2) + (5)	+ (5)	+ (2) + (5)	+ (8)			+ + +	Tsuda and Kato, 1977 Elias et al., 1982 Heidelberger et al., 1983

Table 4: Inorganic compounds tested with the SHE cell transformation assay and classification for carcinogenicity

Inorganic compound CAS number	Point mutations			Cytogenetics (rodent and human cells)			Carcinog. Class.	SHE cell transformation			References SHE cell transformation assay
	Ames	Mammalian cells		*in vitro*	*in vivo*						
		Mouse Lymphoma TK	HPRT		Chrom. Ab.	Micro nucleus		6.7	7.0	7.1-7.35	
										+ +	Hansen and Stern, 1985 Lanfranchi et al., 1988
Quartz Min-U-Sil5 14808-60-7	– (8)		– (8)	+ (8)		– (8)	+ (8)		+	 +	Elias et al., 1996c; 2000;2002a Hersterberg and Barrett, 1984
Refractory ceramic fibre-1,2,3,4 No CAS Nb			– (8)	+ (8)		– (8)	+ (8)		+		Elias et al., 1997,1999b, 2002b
Sodium arsenate 7778-43-0		+ (2*)					+ (8)			+ + +	DiPaolo and Casto, 1979 Heidelberger et al., 1983 Lee et al., 1985
Sodium arsenite 7784-46-5	– (3)	+ (2*) + (3)	? (2[9])			+ (2)	+ (8)			+ +	DiPaolo and Casto, 1979 Lee et al., 1985
Sodium chromate tetrahydrate 10034-82-9	+ (1) +/– (3)						+ (8)			+	DiPaolo and Casto, 1979
Sodium dichromate 10588-01-9	+ (2) + (3) + (5)	+ (7)		+ (5)	+ (5)		+ (8)			+	Elias et al., 1982
Sodium fluoride 7681-49-4	– (1) ? (2) – (3)	+ (1) # (2*) + (3) + (4)	– (3)	+/– (1)	– (1)	– (1) + (2)	EE (1)	+* /-	+/–	 + + +	LeBoeuf et al., 1996 Rivedal et al., 2000 Lasne et al., 1988 Jones et al., 1988 Tsutsui et al, 1984c
Sodium nitrite 7632-00-0	+ (1) + (2)	+ (3) ? (2*)		+ (3)	+ (2)	– (1) + (2) + (10)		+*		+	Heidelberger et al., 1983 Kerckaert et al., 1996b
Sodium o-vanadate 13721-39-6					– (10)	+ (10)	ND		+	 +	Rivedal et al., 1990 Afshari et al., 1993
Strontium chromate 7789-06-2	+ (5)						+ (8)			+	Elias et al., 1991

Table 4: Inorganic compounds tested with the SHE cell transformation assay and classification for carcinogenicity

Inorganic compound CAS number	Point mutations			Cytogenetics (rodent and human cells)			Carcinog. Class.	SHE cell transformation			References SHE cell transformation assay
	Ames	Mammalian cells		*in vitro*	*in vivo*						
		Mouse Lymphoma TK	HPRT		Chrom. Ab.	Micro nucleus		6.7	7.0	7.1-7.35	
Titanium dioxide 13463-67-7	– (1) – (3)	– (1) # (2*) – (4)		– (1)	– (1)	+ (1)	LE (8)	–		–	LeBoeuf et al.,1996 Mikalsen et al., 1988
Titanocene dichloride 1271-19-8	+ (1)			– (1)			–			+	Heidelberger et al., 1983
Tremolite 14567-73-8	– (3)			+(6)			+ (8)			+	Athanasiou et al., 1992
Vanadium (V) pentoxide 1314-62-1	– (1) – (3)	– (3)	– (3)			– (1)	+ (8)	+	 +		Kerckaert et al., 1996(a,b) Rivedal et al., 1990
Zinc chloride 7646-85-7	+ (2)			– (2)			ND	+	 + –		LeBoeuf et al., 1996 Alexandre et al., 2003 Rivedal and Sanner, 1981
Zinc chromate 13530-65-9	+ (3)		+ (5)	+ (5)			+ (8)			+	Elias et al., 1991
Zinc oxide 1314-13-2	– (3)	+ (3) + (4)					ND			+	Suzuki, 1987
Zinc potassium chromates[d] 11103-86-9	+ (1)					+ (1)	+ (8)			+	Elias et al., 1989

a: industrial “molybdate red” or “molybdate orange” pigments;
b: industrial “chromium yellow” pigments;
c : industrial “chromium orange” pigments;
d: industrial “zinc yellow” pigments.
(1) NTP database
(2) GENETOX database
(2*) GENETOX Phase III: review (Mitchell *et al.,* 1997)
(2^{9}) GENETOX Phase III: HPRT/CHO (Li *et al.,* 1988)
(3) CCRIS database
(4) IUCLID database
(5) IARC monographs on the evaluation of carcinogenic risks to humans. Vol 49: Chromium, Nickel and Welding, 1990, IARC, Lyon, France
(6) Kane AB, Boffetta P., Saracci R., Wilbourn J.D. (eds), Mechanisms of Fibre Carcinogenesis. IARC Sc Publ. No140, 1996
(7) Oberly *et al*., J. Toxicol Environ Health, 1982, 9, 367-376
(8) IARC database
(9) Genetic Activity Profiles
(10) Ciranni *et al.,* 1995
ND No data
EE Equivocal evidence
IE Inadequate evidence
LE Limited evidence
+ Positive result
+w Weakly positive result
– Negative result
? Inconclusive result
+/– Diverging results inside a database
Not testable
eq equivocal
* Short term treatment 24 hours or 30 minutes

The BALB/c 3T3 cell transformation assay

State of the art

61. The chemical transformation of 3T3 cells resulting in the induction of morphologically aberrant foci was reported by Kakunaga and Kamahora (1970) who derived subclones from the Aaronson and Todaro 3T3 clone A31 line (1968). The cell lines originated from inbred BALB/c mouse embryo cultures (Aaronson and Todaro, 1968). Later, more extensive studies on the use of cloned 3T3 lines for the quantitative analysis of chemical induction of neoplastic transformations were published (Di Paolo *et al.,* 1972b; Kakunaga, 1973).

62. The subclone A31-714 was used to develop an optimised protocol for the basic transformation assay described by Kakunaga (1973) and served as the basis for 3T3 cell transformation assays employing the currently available A 31 subclones. Several subclones designated A31-1, A31-11, A31-13 have been generated for cell transformation assays. Kakunaga and Crow (1980) established an A31-1-1 clone that is chemical, as well as UV, sensitive which they recommended for use for the cell transformation assay. These clones may differ in their inherent ability to metabolize polycyclic aromatic hydrocarbons (PAHs), and their spontaneous transformation frequencies (Schechtman, 1985b).

63. Like all established mouse cell lines (*e.g.,* L929, A9), BALB/c 3T3 is aneuploid and relatively unstable regarding its karyotype, and other properties such as sensitivity to contact inhibition. Among the advantages of these system assays is the fact that transformation is in most cases associated with malignancy.

Principle of the test

64. The BALB/c 3T3 cell line, like C3H10T1/2, has been shown to be sensitive to tumour-promoting agents (Mondal and Heidelberger, 1976). This is utilized both for enhancing the sensitivity of cells to the transforming potential of chemicals (by subsequent treatment with known tumour-promoting agents) and for screening environmental tumour-promoting agents (Kakunaga, 1985).

65. The original procedure developed by Kakunaga (1973) and recommended by the IARC/NCI/EPA Working Group (1985) is currently used. The cell transformation assay consists of plating target cells expanded from frozen stocks at 10^4 cells per 60-mm dishes in Eagle's minimum essential medium (MEM) containing 10% fetal calf serum. After a 24h period necessary for attachment, the cells are treated for a 3-day exposure period. Cells are cultured for 4-6 weeks with one or two medium changes each week. Then, cells are fixed, stained with Giemsa and scored for morphologically transformed foci. Transformation frequency is expressed as a number of plates with transformed focus among total plates and also an average number of transformed foci per plate.

66. In addition, the cloning efficiency of the treated and control populations (100-500 cells of each treatment set in 60-mm dishes) is determined in the same experimental conditions, carrying these cultures for 7-10 days with renewal of medium similar to the cell transformation assay protocol (IARC/CNI/EPA Working group, 1985).

67. Improvements to the basic protocol were proposed to increase cell transformation frequency, and to shorten the length of the assay:

- Due to the limited metabolic activity associated with BALB/c 3T3 cells, some authors recommended adding an exogenous metabolic activation system, an S9 subcellular enzyme fraction, when non-direct acting chemicals were tested (Schechtman, 1985a). A feeder-layer of X- irradiated primary Rous leukemia (RLC) cells has been used (Matthews *et al.,* 1985).

- The 3T3 cell amplification-transformation (level-II) assay was proposed as an alternative strategy, as in the C3H10T1/2 cell transformation assay. The method consists in harvesting and replating the cells at confluency after chemical treatment (Schechtman, 1985b).

- Matthews et al. (1993a, 1993b) modified the classical assay procedure: the number of seeded cells was increased from 10,000 to 32,000 cells per dish, the cell treatment started 2 days later, instead of the one day in the original procedure, and lasted 48h instead of 72h, so that the number of treated cells was 3 times the number of cells treated in the classical assay. No exogenous metabolic activation system was added to the cells. However, transformation frequency (foci/dish) is reported to be independent on the number of cells plated (Kennedy *et al.,* 1980; Fernandez *et al.,* 1980; Kennedy and Little, 1984; Umeda and Ono, 1986).

- In order to increase the sensitivity of the cell system, a sequential treatment of the cells using TPA as a tumour-promoter was employed (Matthews *et al.,* 1985). After chemical treatment, the test medium was replaced by TPA-added medium continuously applied to the cells until the end of the experiment. A two-stage method was also recommended by Sakai and Sato (1989). This method is now classically used by the Japanese group: the cells are first treated with a known or suspected carcinogen for 72h, cultured in a normal medium for 3 days, exposed in medium with and without TPA for 2 weeks, and cultured in normal medium for an additional 3 weeks.

- Tsuchiya and Umeda (1995) proposed the use of a supplemented medium, the so-called ITES medium containing insulin, transferrin, ethanolamine and sodium selenite, plus 2% FCS, which, in conjunction with a 2 stage protocol, substantially increased the transformation frequency under exposure to chemical carcinogens (Tsuchiya and Umeda, 1997; Fang *et al.,* 2001). Several validation studies confirmed the usefulness of the modified two-stage transformation assay with BALB/c 3T3 cells (Tsuchiya *et al.,* 1999; Kajiwara and Ajimi, 2003; Umeda, 2004). More recently it was shown that ITES could be replaced by insulin and use of 90-mm dishes instead of 60-mm dishes was efficient for practical management. This modified protocol is used for the current international validation study conducted by EC-ECVAM.

- Bhas 42 cells (v-H-ras-transfected BALB/c 3T3 cells) has been introduced as a sensitive cell transformation method that could detect both tumor initiators and promotors distinguishably based on protocols (Ohmori *et al.,* 2004; Asada *et al.,* 2005).

Results

68. The data set of BALB/c 3T3 results (186 chemicals) comprises:

- 165 organic chemicals

  112 classified as rodent carcinogens (table 5)
  53 as rodent non-carcinogens (table 6)

- 21 inorganic chemicals

  15 rodent carcinogens and 6 non-carcinogens (table 7)

Table 5 is restricted to the chemicals (n=110) with BALB/c 3T3 and genotoxicity results available. The chemicals used in the analysis of the performances of the BALB/c 3T3 assay to predict carcinogenicity are presented in table 11 (112 rodent organic carcinogens including PDD and vinclozoline), table 12 (non-carcinogenic and inconclusive organic chemicals) and table 13 (inorganic chemicals,

carcinogens and non-carcinogens).Benzidine and benzidine dihydrochloride were considered as one chemical in the evaluation, as well as 2,6-diaminotoluene and 2,6-diaminotoluene-2HCl.

Rodent carcinogens

69. 66 % of the organic carcinogens and 87% of the inorganic carcinogens are positive in the BALB/c 3T3 assay. Asbestos (crocidolite), silica quartz, potassium chromate, sodium arsenite and arsenate, and vanadium pentoxide are positive. On the other hand, titanium dioxide, a weak carcinogen by inhalation at high doses but negative in NCI studies, gives a negative result in the BALB/c 3T3 system. Examples of organic compounds inducing foci of transformed cells are: aromatic amines, estradiol, diethylstilbestrol, ethyl alcohol, the carcinogenic polycyclic aromatic hydrocarbons, nitrilotriacetic acid, *N*-nitroso-compounds, alkylating agents etc.

70. A number of tumour promoters have been tested using the BALB/c 3T3 cell system by the Japanese group (Sakai and Fujiki, 1991; Sakai, 1997, 2001; Sakai *et al.,* 1997; Sakai and Teshima, 2001; Umeda, 2004): anthralin, BHA, BHT, catechol, okadaïc acid and the phorbol esters PDD and TPA. Positive responses were obtained using a two-stage protocol and the ITES medium.

71. The BALB/c 3T3 assay failed to identify as positive the following chemicals: 2-aminoanthracene, clofibrate, butylbenzylphthalate, DEHP, 1,2-epoxybutane, monuron, procarbazine, ethinyl estradiol, d-limonene, TEHP, 2,4-dinitrotoluene. The chlorinated aliphatic hydrocarbons – chloroethane, dichloroethane, hexachloroethane, and 1,1,1,2-tetrachloroethane were negative in the study of Tu *et al.,* (1985) conducted without metabolic activation : the negative result can be explained by a deficiency in metabolizing capacities of the BALB/c 3T3 cells.

72. Inconclusive and divergent results were registered with the following chemicals : benzanthracene, benzene, cyclophosphamide, NDEA, progesterone and p-rosaniline, 1,1,2,2-tetrachloroethane and tetrachloroethylene.

Rodent non-carcinogens

73. A number of false positives were registered. These included:

- the inorganic chemicals: barium and chromium III chlorides, sodium fluoride, sodium o-vanadate and titanocene dichloride..

- the organic compounds: acetone, 1-naphthylamine, naphthyl isothiocyanate 3-nitropropionic acid, penicillin VK, phenol, p-phenylediamine 2HCl, propyl gallate, HC Red 3, 2,6-toluenediamine 2 HCl, 1,1,1-trichloroethane and disulfiram, all of which were non carcinogenic in the NCI/NTP studies (Gold and Zeiger, 1997, CCRIS or NTP databases), but phenol is classified as tumor promoter by IARC.

74. A number of chemicals behave as promoter-like with the two-stage and improved method developed by Tsuchiya and Umeda (1995):

- 3-aminobenzamide, β-carotene, the biliary lithocholic acid, and 2,6-diaminotoluene;

- the positivity of β-carotene may be surprising, but may be in relation with the toxic effects of carotenoids on development at high doses;

- 3-aminobenzamide (3-AB) is an inhibitor of polyADP-ribose polymerase (PARP) which plays a crucial role in DNA metabolism and especially in DNA repair. Inhibition of DNA repair may

explain why 3-AB treatment produces morphological tranformation in cells pre-initiated by a genotoxic carcinogen (Konishi *et al.,* 1983); and,

- the tumor-promoting activity of the biliary acids is in line with the fact that these endogenous substances are suspected to play a role in the etiology of colorectal cancer (Taguchi, 1995; Hirose *et al.,* 2001).

Chemicals not evaluated for carcinogenicity and non included in the data set

75. Several chemicals, not yet evaluated for rodent carcinogenicity, have been tested on BALB/c 3T3 cells:

- p-nonylphenol (25154-52-3) and sodium o-vanadate (13721-39-6 were found positive by Sakai, (2001).

- MEHP (4376-20-9) was found equivocal by Matthews *et al.,* (1993a).

- dimethyl sulfoxide (67-68-5) was found positive by Matthews *et al.,* (1993b) and negative by Kajiwara *et al.,* (1997).

- vanadium IV oxide (12036-21-4) was found negative (Sabbioni *et al.,* 1991)

Table 5: Rodent carcinogenic organic chemicals tested with the BALB/c 3T3 cell transformation assay

Carcinogen CAS Number	Point mutations			Cytogenetics (rodent and human cells)			BALB/c 3T3 cell transformation	References BALB/c 3T3 cell transformation assays
	Ames	Mammalian cells		*In vitro*	*in vivo*			
		Mouse Lymphoma TK	HPRT		Chrom. Ab.	Micro nucleus		
2-Acetylaminofluorene 53-96-3	+ (2)	+ (1) + (2*) + (3)	+ (2^9) + (3)	+ (1)	+ (1)	+ (2)	+* +	Heidelberger et al., 1983 Matthews et al., 1993b
Acrylamide 79-06-1	–(1) –(3) –(5)	+ (2*) + (3) + (5)		+ (1) + (5)	+ (1) +/– (2) +/– (5)	+ (5)	+	Tsuda et al., 1993
Acrylonitrile 107-13-1	+ (1) + (2) +/– (5)	+ (1) + (2*) – (4) + (5)	+ (5)	+ (1) +/– (5)	– (5)	– (1) – (2) – (5)	+/– +w	Matthews et al., 1985 Matthews et al., 1993b
Actinomycin D 50-76-0	– (3)	+ (2*) + (3)	+ (2^9)	+ (2)		+ (1) ? (2)	+ +	Heidelberger et al., 1983 Lubet et al., 1984
Aflatoxin B_1 1162-65-8	+ (1) + (3) + (5)	+ (3)	+ (2^9) + (3) + (5)	+ (5)	+ (2) +/– (5)	+ (2)	+ + + +	Di Paolo et al., 1972b Cortesi et al., 1983 Friedrich et al., 1985 Lubet et al., 1990
Allyl isovalerate 2835-39-4	– (1) – (3)	+ (1)		+ (1)	– (1)		–	Matthews et al., 1993b
Allyl isothiocyanate 57-06-7	+/– (1) +/– (3)	+ (1) ? (2*)		+ (1)	eq	– (1)	+	Matthews et al., 1993b
3-Amino-1,4-dimethyl-5*H*-pyrido[4,3-*b*]indole (Trp-P-1) 68808-54-8	+ (2) + (3)						+	Sakai and Sato, 1989
3-Amino-1-methyl-5*H*-pyrido[4,3-*b*]indole (Trp-P-2) 72254-58-1	+ (3)						+	Sakai and Sato., 1989
2-Amino-3-methylimidazo [4,5-f] quinoline (IQ) 76180-96-6	+ (3)						+	Cortesi et al.,1983
2-Aminoanthracene 613-13-8	+ (1) + (2)	+ (2*)		? (1)			–	Heidelberger et al., 1983

Table 5: Rodent carcinogenic organic chemicals tested with the BALB/c 3T3 cell transformation assay

Carcinogen CAS Number	Point mutations			Cytogenetics (rodent and human cells)			BALB/c 3T3 cell transformation	References BALB/c 3T3 cell transformation assays
	Ames	Mammalian cells		*In vitro*	*in vivo*			
		Mouse Lymphoma TK	HPRT		Chrom. Ab.	Micro nucleus		
Aniline 62-53-3	– (1) – (2) – (3) – (5)	+ (1) + (2*) + (3) + (5)	+/– (3)	+w (1) +/– (5)		+ (1) ? (2) – (5)	–	Dunkel et al., 1981
Anthralin (Dithranol) 1143-38-0	– (1) – (3)			– (1)		– (2) – (7)	+ (P) + (P) –	Mondal and Heidelberger, 1980 Heidelberger and Mondal, 1982 Viluksela et al., 1994
5-Azacytidine 320-67-2	+ (1) +/– (5)	+ (1) + (2*) + (3) + (5)	+ (3) +/+w (5)	+ (1) +/– (5)		+ (1)	+ + +	Lubet et al., 1990 Matthews et al., 1993b Yasutake et al., 1987
Benz(a)anthracene	+ (1) + (2) + (5)	+ (1) + (2*) +(3) +/– (5)	+ (2^{10}) + (3) – (5)	+/– (5)	+/– (5)	+ (5)	+/– +?	Dunkel et al., 1981 Heidelberger et al., 1983
Benzene 71-43-2	– (1)	– (1) + (2*) +/– (3) +/– (4)	– (3)	– (1) + (2) + (4)	+ (2) + (4)	+ (1) + (2)	+/–	Matthews et al., 1985
Benzidine 92-87-5	+ (1) + (2) +/– (3)	+ (2*) + (3)	+ (2^{9}) +/– (3)	+ (1)		+(2)	+	Cortesi et al., 1983
Benzidine dihydrochloride 531-85-1	+ (1) + (2)	+ (1) + (2*) + (3)		+ (1)	+ (1)	+ (1)	+	Matthews et al., 1993b
Benzo(a)pyrene 50-32-8	+ (1) + (2)	+ (1) + (2*) + (3)	+ (2^{9}) + (3)	+ (1)	+ (1)	+ (2)	+ + + + + +	Atchison et al., 1982 Heidelberger et al., 1983 Friedrich et al., 1985 Baturay and Kennedy, 1986 Sakai and Sato, 1989 Sheu et al., 1994

Table 5: Rodent carcinogenic organic chemicals tested with the BALB/c 3T3 cell transformation assay

Carcinogen CAS Number	Point mutations			Cytogenetics (rodent and human cells)			BALB/c 3T3 cell transformation	References BALB/c 3T3 cell transformation assays
	Ames	Mammalian cells		*In vitro*	*in vivo*			
		Mouse Lymphoma TK	HPRT		Chrom. Ab.	Micro nucleus		
HC Blue 1 2784-94-3	+ (1)	+ (1) ? (2*) +/– (3)		+ (1)	+ (1)	? (2)	?	Matthews et al., 1993b
Butylated hydroxyanisol (BHA) 25013-16-5	– (1) – (2) – (3)					– (1)	–, + (Init) –, + (P)	Sakai and Sato., 1989 Sakai et al., 1997
Butylated hydroxytoluene (BHT) 128-37-0	– (1) – (2) – (3) – (8)	+ (1) + (2*)	+ (8)	– (1) +/– (4)	– (4)	– (1) ? (2) – (4) – (8)	– , – (Init) + (P)	Sakai and Sato., 1989 Sakai et al., 2002
Butylbenzylphtalate 85-68-7	– (1)	– (1) # (2*) + (3) – (4)		– (1) – (4)	+ (1)	– (1)	–	Barber et al., 2000
Catechol 120-80-9	– (1)	+ (2*) + (3) + (4)	+ (3)	+ (4)		– (1) ? (2) ? (3) – (4)	–,+ (P)	Atchison et al., 1982
4-Chloro-o-toluidine hydrochloride 3165-93-3	– (1)	+ (1)		+w (1)			+	Matthews et al., 1993b
Chloroethane (Ethyl chloride) 75-00-3	+ (1) +/– (3)		+ (3)				–	Tu et al., 1985
3 (Chloromethyl)-pyridine HCl 6959-48-4	+ (1) +/– (3)	+ (1) + (2*) + (3)		+ (1)			– +	Heidelberger et al., 1983 Matthews et al., 1993b
Cholic acid 81-25-4	+/– (3)						+w(P)	Umeda et al., 1989
Chrysene 218-01-9	+ (2) +/– (3)		+ (3)				– –	Sala et al., 1987 Sheu et al., 1994
Cinnamyl anthranilate 87-29-6	– (1) – (3)	+ (1) +(2*) +(7)		– (1) – (7)	– (1)	– (1) – (7)	+ + –	Sivak andTu, 1985 Lubet et al., 1990 Matthews et al., 1993b

Table 5: Rodent carcinogenic organic chemicals tested with the BALB/c 3T3 cell transformation assay

Carcinogen CAS Number	Point mutations			Cytogenetics (rodent and human cells)			BALB/c 3T3 cell transformation	References BALB/c 3T3 cell transformation assays
	Ames	Mammalian cells		*In vitro*	*in vivo*			
		Mouse Lymphoma TK	HPRT		Chrom. Ab.	Micro nucleus		
Clofibrate 637-07-0	– (3) – (5)		– (3) – (5)	– (5)			–	Kajiwara and Ajimi, 2003
Cyclophosphamide 50-18-0	+ (2) +/– (3) +/– (5)	+ (1) + (2*) + (3) + (5)	+ (2^{9}) + (5)	+ (2) + (5)	+ (2) +/– (5)	+ (1) + (2) + (5)	– + (+ S9) +	Heidelberger et al., 1983 McCarvill et al., 1990 Sheu et al., 1991
2,4-Diaminotoluene 95-80-7	+ (1) + (2)	+(2*)	– (3)	+ (1)		– (1)	+	Kajiwara and Ajimi., 2003
Dibenz(a,h)anthracene 53-70-3	+ (2) +/– (3) + (5)	+ (2*) + (5)	+ (2^{10}) + (3) + (5)	+ (5)			+	Heidelberger et al., 1983
1,2-Dibromoethane 106-93-4	+ (1) + (3)	+ (1) + (2) + (3)	+ (3) + (2)	+ (1)	– (1)	+ (1) ? (2)	+ + (Init),+(II) + (P)	Perocco et al., 1991 Colacci et al., 1995 Colacci et al., 1996
Dichlorodiphenyl trichloroethane (DDT) 50-29-3	– (1) – (2) – (3)	– (1)	+ (2^{10})	– (1) + (2) – (4)		? (2)	+ +	Fitzgerald et al., 1989 Yamasaki et al, 1996
1,2-Dichloroethane 107-06-2	+ (1) +w (2)		+ (2^{9})	+ (1)		– (1)	–	Tu et al., 1985
Dichlorvos 62-73-7	+ (1) + (2)	+ (1) +(2*) + (3)	+ (3)	+ (1)	– (1) – (2)	– (1) ? (2)	eq	Matthews et al., 1993b
Diepoxybutane, 1,2 :3,4 1464-53-5	+ (2) + (3) +/– (5)	+ (2*) + (5)	+ (5)	+ (5)	+ (5)	+ (5)	+	Heidelberger et al., 1983
Di(2-ethylhexyl)phthalate (DEHP) 117-81-7	– (1) – (3) – (5)	– (1) # (2*) +/– (3) +/– (4) +/– (5)	– (5)	– (1) – (4) – (5)	– (1) – (4) – (5)	– (1) + (4) – (5)	– – –	Matthews et al., 1985, Matthews et al., 1993b Kajiwara and Ajimi., 2003

Table 5: Rodent carcinogenic organic chemicals tested with the BALB/c 3T3 cell transformation assay

Carcinogen CAS Number	Point mutations			Cytogenetics (rodent and human cells)			BALB/c 3T3 cell transformation	References BALB/c 3T3 cell transformation assays
	Ames	Mammalian cells		*In vitro*	*in vivo*			
		Mouse Lymphoma TK	HPRT		Chrom. Ab.	Micro nucleus		
Diethylstilbestrol (DES) 56-53-1	– (1) – (2) – (3) – (5)	+ (1) + (2*) +/– (3) +/– (5)	– (5)	+ (1) +/– (5)	+ (5)	+/– (1) + (2) – (5)	– + + + + + (init)	Heidelberger et al., 1983 Friedrich et al., 1985 Matthews et al., 1985 Fitzgerald et al., 1989 Matthews et al., 1993b Kajiwara and Ajimi., 2003
N,N-Dimethyl-4-aminoazobenzene 60-11-7	+ (1) + (2) +/– (3) +/– (5)	– (2*) +/– (3) + (5)	– (3) – (5)	+/– (5)		+ (2) – (5)	– –	Heidelberger et al., 1983 Sivak and Tu, 1985
7,12-Dimethyl benzanthracene 57-97-6	+ (1) + (2) + (3)	+ (1) + (2*) + (3)	+ (2^{9}) + (3)	+ (2)	+ (2)	+ (1) + (2)	+	Heidelberger et al., 1983
Dimethylvinyl chloride 513-37-1	– (1) – (3)	+ (1) + (2*) + (3)		– (1)		+ (1)	+	Matthews et al., 1993b
2,4-Dinitrotoluene 121-14-2	+ (1)	+ (1)	? (2^{9})	– (1)		– (2)	–	Matthews et al., 1993b
1,4-dioxane 123-91-1	– (1) – (3)	– (1) ? (2*) – (3)		– (1)		– (1)	+	Sheu et al., 1988
Epichlorhydrin 106-89-8	+ (5) + (2) +/– (3)	+ (2*) + (5)	– (3) +/– (5)	+ (2) +/– (5)	+/? (2) +/– (5)	– (5) ? (2^{12})	+	Matthews et al., 1993b
1,2-Epoxybutane 106-88-7	+/– (1) + (2) + (3)	+ (1) + (2*) + (3)		+ (1)		– (1)	–	Heidelberger et al., 1983
Estradiol 50-28-2	– (1) – (3) – (5)	? (2*)	+/– (3) – (5)		– (7)	– (1)	+	Liehr et al., 1987
Ethinylestradiol 57-63-6	– (1) – (3)					– (1)	–	Heidelberger et al., 1983
l-Ethionine 13073-35-3	– (2)					+ (2)	–	Friedrich et al., 1985

Table 5: Rodent carcinogenic organic chemicals tested with the BALB/c 3T3 cell transformation assay

Carcinogen CAS Number	Point mutations			Cytogenetics (rodent and human cells)			BALB/c 3T3 cell transformation	References BALB/c 3T3 cell transformation assays
	Ames	Mammalian cells		*In vitro*	*in vivo*			
		Mouse Lymphoma TK	HPRT		Chrom. Ab.	Micro nucleus		
Ethyl acrylate 140-88-5	– (1) – (3)	+ (1) + (2*) + (3)	+ (3)	+ (1)	– (1)	+ (2)	+	Matthews et al., 1993b
Ethyl Alcohol Ethanol 64-17-5	– (1) – (2) – (3) – (5)	– (2*) – (3) + (4) – (5)		– (2) – (4) +/– (5)	? (4) – (5)	– (2) – (4) +/– (5)	+	Matthews et al., 1993b
Ethyl methanesulfonate (EMS) 62-50-0	+/? (1) + (2) +/– (3) +/– (5)	+ (2*) + (3)	+ (2^{9}) + (2^{10}) + (2^{11}) + (3)	+ (1) + (5)	+ (1) +/? (2) + (5)	+ (2) + (5)	+ +	Lubet et al., 1984 Kajiwara et al., 1997
Ethylene thiourea 96-45-7	+w/–(1) + (2)	+ (1) # (2*) + (3)		– (1)		? (2)	+/–	Matthews et al., 1993b
Furyl furamide (2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide (AF-2)) 3688-53-7	+ (1) + (2) + (3)	+ (2*) + (3)	+ (2^{10})	+ (1)		? (2^{12})	+	Sakai and Sato., 1989
Glycidaldehyde 765-34-4	+ (1) + (2) + (3)	+ (2*)					+ +	Heidelberger et al., 1983 Matthews et al., 1993b
Hexachloroethane 67-72-1	– (1) – (2) – (3)			– (1)			–	Tu et al., 1985
Hexamethyl phosphoramide (HMPA) 680-31-9	– (1) – (5)	+ (1) + (2*) +/– (3) + (5)	+/– (3)	– (1) – (5)	+ (1) +/– (5)	+ (2) + (5)	+	Matthews et al., 1993b
N-Hydroxy-2-acetylaminofluorene 53-95-2	+ (2) + (3)		+ (2)				+	Heidelberger et al., 1983
Isophorone 78-59-1	– (1) – (3)	+ (1) + (2*) – (3)		– (1)	– (1)		+	Matthews et al., 1993b

Table 5: Rodent carcinogenic organic chemicals tested with the BALB/c 3T3 cell transformation assay

Carcinogen CAS Number	Point mutations			Cytogenetics (rodent and human cells)			BALB/c 3T3 cell transformation	References BALB/c 3T3 cell transformation assays
	Ames	Mammalian cells		*In vitro*	*in vivo*			
		Mouse Lymphoma TK	HPRT		Chrom. Ab.	Micro nucleus		
d-Limonene 5989-27-5	– (1) – (3)	– (1) ? (2*) + (3)		– (1)			–	Matthews et al., 1993b
Melphalan 148-82-3	+ (1) + (2) + (3) +/– (5)	+ (5)		+ (1) + (5)	+ (5)	+ (1)	+	Matthews et al., 1993b
Methapyrilene Hydrochloride 135-23-9	– (1)	– (1) + (2*) +/– (3)		+ (1)			–	Kajiwara and Ajimi., 2003
Methyl methane sulfonate (MMS) 66-27-3	+ (1) + (2) +/– (3) +/– (5)	+ (2*) + (3) + (5)	+ (2^{9}) +/– (5)	+ (2)	+ (2) +/– (5)	+ (2) + (5)	+	Kajiwara et al., 1997
Methylazoxymethanol acetate 592-62-1	+ (1) + (3)	+ (2*) + (3)					+	Heidelberger et al., 1983
Methylcarbamate 598-55-0	– (1) – (2) – (3)	– (1) # (2*) – (3)		– (1)		– (1)	+ + (init)	Kajiwara et al., 1997 Kajiwara and Ajimi., 2003
3-Methylcholanthrene (MCA) 56-49-5	+ (1) + (2)	+ (2*)	+ (2^{9}) + (2^{10}) + (3)	– (1) – (2)		? (2)	+ + + (init) +w +	Heidelberger et al., 1983 Cortesi et al., 1983 Friedrich et al., 1985 Tsuchiya et al., 1999 Kajiwara and Ajimi., 2003
4,4'-Methylene bis-(N,N'-dimethylaniline) 101-61-1	+/– (1) + (2) + (9)	+ (1) + (2*) + (9)		– (1)			+	Sivak and Tu, 1985
Methylthiophanate 23564-05-8	– (3) + (1)						+	Perocco et al., 1997
Mezerein 34807-41-5	– (1) – (3)						+	Matthews et al., 1993b

Table 5: Rodent carcinogenic organic chemicals tested with the BALB/c 3T3 cell transformation assay

Carcinogen CAS Number	Point mutations			Cytogenetics (rodent and human cells)			BALB/c 3T3 cell transformation	References BALB/c 3T3 cell transformation assays
	Ames	Mammalian cells		*In vitro*	*in vivo*			
		Mouse Lymphoma TK	HPRT		Chrom. Ab.	Micro nucleus		
Mitomycin C 50-07-7	+/– (3) +/– (5)	+ (2*) + (3)	+ (2^{9}) + (3)	+ (2) + (5)	+ (1) + (2) + (5)	+ (1) + (2) + (5)	+	Kajiwara and Ajimi, 2003
Monuron 150-68-5	– (1) – (3)	– (1) + (2*)		+ (1)	– (1)	+ (1)	–	Matthews et al., 1993b
Nalidixic acid 389-08-2	+/– (1) – (3)	– (1) – (3)	– (3)	– (1)			–	Kaneko and Horikoshi., 1988
2-Naphthylamine 91-59-8	+ (1) + (2) +/– (3) + (6) +/– (5)	+ (1) + (2*) + (5)	+ (2^{9}) +/– (5)	+ (1) + (5)	? (1)	– (1) + (2) – (5) + (2^{12})	– – + (+ S9) +	Heidelberger et al., 1983 Sivak and Tu, 1985 McCarvill et al., 1990 Matthews et al., 1993b
Nitrilotriacetic acid 139-13-9	– (1) – (3)	– (3)		– (1)			+	Matthews et al., 1993b
2-Nitro-1,4-phenylenediamine 5307-14-2	+ (1) + (2) +/– (5)	+ (1) + (2*) + (3) + (5)	+ (3)	+ (1) + (2) + (5)	– (5)	? (2) – (5)	+	Kajiwara and Ajimi., 2003
1-Nitropyrene 5522-43-0	+/– (3)		+ (2^{9})				+	Sheu et al., 1994
2-Nitropyrene 789-07-1	+/– (3)						+	Sheu et al., 1994
4-Nitroquinoline-N-oxide 56-57-5	+ (2) + (3)	+ (1) + (2*) + (3)	+ (2^{9}) + (3)	+ (1) + (2)	+ (1) + (2)	+ (2)	+	Heidelberger et al., 1983
N-Nitroso-*N*-ethylaniline 612-64-6	– (3)						– +	Heidelberger et al., 1983 Sivak and Tu , 1985
N-Nitroso-*N*-ethylurea (ENU) 759-73-9	+ (1) + (2) + (3) + (5)		+ (2^{9}) + (3)	+ (1) + (2) + (5)	+ (2) + (5)	+ (1) + (2) + (5)	+ +	Kajiwara et al., 1997 Kajiwara and Ajimi, 2003

Table 5: Rodent carcinogenic organic chemicals tested with the BALB/c 3T3 cell transformation assay

Carcinogen CAS Number	Point mutations			Cytogenetics (rodent and human cells)			BALB/c 3T3 cell transformation	References BALB/c 3T3 cell transformation assays
	Ames	Mammalian cells		*In vitro*	*in vivo*			
		Mouse Lymphoma TK	HPRT		Chrom. Ab.	Micro nucleus		
N-Nitroso-*N*-methylnitrosoguanidine (MNNG) 70-25-7	+ (2) + (3)	+ (2*) + (3)	+ (2^{9}) + (3)	+/– (2)	? (2)	+ (2)	+ + + + + + + +	Cortesi et al., 1983 Heidelberger et al., 1983 Friedrich et al., 1985 Lu et al., 1986 Matthews et al., 1993b Tsuchiya and Umeda, 1995 Tsuchiya and Umeda, 1997 Kajiwara and Ajimi, 2003
N-Nitroso-*N*-methylurea (MNU) 684-93-5	+ (2) + (3) + (5)	+ (2*)	+ (2^{9}) + (5)	+ (2) + (5)	+ (2) + (5)	+ (1) ? (2)	+ +	Fitzgerald et al.,1989 Kajiwara et al., 1997
N-Nitrosodiethylamine (NDEA) 55-18-5	+/– (1) + (2) +/– (3)	+ (2*) + (3)	+ (2^{9}) +/– (3)	+ (2)		? (2)	+/–	Dunkel et al., 1981
N-Nitrosodimethylamine (NDMA) 62-75-9	+/– (1) + (2) +/– (3) + (5)	+ (2*) +(3) + (5)	+ (2^{9}) + (3) + (5)	+/– (1) + (2) + (5)	– (2) + (6)	+ (2)	+ + +	Fitzgerald et al., 1989 Matthews et al., 1993b Tsuchiya and Umeda, 1995
N-Nitrosodiphenylamine Diphenyl nitrosamine 86-30-6	– (1) – (2) – (3)	+ (1) – (2*)	– (2^{10})	– (1)		? (2)	– +	Heidelberger et al., 1983 Matthews et al., 1993b
Okadaic acid 78111-17-8	– (3)	+ (3)					+ (P) –, + (P) + (P)	Katoh et al., 1990 Sakai and Fujiki, 1991 Tsuchiya and Umeda, 1995
4,4'-Oxydianiline 101-80-4	+ (1)	+ (1) + (2*)		+ (1)	+ (1)	+ (1)	+	Matthews et al., 1993b
Phenobarbital 50-06-6	+w (1) – (5)	+ (1) ? (2*) +/– (3) – (5)	+ (3) + (5)	+ (1)		+ (2)	+ –	Matthews et al., 1993b Kajiwara and Ajimi, 2003
Polychlorobiphenyl (PCB) 1336-36-3					? (2)	– (2)	+	Matthews et al, 1993b

Table 5: Rodent carcinogenic organic chemicals tested with the BALB/c 3T3 cell transformation assay

Carcinogen CAS Number	Point mutations			Cytogenetics (rodent and human cells)			BALB/c 3T3 cell transformation	References BALB/c 3T3 cell transformation assays
	Ames	Mammalian cells		*In vitro*	*in vivo*			
		Mouse Lymphoma TK	HPRT		Chrom. Ab.	Micro nucleus		
Procarbazine HCl Natulan 366-70-1	– (1) – (2) – (3)	+ (2*) + (3)	+ (3)			+ (2)	–	Dunkel et al., 1981
Progesterone 57-83-0	– (1) – (3)	– (1) eq (2*)					+/–	Dunkel et al., 1981 Heidelberger et al., 1983
β-Propionolactone 57-57-8	+ (1) + (3) +/– (5)	+ (2*) + (3) + (5)	+ (2^9) + (5)	+ (1) + (5)	+ (5)	? (2) +/– (5)	+ + +	Dunkel et al., 1981 Atchison et al., 1982 Baturay and Kennedy, 1986
1,2-Propyleneimine 75-55-8	+ (1) + (2) + (3) +/– (5)						+	Heidelberger et al., 1983
Pyrilamine maleate 59-33-6	– (3)	+ (2*) +/– (3)				– (1)	–	Kajiwara and Ajimi, 2003
Quercetin 117-39-5	+ (1) + (2) +/– (3) +/– (5)	+ (2*)	? (2^9)	+ (1) + (5)	+ (5)	+ (2) + (5)	+w +w	Meltz and MacGregor, 1981 Tanaka et al.,1987
Reserpine 50-55-5	– (1) – (3)	– (1) # (2*)		– (1)	+ (1)	– (1)	– + + (init)	Matthews et al., 1993b Kajiwara et al., 1997 Kajiwara and Ajimi., 2003
p-Rosaniline hydrochloride CI Basic Red 9 569-61-9	+/? (1) – (2) +/– (3) +/– (5)	+ (2*) + (5)	– (1) – (6)	– (1) – (5)		– (1)	+ + –	Heidelberger et al., 1983 Sivak and Tu, 1985 Matthews et al., 1993b
Safrole 94-59-7	– (1) – (2)	+ (1) + (2*) +/– (3)	+/– (3)	– (1)		? (2)	+ (+S9)	Matthews et al., 1985
Sodium saccharin 128-44-9	– (2) – (3)	? (2*)				? (2)	+	Matthews et al., 1993b
1,1,1,2-Tetrachloroethane 630-20-6	– (1) + (3)	+/– (1) ? (2*)		– (1)	+ (1)	+ (1)	–	Tu et al., 1985
1,1,2,2-Tetrachloroethane 79-34-5	– (1) + (3)	– (1)		– (1)		+ (1)	– +	Tu et al., 1985 Colacci et al., 1990

Table 5: Rodent carcinogenic organic chemicals tested with the BALB/c 3T3 cell transformation assay

Carcinogen CAS Number	Point mutations			Cytogenetics (rodent and human cells)			BALB/c 3T3 cell transformation	References BALB/c 3T3 cell transformation assays
	Ames	Mammalian cells		*In vitro*	*in vivo*			
		Mouse Lymphoma TK	HPRT		Chrom. Ab.	Micro nucleus		
Tetrachlorethylene 127-18-4	– (1) +/– (3)	eq (1) ? (2*)	– (3)	– (1)	– (1)	– (1)	+ –	Kajiwara et al., 1997 Kajiwara and Ajimi., 2003
12-*o*-Tetradecanoyl phobol 13-acetate (TPA) 16561-29-8	– (2)		– (2^{10})				–,+ (P) –,+ (P) –,+ (P) + (P) + (P)	Mondal et al., 1976,1978 Boreiko et al., 1982 Kuroki and Sasaki, 1985 Lu et al., 1986 Tsuchiya et al., 1999
o-Toluidine 95-53-4	+ (1) ? (2) – (5)	+ (1) ? (2*) – (3) +/– (4) +/– (5)	+/– (3) +/– (5)	+ (1) +/– (5)		+/– (1) – (5)	+	Matthews et al., 1993b
1,1,2-Trichloroethane 79-00-5	–/eq (1) – (3)			+ (1)			+	Tu et al., 1985
Trichlorethylene 79-01-6	– (1) ? (2) +/– (3)	+ (1) + (2*) +/– (3)		– (1)	– (1)	– (1) + (2)	+	Tu et al., 1985
Tris(2-ethylhexyl)phosphate (TEHP) 78-42-2	– (1) – (3)	– (1) # (2*)		– (1)		– (1)	–	Matthews et al., 1993b
Vinyl chloride 75-01-4	+ (2) + (3)					+ (2)	+	Tu et al., 1985

(1) NTP database
(2) GENETOX database
(3) CCRIS database
(4) IUCLID database
(5) Genetic Activity Profile
(6) CICADS (IPCS)
(7) IARC Monographs
(8) Great Lakes Water Quality Agreement Genetic Activity Profiles
(2*) GENETOX Phase III: review (Mitchell *et al.,* 1997)
(2^{9}) GENETOX Phase III: HPRT/CHO (Li *et al.,* 1988)
(2^{10}) in GENETOX : HPRT/V79 (Bradley *et al.,* 1981)
(2^{11}) in GENETOX : HPRT/CHO (Hsie *et al.,* 1981)
(2^{12}) in GENETOX : (Mavournin *et al.,* (1990)
(ST) Sequential treatments
(Init) *in vitro* initiation assay in two-stage transformation
(P) *in vivo* tumor promoter; *in vitro* promotion assay in two-stage transformation
(II) Level II replating assay

\+ Positive result
+u Positive unconfirmed
– Negative result
+w Weakly positive result
? Inconclusive result
+? Positive at one concentration or at two non-consecutive concentrations
+/– Diverging results inside a database
Not testable
eq equivocal
IC Inconclusive for carcinogenicity
* Single judgement and the first judgement before comma without subsequent notice denote the results of one-stage transformation assay.

Table 6: Non-carcinogenic and inconclusive for carcinogenicity organic chemicals tested with the BALB/c 3T3 cell transformation assay

Carcinogen CAS Number	Point mutations			Cytogenetics (rodent and human cells)			BALB/c 3T3 cell transformation	References BALB/c 3T3 cell transformation assays
	Ames	Mammalian cells		*In vitro*	*in vivo*			
		Mouse Lymphoma TK	HPRT		Chrom. Ab.	Micro nucleus		
Acetone 67-64-1	– (1) – (2) – (3)	? (2*) – (4)		– (1) – (2)	– (2)	– (1) – (2) – (3)	+	Matthews et al., 1993b
4-Acetylaminofluorene 28322-02-3	+ (2)	+/– (1) + (2*) +/– (3)		– (1)	– (1)	? (2)	+ ?	Heidelberger et al., 1983 Matthews et al., 1993b
Acid red 14 (IC) 3567-69-9	– (1) – (2)	– (1) – (2*)		– (1)		(1)	+	Matthews et al., 1993b
3-Aminobenzamide 3544-24-9			? (2^{9})	? (2)			+ (P)	Lubet et al., 1984
Anilazine 101-05-3	– (1) – (3)	+ (1) # (2*) + (3)	– (3)	– (1)			eq	Matthews et al., 1993b
Anthracene 120-12-7	+w (1) – (2) +/– (3)	+ (1) + (2*) + (3) +/– (4)		– (2)		? (2)	–	Heidelberger et al., 1983
Anthranilic acid 118-92-3	– (1) – (3)	+ (2*) +/– (3)	– (3)	+w (1)	eq (1)		–	Umeda et al., 1989
l-Ascorbic acid 50-81-7	–/+w (1) ? (2) – (3)	? (1) # (2*)		– (1)	+ (1)	+ (1) ? (2)	– – –	Matthews et al., 1993b Kajiwara and Ajimi, 2003 Tsuchiya et al., 1995
Benzo(e)pyrene 192-97-2	+ (1) + (2) +/– (3)	+ (1) + (2*) +/– (3)	– (2^{10})				– – –	Schechtman et al., 1979 Heidelberger et al., 1983 Tsuchiya and Umeda, 1995
Benzoin 119-53-9	–/+w (1) +/– (3)	+ (1) – (2*) +/– (3)	+/– (3)	– (1)	– (1)	– (1)	– –	Matthews et al., 1993b Kajiwara and Ajimi, 2003
Benzyl alcohol 100-51-6	– (1) – (3)	eq (1) ? (2*) +/– (3)		+(1)			+	Matthews et al., 1993

Table 6: Non-carcinogenic and inconclusive for carcinogenicity organic chemicals tested with the BALB/c 3T3 cell transformation assay

Carcinogen CAS Number	Point mutations			Cytogenetics (rodent and human cells)			BALB/c 3T3 cell transformation	References BALB/c 3T3 cell transformation assays
	Ames	Mammalian cells		*In vitro*	*in vivo*			
		Mouse Lymphoma TK	HPRT		Chrom. Ab.	Micro nucleus		
Bisphenol A 80-05-7	– (1) – (3)	– (1) – (2*) – (3) – (4)		– (1)		– (1)	–	Matthews et al., 1993
HC Blue 2 33229-34-4	+ (1)	+ (1) ? (2*) + (3)		– (1)	– (1)		–	Matthews et al., 1993b
2-tert-Butyl-1,4-hydroquinone 1948-33-0	– (1) – (3)			+ (1)	– (1)	– (1)	–	Sakai and Teshima, 2001
Caprolactam 105-60-2	– (1) – (3)	– (1) – (2*) – (3) – (4)	– (2^{9}) – (3)	– (1)	– (1)	– (1) – (4)	– –	Matthews et al., 1993a Kajiwara and Ajimi, 2003
β-Carotene 7235-40-7	+ (1) – (3)					? (2)	+(P)	Perocco et al., 1999
o-Chloroaniline 95-51-2	– (1) – (3)	+ (1) + (2*) + (3) + (4)				+/– (1)	–	Sivak and Tu, 1985
2-Chloroethanol 107-07-3	+ (1) + (2) +/– (3)	+ (1) + (2*)		+ (1)	– (1)	– (1)	+ –	Matthews et al., 1993b Kajiwara et al., 1997
Cyanazine 21725-46-2				– (Ka 2000)		– (Kb 2000)	+	Perocco et al., 1993
Cytosine arabinoside 147-94-4	– (3)	+ (2*) + (3)	+ (2^{10}) +/– (3)	+ (2)		+ (2)	+	Matthews et al., 1993b
2,6-Diaminotoluene 823-40-5	+ (3)						+ (init)	Kajiwara andAjimi, 2003
1,1-Dichloroethane 75-34-3	– (1) – (3)						–	Tu et al., 1985
2,4-Dimethoxyaniline HCl 54150-69-5	+ (1)	+ (1) ? (2*)		+ (1)		– (1)	+	Sivak and Tu, 1985

Table 6: Non-carcinogenic and inconclusive for carcinogenicity organic chemicals tested with the BALB/c 3T3 cell transformation assay

Carcinogen CAS Number	Point mutations			Cytogenetics (rodent and human cells)			BALB/c 3T3 cell transformation	References BALB/c 3T3 cell transformation assays
	Ames	Mammalian cells		*In vitro*	*in vivo*			
		Mouse Lymphoma TK	HPRT		Chrom. Ab.	Micro nucleus		
Ephedrine sulfate 134-72-5	– (1) – (3)	– (1) ? (2*)		– (1)			–	Matthews et al., 1993b
Ethylenediaminetetracetic acid (EDTA) 150-38-9	– (1) – (3)	– (1) – (2*)		– (1)			–	Matthews et al., 1993b
Eugenol 97-53-0	– (1)	+ (1) + (2*) + (3)		+ (1)	? (1)	– (1) + (2)	?	Matthews et al., 1993b
Geranyl acetate 105-87-3	– (1)	+ (1) # (2*)		– (1)	– (1)	– (1)	–	Matthews et al., 1993b
Hydrocortisone 50-23-7				+ (3)			–	Umeda et al., 1983
8-Hydroxyquinoline 148-24-3	+ (1) + (2)	+ (1) + (2*)		+w (1)	– (1)	– (1) ? (2)	eq –	Matthews et al., 1993b Kajiwara et al., 1997
Lithocholic acid 434-13-9	– (1) – (3)	+/ – (1) ? (2*) +/– (3)		– (1)			– + (P)	Sivak andTu, 1985 Umeda et al., 1989
d-Mannitol 69-65-8	– (1) – (3)	– (1) – (2*)		– (1)	– (1)	– (1)	+ –	Matthews et al., 1993b Kajiwara and Ajimi, 2003
dl-Menthol 15356-70-4	– (1)	– (1) ? (2*)		– (1)	eq (1)	– (1)	– –	Matthews et al., 1993b Kajiwara and Ajimi, 2003
Methoxychlor 72-43-5	– (1) – (2)	+ (1) # (2*) +/– (3)	– (3)	– (1)			+ + –	Dunkel et al., 1981 Heidelberger et al., 1983 Matthews et al., 1993b
1-Naphthylamine 134-32-7	+ (1) + (2) +/– (3)	? (2*)		+ (1) +/– (3)		+(2) +(2[12])	+	Matthews et al., 1993b
Naphthylisothiocyanate 551-06-4	+ (1)						+	Heidelberger et al., 1983
4-Nitro-*o*-phenylenediamine 99-56-9	+ (1) + (2) +/– (3)	+ (1) eq (2*) + (3)	– (3)	– (1) + (2)	– (1)	eq (1) ? (2)	– + + (init)	Sivak et Tu ,1985 Matthews et al., 1993b Kajiwara et al., Ajimi, 2003

Table 6: Non-carcinogenic and inconclusive for carcinogenicity organic chemicals tested with the BALB/c 3T3 cell transformation assay

Carcinogen CAS Number	Point mutations			Cytogenetics (rodent and human cells)			BALB/c 3T3 cell transformation	References BALB/c 3T3 cell transformation assays
	Ames	Mammalian cells		*In vitro*	*in vivo*			
		Mouse Lymphoma TK	HPRT		Chrom. Ab.	Micro nucleus		
3-Nitropropionic acid 504-88-1	+ (1) +/– (3) +/– (5)	+ (1) # (2*) + (3) + (5)	– (3) – (5)	+w (1)			+	Matthews et al., 1993b
Oxytetracycline-HCl 2058-46-0	– (1) – (3)	+ (1) + (2*)		– (1)			–	Matthews et al., 1993b
Penicilline VK 132-98-9	– (1) – (3)	+/– (3) + (2*)		– (1)			+	Matthews et al., 1993b
Phenanthrene 85-01-8	+w (1) – (2) +/– (3)			– (2)		? (2)	–	Heidelberger et al., 1983
Phenol 108-95-2	– (1)	+ (1) # (2*) + (3) ?/+ (4)	– (3)	+ (1) + (4)	+ (1)	+ (1) ? (2) ? (4)	+ + +	Matthews et al., 1993b Kajiwara et al., 1997 Kajiwara and Ajimi., 2003
p-Phenylenediamine dihydrochloride 624-18-0	+ (1)	+ (1) eq (2*) + (3)	– (3)	+ (1)			+ +	Matthews et al., 1993b Sivak and Tu, 1985
Phtalic anhydride 85-44-9	– (1) – (3)	+ (1)		– (1)			–	Matthews et al., 1993b
Propyl gallate 121-79-9	– (1) – (3)	+ (1) + (2*)		+ (1)	+ (1)	+/– (1)	+	Matthews et al., 1993b
Pyrene 129-00-0	+/– (1) – (2) +/– (3)	+ (1) + (2*) + (3)	– (2^{10}) – (3)	– (1) – (2)	– (1)	? (2)	– +/–	Heidelberger et al., 1983 Sheu et al., 1994
HC Red 3 2871-01-4	+ (1)	+ (1)		+ (1)			+	Matthews et al., 1993b
Rotenone 83-79-4	– (1) – (3)	+ (1) + (2*) + (3)		– (1)			–	Matthews et al., 1993b
Saccharin 81-07-2	– (1)	? (2)					–	Sakai and Sato., 1989

Table 6: Non-carcinogenic and inconclusive for carcinogenicity organic chemicals tested with the BALB/c 3T3 cell transformation assay

Carcinogen CAS Number	Point mutations			Cytogenetics (rodent and human cells)			BALB/c 3T3 cell transformation	References BALB/c 3T3 cell transformation assays
	Ames	Mammalian cells		*In vitro*	*in vivo*			
		Mouse Lymphoma TK	HPRT		Chrom. Ab.	Micro nucleus		
Tetracycline HCl 64-75-5	– (1) – (3)	? (1) – (2*) +/– (3)		– (1)			– –	Matthews et al., 1993b Kajiwara and Ajimi, 2003
Tetraethylthiuramdisulfide 97-77-8	– (1)	+ (1) eq (2*) + (3)		+ (1)		? (2)	+	Matthews et al., 1993b
2,6-Toluenediamine-2HCl 15481-70-6	+ (1) +/– (3)	+ (1)		+ (1)	– (1)	+ (1)	+	Matthews et al., 1993b
1,1,1-Trichloroethane 71-55-6	– (1) +/– (3)	– (1) + (2*)		+ (1)		eq (1)	+	Tu et al., 1985
Triphenyltin Hydroxide 76-87-9	– (1) – (3)	+ (1)	– (3)	– (1)			?	Matthews et al., 1993b

(1) NTP database
(2) GENETOX database
(3) CCRIS database
(4) IUCLID database
(5) Genetic Activity Profile
(2*) GENETOX Phase III: review (Mitchell *et al.,* 1997)
(2^{9}) GENETOX Phase III: HPRT/CHO (Li *et al.,* 1988)
(2^{10}) in GENETOX : HPRT/V79 (Bradley *et al.,* 1981)
(2^{12}) in GENETOX : (Mavournin *et al.,* 1990)
Ka Kligerman *et al.,* 2000a
Kb Kligerman *et al.,* 2000b
(ST) Sequential treatment
(P) *in vivo* tumor promoter; in vitro promotion assay in two-stage transformation
(Init) *in vitro* initiaton assay in two-stage transformation assay
\+ Positive result
– Negative result
+w Weakly positive result
eq equivocal result
? Inconclusive result
IC Inconclusive for carcinogenicity
+/– Divergent results inside a database
\# Not testable

Table 7: Inorganic compounds tested with the BALB/c 3T3 cell transformation assay, and their classification for carcinogenicity

Inorganic compound CAS number	Point mutations			Cytogenetics (rodent and human cells)			Carcinog. Class	BALB/c 3T3 cell transformation	References BALB/c 3T3 cell transformation assay
	Ames	Mammalian cells		*In vitro*	*In vivo*				
		Mouse Lymphoma TK	HPRT		Chrom. Ab.	Micro nucleus			
Barium chloride dihydrate 10326-27-9	– (1) – (3)	+ (1)		– (1)			– (1)	+	Matthews et al., 1993a
Barium chromate 10294-40-3	– (5)						IE (7)	+	Matthews et al., 1993b
Beryllium sulfate 13510-49-1	– (1) – (8)			+/– (8)		– (8)	+ (7)	+ +	Heidelberger et al., 1983 Keshava et al., 2001
Calcium chromate 13765-19-0	+ (1) + (2) + (5)	+ (1) + (2*) + (3)	+ (3) + (5)	– (1) + (5)		– (5)	+ (7)	+	Dunkel et al., 1981
Chromium [III] chloride 10025-73-7	? (2) – (5)		+ (3)	+ (5)	– (2)		IE (7)	+	Saffiotti and Bertolero., 1989
Chrysotile 12001-29-5	– (6)		+w (6)	+ (6)			+ (7)	+ +	Lu et al., 1988 Kajiwara and Ajimi, 2003
Crocidolite 12001-28-4	– (3)		+/– (3)	+ (6)			+ (7)	+	Lu et al., 1988
Lead acetate 301-04-2	– (1)	– (1) # (2*) – (3)	+ (3)	– (2)	– (2)	– (1) + (2)	+ (7)	+ –	Dunkel et al., 1981 Kajiwara and Ajimi., 2003
Potassium chromate 7789-00-6	+ (2)	+ (2*) + (5)	+ (3)	+ (5)		+ (2) + (5)	+ (7)	+	Saffiotti and Bertolero, 1989
Selenium sulfide 7446-34-6	+ (1) + (3)	+ (1)		+ (1)	+ (1)	– (1)		+	Matthews et al., 1993b
Silica quartz 14808-60-7	– (7)		– (7)	+ (7)		– (7)	+ (7)	+	Saffiotti and Ahmed, 1995
Sodium arsenate 7778-43-0		+ (2*)					+ (7)	+ + +	Bertolero et al., 1987 Saffiotti and Bertolero., 1989 Tsuchiya et al., 2005
Sodium arsenite 7784-46-5	– (3)	+ (3) + (2*)	? (2[9])			+ (2)	+ (7)	+ + + +	Bertolero et al, 1987 Saffiotti and Bertolero., 1989 Kajiwara and Ajimi., 2003 Tsuchiya et al., 2005

Table 7: Inorganic compounds tested with the BALB/c 3T3 cell transformation assay, and their classification for carcinogenicity

Inorganic compound CAS number	Point mutations			Cytogenetics (rodent and human cells)			Carcinog. Class	BALB/c 3T3 cell transformation	References BALB/c 3T3 cell transformation assay
	Ames	Mammalian cells		*In vitro*	*In vivo*				
		Mouse Lymphoma TK	HPRT		Chrom. Ab.	Micro nucleus			
Sodium chloride 7647-14-5	– (1)	+/– (1) ? (2*)		? (1)		? (2)		?	Matthews et al., 1993b
Sodium chromate tetrahydrate 10034-82-9	+ (1) +/– (3)						+ (7)	+	Kajiwara and Ajimi, 2003
Sodium fluoride 7681-49-4	– (1) ? (2) – (3)	+ (1) # (2*) + (3) + (4)	– (3)	+/– (1)	– (1)	– (1) + (2)	EE (1)	+	Lasne et al., 1988
Sodium nitrite 7632-00-0	+ (1) + (2)	+ (3) ? (2*)		+ (3)	+ (2)	– (1) + (2)		+ + (init)	Tsuda and Hasegawa., 1990 Sakai and Sato., 1989
Sodium o-vanadate 13721-39-6				+ (8)			ND	+(P)	Sakai, 1997
Titanium dioxide 13463-67-7	– (1) – (3)	– (1) # (2*) – (4)		– (1)	– (1)	+ (1)	LE (7)	–	Matthews et al., 1993b
Titanocene dichloride 1271-19-8	+ (1)			– (1)			–	+	Heidelberger et al., 1983
Vanadium (V) pentoxide 1314-62-1	– (1) – (3)	– (3)	– (3)			– (1)	+ (7)	+	Sabbioni et al., 1991

(1) NTP database
(2) GENETOX database
(2*) GENETOX Phase III: review (Mitchell *et al.,* 1997)
(2^9) GENETOX Phase III: HPRT/CHO (Li *et al.,* 1988)
(3) CCRIS database
(4) IUCLID database
(5) IARC monographs on the evaluation of carcinogenic risks to humans. Vol 49: Chromium, Nickel and Welding, 1990, IARC, Lyon, France
(6) Kane AB, Boffetta P., Saracci R., Wilbourn J.D. (eds), Mechanisms of Fibre Carcinogenesis. IARC Sc Publ. N°140, 1996
(7) IARC database
(8) Migliore *et al.,* 1993. Mutat Res, *319, 205-13*

(Init) *in vitro* initiation assay in two-stage transformation assay
(P) *in vivo* tumor promoter; *in vitro* promotion assay in two-stage transformation
EE Equivocal evidence, IE Inadequate evidence, LE Limited evidence
\+ Positive result
– Negative result
+w Weakly positive result
? Inconclusive result
Not testable
ND No data
+/– Divergent results inside a database

The C3H/10T1/2 cell transformation assay

State of the art

76. The C3H/10T1/2 mouse embryo cell line was established with the property of a high degree of post-confluence inhibition of cell division *in vitro*, which was accomplished by maintaining the primary culture of inbred C3H mouse embryos at a low cell density by means of a rigid 10-day transfer schedule of 0.5 x 10^5 cells in 60 mm culture dishes (Reznikoff *et al.,* 1973a). A single clonal isolate (clone 8) was chosen for subsequent work. This was the third embryonic mouse cell line established, following BALB/c 3T3 and Swiss 3T3 cells, displaying a stable phenotype of post-confluence inhibition of cell division, and very low incidence of spontaneous morphological transformation, and the absence of tumorigenicity in isologous host animals.

77. C3H/10T1/2 cells are fibroblastic in shape, but with suitable stimuli will reveal their embryonic origin by displaying a pluripotential, stem cell-like behaviour with an ability to differentiate into cells with myocyte, adipocyte, chondrocyte or endothelial cell characteristics (Taylor and Jones, 1979; Hirschi, *et al.,* 1998). C3H/10T1/2 cells are hypertetraploid and highly sensitive to a wide range of chemical and physical carcinogens which induce the development of defined foci of transformed cells overlying the normal cell monolayer. These foci exhibit a typical pattern of disoriented and piled-up growth.

Principle of the test

78. The standard C3H/10T1/2 test most often employed is similar to that described for the BALB/c 3T3 assay. Cells from frozen stocks are revived and seeded into 60mm culture dishes (1000-2000 cells per dish) for a 24 hour for attachment period. Test chemicals are added for 48 h, after which the medium is removed and the cells refed with fresh medium without the test agent. The cells are maintained in culture for up to six weeks or longer, then fixed with methanol and stained with 10% Giemsa. Foci are scored by macroscopic and microscopic examination.

79. Both type II and type III foci are usually scored with the C3H/10T1/2 cell transformation system (Reznikoff *et al.,* 1973b). Cells from type III foci formed tumors at a frequency of 80-90% upon re-injection into syngeneic hosts, while a frequency of 50% is obtained with type II cells (Reznikoff *et al.,* 1973b). This explains why some laboratories score only type III foci as representing true transformation, while type II foci are also included by other authors. In either case, the transformants are much more likely to display a neoplastic or malignant phenotype than the usual preneoplastic phenotype of morphologically transformed SHE cells.

80. It is generally recognized that culture conditions, particularly serum preparations and serum concentration, influence the outcome of the basic and TPA-promoted assay (Frazelle *et al.,* 1983; Oshiro *et al.,* 1982; Bertram, 1977). These may be mediated through the inhibitory influence of the normal C3H/10T1/2 monolayer cells on the transformed cells within the population.

81. Several modifications of the standard assay have been employed to increase its sensitivity to transforming agents and are often employed when test agents fail to induce significant numbers of transformants in the standard assay.

82. A level II replating assay was developed (Schechtman *et al.,* 1987) to increase transformation frequencies by releasing transformed cells from the cell monolayer inhibitory effect. Allowing further cell replications by this means may provide conditions necessary for full conversion to and expression of the transformed phenotype. Replating confluent cultures at lower density (2 x 10^5 cells/ 60mm dish) enhanced the numbers of transformed foci detected following treatment with a variety of chemicals.

83. A two-stage initiation-promotion protocol was established by Mondal *et al.,* (1976 a, b), using the phorbol ester TPA, which has subsequently been useful for enhancing the yield of transformants from short-term treatment with many initiating agents. Transformed cells created by certain initiators in two-stage protocols may be less overtly transformed than those selected by conditions in the standard assay, since in these cases removal of the promoter causes many, but not all, of the transformants to revert to non-transformed morphologies (Sanchez *et al.,* 1986; Chen and Herschman, 1988).

84. Several other minor variations have been reported to enhance transformation frequencies in the standard assay with certain chemicals. These include: 1) cell cycle synchronization by release from confluence prior to treatment (McCormick and Bertram, 1982); and 2) increasing the numbers of cells at risk either by later exposure of asynchronous cells (5 days after seeding instead of 1 day) or by initially seeding larger numbers of cells (Schechtman *et al.,* 1987; Nesnow *et al.,* 1982) and; 3) a longer exposure period (6 days) of the asynchronous cultures (Landolph, 1985; Oshiro and Balwierz, 1982). It has been suggested that the modified assays be applied in a sequential manner to chemicals that are weakly active or inactive in the standard assay (Landolph, 2006). In this scheme, assay protocols that double the cell number and exposure time would be employed initially, but if results remain negative, protocols with long exposure times or with greater cell numbers would be applied.

85. C3H/10T1/2 cells do not have a broad repertoire of xenobiotic metabolic capability. The ability of C3H/10T1/2 cells to activate polycyclic aromatic hydrocarbons to mutagenic and transforming forms through activities of inducible microsomal cytochrome P450 1B1 has been well described (Alexander *et al.,* 1999; Nesnow *et al.,* 2000). Other pro-carcinogens were not metabolised or metabolised slowly to transforming intermediates (Oshiro and Balwierz, 1982). Consequently, exogenous metabolic activation systems such as rodent liver homogenate S9 fractions have been used to advantage during chemical treatment of these cells (Benedict *et al.,* 1978; Billings *et al.,* 1985; Tu *et al.,* 1984). More recently, transfection of an expression vector encoding a phase I metabolic enzyme (CYP2A6) was employed to greatly enhance C3H/10T1/2 cell transformation by nitrosamines in a standard assay format (Nesnow *et al.,* 1994).

86. Several alternative transformation assays have been studied, but have not been widely adopted methodologies. Analogous to the SHE colony transformation assay, C3H/10T1/2 cultures were shown to generate transformed colonies when cells at clonal densities were treated with carcinogens (Nelson and Garry, 1983). No cell feeder layer is required and transformed colonies may be scored within 2 weeks, compared to the 6 to 8 week time normally required in the standard transformation assay. Despite these advantages, no further development or validation of this assay variation has occurred since the 1983 publication.

Results

87. The data set of C3H/10T1/2 results (141 chemicals) comprises:

- 121 organic chemicals

  98 classified as rodent carcinogens (table 8)
  23 as non-carcinogens (table 9)

- 20 inorganic chemicals

  19 rodent carcinogens and 1 non-carcinogen, ammonium metavanadate (table 10)

Tables 8-10 are restricted to the chemicals with C3H/10T1/2 and genotoxicity results available. The chemicals used in the analysis of the performances of the SHE assay to predict carcinogenicity are

presented in table 11 (rodent organic carcinogens), table 12 (non-carcinogenic and inconclusive organic chemicals) and table 13 (inorganic chemicals, carcinogens and non-carcinogens).

88. A good correlation between transformation assay outcome and rodent carcinogenicity was demonstrated among the chemicals tested. Further research into this promising approach is required to address the mechanisms by which increased focus-formation is produced by diverse chemicals, and how the sensitivities of the variously transformed C3H10T1/2 cells differ from each other.

Rodent carcinogens

89. 68 % of the organic carcinogens and 53% of the inorganic carcinogens were positive in the C3H assay. Different forms of asbestos (crocidolite, chrysotile, amosite) were positive, as well as hydrogen peroxide, ozone, some nickel salts, ethyl alcohol, epichlorhydrin, aromatic amines and N-nitroso compounds induced transformed foci of C3H10T1/2 cells.

90. Improvements resulted from the development of the level II assay compared with the initial protocol. The level II assay response, though not fully validated across a wide range of carcinogens and noncarcinogens, is regarded as being more sensitive than the standard assay (Landolph, 2006). Therefore in evaluations of agents based on overall calls of transformation results, it would be prudent favor a positive level II result over a negative result in the standard assay.

91. A high number of tumor promoters were also detected as positive by the test using a two-stage treatment. These included anthralin, BHT, mezerein, phorbol esters, clofibrate and dioxines (hexa and tetra chlorinated). It should be noted that these chemicals were either negative or ambiguous in genotoxicity assays.

92. The following chemicals were false negative: lead acetate, potassium dichromate, nickel chloride and sulphate and sodium arsenate as inorganic compounds, and bromodeoxyuridine, phenobarbital, propyleneimine, styrene and thioacetamide as organics. Divergent responses were obtained for DEHP, DES, hexamethyl phosphoramide and 5-nitrotoluidine with the classical protocol, which may explain the negative responses, as the level II assay was not used for these chemicals.

Rodent non-carcinogens

93. Most non-carcinogenic chemicals were negative. However, a few positive responses were obtained in the standard transformation assay (caffeine, cytarabine), the level II replating assay (6-aminochrysene, diazanon), or as promoters of transformation (lithocholic acid, saccharin-acid form).

Chemicals not evaluated for rodent carcinogenicity and not included in the data set

94. Several chemicals not included in the data set due to lack of information on rodent carcinogenicity have been tested in the C3H/10T1/2 assay. Positive responses were obtained for 5-fluorodeoxy-uridine (50-91-9) by Heidelberger *et al.,* (1983), and tri-n-butyl chloride (1461-22-9) by Parfett and Pilon (1993). Negative responses were obtained for trenbolone (10161-33-8) by Schiffman *et al.,* (1988) and 4-*O*-methyl TPA (57716-89-9) by Boreiko *et al.,* (1986).

Table 8: Rodent carcinogenic organic chemicals tested with the C3H10T1/2 cell transformation assay

Carcinogen CAS number	Point mutations			Cytogenetics (rodent and human cells)			C3H/10T1/2 cell transformation	References C3H/10T1/2 cell transformation assays
	Ames	Mammalian cells		*In vitro*	*In vivo*			
		Mouse Lymphoma TK	HPRT		Chrom. Ab.	Micro nucleus		
Acetaldehyde 75-07-0	– (1) – (3) – (5)	+ (2*) + (3) + (5)	+ (5) + (3)	+ (1) + (5)		– (5)	– (init)	Abernethy et al., 1983
Acetaminophen (paracetamol) 103-90-2	– (1)			+ (1) + (5)	+ (5)	+/– (5)	+ – (P)	Patierno et al., 1989
2-Acetylaminofluorene 53-96-3	+ (2)	+ (1) + (2*) + (3)	+ (2^9) + (3)	+ (1)	+ (1)	+ (2)	+ –/– – +	Lawrence and McGregor, 1985 Dunkel et al., 1988 Oshiro and Balwierz, 1982 Poole and McGregor, 1982
Acrylamide 79-06-1	– (1) – (3) – (5)	+ (2*) + (3) + (5)		+ (1) + (5)	+ (1) +/– (2) +/– (5)	+ (5)	+	Banerjee and Segal, 1986
Acrylonitrile 107-13-1	+ (1) + (2) +/– (5)	+ (1) + (2*) – (4) + (5)	+ (5)	+ (1) +/– (5)	– (5)	– (1) – (2) – (5)	+/– + +	Lawrence and McGregor, 1985 Swierenga and Yamasaki, 1992 Banerjee and Segal, 1986
Actinomycin D 50-76-0	– (3)	+ (2*) + (3)	+ (2^9)	+ (2)		+ (1) ? (2)	+	Heidelberger et al., 1983
Aflatoxin B1 1162-65-8	+ (1) + (3) + (5)	+ (3)	+ (2^9) + (3) + (5)	+ (5)	+ (2) +/– (5)	+ (2)	+ –/+(init) + + +/– –/+(II)	Amstad et al., 1983 Boreiko et al., 1982 Nesnow et al., 1982 Oshiro and Balwierz, 1982 Dunkel et al., 1988 Schechtman et al., 1987
4-amino-1,1'-biphenyl 92-67-1	+ (1) + (2) +/– (3) +/– (5)	+ (2*) + (5)	+ (3) + (5)	+ (1)		+ (1) ? (2)	–/–(II)	Schechtman et al., 1987
4-Amino-2-nitrophenol 99-57-0	+ (1) +/– (3) +/– (5)	+ (2*) +(5)		+ (1) +(5)	– (5)	– (1) ? (2) – (5)	+	Dunkel et al., 1988

Table 8: Rodent carcinogenic organic chemicals tested with the C3H10T1/2 cell transformation assay

Carcinogen CAS number	Point mutations			Cytogenetics (rodent and human cells)			C3H/10T1/2 cell transformation	References C3H/10T1/2 cell transformation assays
	Ames	Mammalian cells		*In vitro*	*In vivo*			
		Mouse Lymphoma TK	HPRT		Chrom. Ab.	Micro nucleus		
2-Aminoanthracene 613-13-8	+ (1) + (2)	+ (2*)		? (1)			–/+(II) +	Schechtman et al., 1987 Poole and McGregor, 1982
Amsacrine 51264-14-3	+/– (5) +/– (3)	+ (2*) + (3) +(5)	+ (5) + (3)	+(5)	+(5)	+(2) +(5)	+	Ferguson et al., 1986
Aniline 62-53-3	– (1) – (2) – (3) – (5)	+ (1) + (2*) + (3) + (5)	+/– (3)	+w (1) +/– (5)		+ (1) ? (2) – (5)	–/+(II) –/–	Schechtman et al., 1987 Dunkel et al., 1988
Anthralin (Dithranol) 1143-38-0	– (1) – (3)			– (1)		– (2) – (7)	+ (P) + (P) –	Mondal and Heidelberger, 1980 Heidelberger and Mondal, 1982 Viluksela et al., 1994
5-Azacytidine 320-67-2	+ (1) +/– (5)	+ (1) + (2*) + (3) + (5)	+ (3) +/+w (5)	+ (1) +/– (5)		+ (1)	+ +	Heidelberger et al., 1983 Frazelle et al., 1984
Benz(a)anthracene 56-55-3	+ (1) + (2) + (5)	+ (1) + (2*) +(3) +/– (5)	+ (2^{10}) + (3) – (5)	+/– (5)	+/– (5)	+ (5)	+	Oshiro and Balwierz, 1982
Benzene 71-43-2	– (1)	– (1) + (2*) +/– (3) +/– (4)	– (3)	– (1) + (2) + (4)	+ (2) + (4)	+ (1) + (2)	+ –/–	Nesnow et al., 1985 Lawrence and McGregor, 1985
Benzidine 92-87-5	+ (1) + (2) +/– (3)	+ (2*) + (3)	+/– (3)	+ (1)		+(2)	–/+(II)	Schechtman et al., 1987
Benzo(a)pyrene 50-32-8	+ (1) + (2)	+ (1) + (2*) + (3)	(2^{9}) + (3)	+ (1)	+ (1)	+ (2)	+ +/+	Heidelberger et al., 1983 Dunkel et al., 1988

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	Ames	Mammalian cells		*In vitro*	*In vivo*			
		Mouse Lymphoma TK	HPRT		Chrom. Ab.	Micro nucleus		
1,4-Benzoquinone dioxime p-Quinonedioxime 105-11-3	+ (1) + (3) + (5)	+ (1) +/– (3) + (5)		+ (1)		– (5)	–(II) +/–	Schechtman et al., 1987 Dunkel et al., 1988
Benzyl chloride 100-44-7	+ (1) +/– (5)	+ (2*) + (5)	? (2^9) + (5)	+/– (5)		– (2) – (5)	–	Poole and McGregor, 1982
Bleomycin 11056-06-7	– (5) +/– (3)		+/– (5) + (2^9) – (3)	+ (5) + (2)	+ (5) + (2)	+/– (5) + (2)	+	Heidelberger et al., 1983
5-Bromo-2'-deoxyuridine 59-14-3	– (3)		+ (2^9) + (3)	+ (2)		+ (2)	–	Heidelberger et al., 1983
Butylated hydroxytoluene 128-37-0	– (1) – (2) – (3) – (8)	+ (1) + (2*)	+ (8)	– (1) +/– (4)	– (4)	– (1) ? (2) – (4) – (8)	+ (P) + (P)	Mondal and Heidelberger, 1980 Heidelberger and Mondal, 1982
3-Chloro-4-(dichloromethyl)-5-hydroxy-2(5*H*)-furanone (MX) 77439-76-0	+/– (5)	+ (3)	+/– (3)			– (2) – (3)	+ (P)	Laaksonen et al., 2001
3-Chloro-4-methyl-5-hydroxy-2(5H)-furanone (MCF) 112309-61-2	+ (3)						–	Laaksonen et al., 2003
p-Chloroaniline 106-47-8	+/– (1) +/– (5)	+ (1) + (2*) + (3) + (5)		+ (1) + (5)			+	Dunkel et al., 1988
3 (Chloromethyl)-pyridine HCL 6959-48-4	+/– (3)	+ (2*) + (3)					–	Dunkel et al., 1988
Cholic acid 81-25-4	+/– (3)						+ (P)	Kaibara et al., 1984
Cigarette smoke condensate	+ (7)	+ (7)					+	Benedict et al., 1975

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	Ames	Mammalian cells		*In vitro*	*In vivo*			
		Mouse Lymphoma TK	HPRT		Chrom. Ab.	Micro nucleus		
Cinnamyl anthranilate 87-29-6	– (1) – (3)	+ (1) + (2*) + (7)		– (1) – (7)	– (1)	– (1) – (7)	–/+ (II) –/?	Schechtman et al., 1987 Dunkel et al., 1988
Clofibrate 637-07-0	– (3) – (5)		– (3) – (5)	– (5)			+ (P)	Lillehaug et al., 1986
Cyclopenta[cd]-pyrene 27208-37-3	+ (2)	+ (2*)					+	Krolewski et al., 1986
Cyclophosphamide 50-18-0	+ (2) +/– (3) +/– (5)	+ (1) + (2*) + (3) + (5)	+ (2^{9}) + (5)	+ (2) + (5)	+ (2) +/– (5)	+ (1) + (2) + (5)	+ ? + w (+S9)	Heidelberger et al., 1983 Dunkel et al., 1988 Patierno et al, 1989
Daminozide 1596-84-5	– (1) – (5)	? (1)		– (1) + (5)	+ (5)	? (1)	–	Dunkel et al., 1988
Dibenz(a,h)anthracene 53-70-3	+ (2) +/– (3) + (5)	+ (2*) + (5)	+ (2^{10}) + (3) + (5)	+ (5)			+ – +/– –/+(II)	Heidelberger et al., 1983 Lubet et al., 1983 Dunkel et al, 1988 Schechtman et al., 1987
p,p'-Dichlorodiphenyl dichloroethane *(p,p'*-DDD) 72-54-8	– (1)			– (1) + (2)			+	Langenbach and Gingell, 1975
p,p'-Dichlorodiphenyl dichloroethylene *(p,p'*-DDE) 72-55-9	– (1) – (2) – (3)	+ (1) + (2*) + (3)		– (1) + (2)			+	Langenbach and Gingell, 1975
p,p'-Dichlorodiphenyl trichloroethane (DDT) 50-29-3	– (1) – (2) – (3)	– (1)	+ (2^{10})	– (1) + (2) – (4)		? (2)	+	Langenbach and Gingell, 1975
Diepoxybutane, 1,2 :3,4 1464-53-5	+ (2) + (3) +/– (5)	+ (2*) + (5)	+ (5)	+ (5)	+ (5)	+ (5)	+	Nelson and Garry, 1983

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	Ames	Mammalian cells		*In vitro*	*In vivo*			
		Mouse Lymphoma TK	HPRT		Chrom. Ab.	Micro nucleus		
Di(2-ethylhexyl)phthalate (DEHP) 117-81-7	– (1) – (3) – (5)	– (1) # (2*) +/– (3) +/– (4) +/– (5)	– (5)	– (1) – (4) – (5)	– (1) – (4) – (5)	– (1) + (4) – (5)	– +w/+w	Sanchez et al., 1987 Lawrence and McGregor, 1985
Diethylstilbestrol (DES) 56-53-1	– (1) – (2) – (3) – (5)	+ (1) + (2*) +/– (3) +/– (5)	– (5)	+ (1) +/– (5)	+ (5)	+/– (1) + (2) – (5)	+ (P) –/ ? –/+	Lillehaug and Djurhuus, 1982 Dunkel et al., 1988 Lawrence and McGregor, 1985
N,N-Dimethyl-4-aminoazobenzene 60-11-7	+ (1) + (2) +/– (3) +/– (5)	– (2*) +/– (3) + (5)	– (3) – (5)	+/– (5)		+ (2) – (5)	+ –	Dunkel et al., 1988 Nesnow et al., 1982
7,12-Dimethyl benzanthracene 57-97-6	+ (1) + (2) + (3)	+ (1) + (2*) + (3)	+ (2^{9}) + (3)	+ (2)	+ (2)	+ (1) + (2)	+ + –/+(II) +/+	Saxholm, 1979 Heidelberger et al., 1983 Schechtman et al, 1987 Dunkel et al., 1988
Epichlorhydrin 106-89-8	+ (5) + (2) +/– (3)	+ (2*) + (5)	– (3) +/– (5)	+ (2) +/– (5)	+/? (2) +/– (5)	– (5) ? (2^{12})	+ + +	Nourse et al., 1983 Kolman et al., 1994 Kolman and Dusinska, 1995
Estradiol 50-28-2	– (1) – (3) – (5)	? (2*)	+/– (3) – (5)		– (7)	– (1)	+	Kennedy and Weichselbaum, 1981
Ethyl Alcohol Ethanol 64-17-5	– (1) – (2) – (3) – (5)	– (2*) – (3) + (4) – (5)		– (2) – (4) +/– (5)	? (4) – (5)	– (2) – (4) +/– (5)	+	Swierenga and Yamasaki, 1992
Ethyl methanesulfonate (EMS) 62-50-0	+/? (1) + (2) +/– (3) +/– (5)	+ (2*) + (3)	+ (2^{9}) + ($2^{10,11}$) + (3)	+ (1) + (5)	+ (1) +/? (2) + (5)	+ (2) + (5)	+ + –/+(II) +/+ + (init) +	Evans et al., 1981 Lubet et al., 1983 Schechtman et al., 1987 Dunkel et al., 1988 Frazelle et al., 1984 Borek et al., 1984

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Carcinogen CAS number	Point mutations			Cytogenetics (rodent and human cells)			C3H/10T1/2 cell transformation	References C3H/10T1/2 cell transformation assays
	Ames	Mammalian cells		*In vitro*	*In vivo*			
		Mouse Lymphoma TK	HPRT		Chrom. Ab.	Micro nucleus		
Ethylene Oxide 75-21-8	+ (2) + (3) +/– (5)		+ (2^9) + (5)	+ (5)	+ (5)	+ (2) + (5)	+ +	Kolman et al., 1990. Kolman et al., 1989
5-Fluorouracil 51-21-8	– (1) – (3) +/– (5)	+ (3)		+ (5)	– (5)	+ (2) + (5)	+	Heidelberger et al., 1983
Formaldehyde 50-00-0	+(1) +/– (3) +/– (5)	+ (2*)	+ (5) + (6)	+ (1) + (5)	+/– (5)	? (2) +/– (5)	+ (P) + (init)	Frazelle et al., 1983 Ragan and Boreiko, 1981
Hexamethyl phosphoramide (HMPA) 680-31-9	– (1) – (5)	+ (1) + (2*) +/– (3) + (5)	–/+ (3)	– (1) – (5)	+ (1) +/– (5)	+ (2) + (5)	+/– –	Lawrence and McGregor, 1985 Nesnow et al., 1985
N-Hydroxy-2-acetylaminofluorene 53-95-2	+ (2) + (3)		+ (2^9)				+ –/+(II)	Oglesby et al., 1982 Schechtman et al., 1987
Melphalan 148-82-3	+ (1) + (2) + (3) +/– (5)	+ (5)		+ (1) + (5)	+ (5)	+ (1)	+ + +	Murnane and Byfield, 1981 Hall et al., 1982 Heidelberger et al., 1983
8-Methoxypsoralen + UVA 298-81-7	+ (5)	+ (5)	+ (5)	+ (5)	+ (5)	+ (5)	+	Uwaifo et al., 1983
Methyl iodide 74-88-4	+ (3) +/– (5)	? (2*) + (3)					–	Oshiro et al., 1981
Methyl methane sulfonate (MMS) 66-27-3	+ (1) + (2) +/– (3) +/– (5)	+ (2*) + (3) + (5)	+ (2^9) +/– (5)	+ (2)	+ (2) +/– (5)	+ (2) + (5)	+ + (init) +	Oshiro et al., 1981 Frazelle et al., 1984 Borek et al., 1984
Methylazoxymethanol acetate 592-62-1	+ (1) + (3)	+ (2*) + (3)					–/+(II)	Schechtman, 1987

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	Ames	Mammalian cells		*In vitro*	*In vivo*			
		Mouse Lymphoma TK	HPRT		Chrom. Ab.	Micro nucleus		
3-Methylcholanthrene (MCA) 56-49-5	+ (1) + (2)	+ (2*)	+ (2^{9}) + (3)	– (1) – (2)		? (2)	+ +/+	Heidelberger et al., 1983 Dunkel et al., 1988
4,4'-Methylene bis-(N,N'-dimethylaniline) 101-61-1	+/– (1) + (2) + (9)	+ (1) + (2*) + (9)		– (1)			+/+	Dunkel et al., 1988
4-Methylnitrosoamino-1-(3-pyridyl)-1-butanone (NNK) 64091-91-4	+ (3)		+ (3)			+ (1)	–/+ (tf) –/+ (tf)	Tiano et al., 1993 Nesnow et al, 1994
Metronidazole 443-48-1	+ (1) + (2) + (3) +/– (5)		+/– (5)	– (5)	– (5)	+ (2) – (5) – ? (2^{12})	+	Miller et al., 1982
Mezerein 34807-41-5	– (1) – (3)						+ (P)	Boreiko et al., 1986
Michler's ketone, 4,4'-bis-(dimethylamino) benzophenone 90-94-8	+/– (1) +/– (3)	+ (1) + (2*)		+/– (1)			+/+ +/+(II)	Dunkel et al., 1988 Schechtman et al., 1987
Mitomycin C 50-07-7	+/– (3) +/– (5)	+ (2*) + (3)	+ (2^{9}) + (3)	+ (2) + (5)	+ (1) + (2) + (5)	+ (1) + (2) + (5)	+ (init)	Frazelle et al., 1984
2-Naphthylamine 91-59-8	+ (1) + (2) +/– (3) + (6) +/– (5)	+ (1) + (2*) + (5)	+ (2^{9}) +/– (5)	+ (1) + (5)	? (1)	– (1) + (2) – (5) + (2^{12})	–/+(II)	Schechtman et al., 1987
Nitrilotriacetic Acid 139-13-9	– (1) – (3)	– (3)		– (1)			+	Dunkel et al., 1988
2-Nitro-1,4-phenylenediamine 5307-14-2	+ (1) + (2) +/– (5)	+ (1) + (2*) + (3) + (5)	+ (3)	+ (1) + (2) + (5)	– (5)	? (2) – (5)	+ –/+(II) +/?	Heidelberger et al., 1983 Schechtman et al., 1987 Dunkel et al., 1988

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	Ames	Mammalian cells		*In vitro*	*In vivo*			
		Mouse Lymphoma TK	HPRT		Chrom. Ab.	Micro nucleus		
5-Nitro-o-toluidine 99-55-8	+ (1) +/– (3) +/– (5)			+ (1)			+	Dunkel et al., 1988
Nitrogen mustard 51-75-2	+ (2) +/– (5)	+ (2*)	+ (5)	+ (2) + (5)	+ (2)	+ (2) + (5)	+ +	Poole and McGregor; 1982 Murnane and Byfield, 1981
4-Nitroquinoline-N-oxide 56-57-5	+ (2) + (3)	+ (1) + (2*) + (3)	+ (2^{9}) + (3)	+ (1) + (2)	+ (1) + (2)	+ (2)	+ +	Oshiro and Balwierz, 1982 Poole and McGregor, 1982
N-Nitroso-*N*-ethylurea (ENU) 759-73-9	+ (1) + (2) + (3) + (5)		+ (2^{9}) + (3)	+ (1) + (2) + (5)	+ (2) + (5)	+ (1) + (2) + (5)	+ (init) + (init), +(II) +	Frazelle et al., 1984 De Kok et al., 1986 Borek et al., 1984
N-Nitroso-*N*-methylnitroguanidine (MNNG) 70-25-7	+ (2) + (3)	+ (2*) + (3)	+ (2^{9}) + (3)	+/– (2)	? (2)	+ (2)	+ + + + + +/–	Heidelberger et al., 1983 Kaibara et al., 1984 Abernethy et al., 1985 Frazelle et al., 1984 Borek et al., 1984 Dunkel et al., 1988
N-Nitroso-*N*-methylurea (MNU) 684-93-5	+ (2) + (3) + (5)	+ (2*)	+ (2^{9}) + (5)	+ (2) + (5)	+ (2) + (5)	+ (1) ? (2)	+ + (init)	Oshiro et al., 1981 Frazelle et al., 1984
N-Nitrosodiethylamine (NDEA) 55-18-5	+/– (1) + (2) +/– (3)	+ (2*) + (3)	+ (2^{9}) +/– (3)	+ (2)		? (2)	–/+(II) –/+ (tf)	Schechtman et al., 1987 Nesnow et al., 1994
N-Nitrosodimethylamine (NDMA) 62-75-9	+/– (1) + (2) +/– (3) + (5)	+ (2*) +(3) + (5)	+ (2^{9}) + (3) + (5)	+/– (1) + (2) + (5)	– (2) + (6)	+ (2)	–/+(II) –/–	Schechtman et al., 1987 Lawrence and McGregor, 1985
N-Nitrosodiphenylamine Diphenyl nitrosamine 86-30-6	– (1) – (2) – (3)	+ (1) – (2*)	– (2^{10})	– (1)		? (2)	+/–	Dunkel et al., 1988
Okadaic acid 78111-17-8	– (3)	+ (3)					– (Inh)	Mordan et al., 1990

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	Ames	Mammalian cells		*In vitro*	*In vivo*			
		Mouse Lymphoma TK	HPRT		Chrom. Ab.	Micro nucleus		
Phenacetin 62-44-2	– (1) +/– (3) +/– (5)	– (1) # (2*)		+/– (5)	+ (5)	+ (2) +/– (5)	+ +	Patierno et al., 1989 Dybing et al., 1981
Phenobarbital 50-06-6	+w (1) – (5)	+ (1) ? (2*) +/– (3) – (5)	+ (3) + (5)	+ (1)		+ (2)	–/– (P) –/– (P) –/– (P)	Lawrence and McGregor, 1985 Mondal and Heidelberger, 1980 Heidelberger and Mondal, 1982
Procarbazine HCl Natulan 366-70-1	– (1) – (2) – (3)	+ (2*) + (3)	+ (3)			+ (2)	–/?	Dunkel et al., 1988
1,3-Propane sultone 1120-71-4	+ (2) + (3) + (5)			+ (5)			+ +w	Nesnow et al., 1982 Oshiro et al., 1981
β-Propionolactone 57-57-8	+ (1) + (3) +/– (5)	+ (2*) + (3) + (5)	+ (2^9) + (5)	+ (1) + (5)	+ (5)	? (2) +/– (5)	+	Oshiro et al., 1981
Propylene Oxide 75-56-9	+ (1) + (3) +/– (5)	+ (1) + (2*) + (3) + (5)	+ (5)	+ (1) + (5)	+ (1) +/– (5)	– (1) +/– (5)	+ +	Kolman et al., 1994 Kolman and Dusinska, 1995
Propyleneimine, 1,2- 75-55-8	+ (1) + (2) + (3) +/– (5)						–/–(II)	Schechtman et al., 1987
Quercetin 117-39-5	+ (1) + (2) +/– (3) +/– (5)	+ (2*)	? (2^9)	+ (1) + (5)	+ (5)	+ (2) + (5)	+	Panse et al., 1997
p-Rosaniline hydrochloride CI Basic Red 9 569-61-9	+/? (1) – (2) +/– (3) +/– (5)	+ (2*) + (5)	– (1) – (6)	– (1) – (5)		– (1)	–/– –/–(II)	Dunkel et al., 1988 Schechtman et al., 1987

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	Ames	Mammalian cells		*In vitro*	*In vivo*			
		Mouse Lymphoma TK	HPRT		Chrom. Ab.	Micro nucleus		
Safrole 94-59-7	– (1) – (2)	+ (1) + (2*) +/– (3)	+/– (3)	– (1)		? (2)	+/– +/–	Lawrence and McGregor, 1985 Nesnow et al., 1985
Styrene 100-42-5	– (1) + (2) +/– (3) +/– (5)		– (2^{10}) +/– (5)	– (1) + (5)	+ (2) +/– (5)	+ (2) +/– (5)	–/–(P)	Male et al., 1985
Styrene oxide (96-09-3)	+ (1) + (2) +/– (3) +/– (5)	+ (1) + (2*) + (5)	+ (2^{10}) + (5)	+ (5)	+/– (5)	? (2) – (5)	–/+(P)	Male et al., 1985
2,3,7,8-Tetrachlorodibenzodioxin (TCDD) 1746-01-6	– (1) – (3) – (5)	– (1) # (2*) – (3) + (5)		– (1) + (5)	– (1)	? (2)	+(P)	Abernethy et al., 1985
12-*O*-Tetradecanoyl phorbol 13-acetate (TPA) 16561-29-8	– (2)		– (2^{10})				+(P) +(P) +(P)	Sivak and Tu, 1980 Boreiko et al., 1982 Cain et al., 1993
Thioacetamide 62-55-5	– (1) – (2) – (3)	+ (1) ? (2*) +/– (3)				+ (2)	–	Dunkel et al., 1988
Thiotepa 52-24-4	+ (5)		+ (5)	+ (5)	+ (5)	+ (5)	+ +	Heidelberger et al., 1983 Swierenga and Yamasaki, 1992
o-Toluidine 95-53-4	+ (1) ? (2) – (5)	+ (1) ? (2*) – (3) +/– (4) +/– (5)	+/– (3) +/– (5)	+ (1) +/– (5)		+/– (1) – (5)	+/– +	Lawrence and McGregor, 1985 Nesnow et al., 1985
Tris (2,3-dibromopropyl phosphate) 126-72-7	+ (1) + (2) + (3) +/– (5)	+ (5)	+ (5)	+/– (5)	+/– (5)	+ (2) + (5)	–/+w ?/– –/+(II)	Sala et al., 1982 Dunkel et al., 1988 Schechtman et al., 1987

Table 8: Rodent carcinogenic organic chemicals tested with the C3H10T1/2 cell transformation assay

Carcinogen CAS number	Point mutations			Cytogenetics (rodent and human cells)			C3H/10T1/2 cell transformation	References C3H/10T1/2 cell transformation assays
	Ames	Mammalian cells		*In vitro*	*In vivo*			
		Mouse Lymphoma TK	HPRT		Chrom. Ab.	Micro nucleus		
Tris (2-chloroethyl) phosphate 115-96-8	– (1) – (2) +/– (3) +/– (5)		– (5)	– (1) – (5)		+ (2)	–/+w	Sala et al., 1982

NTP database
GENETOX database
CCRIS database
IUCLID database
Genetic Activity Profile
CICADS (IPCS)
IARC monographs
Great Lakes Water Quality Agreement Genetic Activity Profiles
IRIS (US EPA)
(2*) GENETOX Phase III: review (Mitchell *et al.,* 1997)
(2^{9}) GENETOX Phase III: HPRT/CHO (Li *et al.,* 1988)
(2^{10}) in GENETOX : HPRT/V79 (Bradley *et al.,* 1981)
(2^{11}) in GENETOX : HPRT/CHO (Hsie *et al.,* 1981)
(2^{12}) in GENETOX : (Mavournin *et al.,* (1990)
(P) tested as a promoter of transformation (+(P); -(P): positive or negative results, respectively)

+	Positive result
+/+	Positive standard transformation in independent assays
–	Negative result
–/–	Negative standard transformation result in independent assays
+w	Weakly positive result
?	Inconclusive result
+/–	Diverging results inside a database or between independent transformation assays within a single report
#	not testable
eq	equivocal
init	tested as an initiator of transformation
(II)	Level 2 replating assay (+(II) or –(II) positive or negative results, respectively)
Inh	Inhibitory
Sync Cell	cycle-synchronized culture
(tf)	transfected with an exogenous gene for metabolic activation

Table 9: Non-carcinogenic and inconclusive for carcinogenicity organic chemicals tested with the C3H10Ht1/2 cell transformation assay

Non-Carcinogen CAS number	Point mutations			Cytogenetics (rodent and human cells)			C3H/10T1/2 cell transformation	References C3H/10T1/2 cell transformation assays
	Ames	Mammalian cells		*in vitro*	*in vivo*			
		Mouse Lymphoma TK	HPRT		Chrom. Ab.	Micro nucleus		
6-Aminochrysene 2642-98-0	+ (2) +/– (3) + (6)		+/– (3) + (6)				–/+(II)	Schechtman et al., 1987
Anilazine 101-05-3	– (1) – (3)	+ (1) # (2*) + (3)	– (3)	– (1)			+/– –/– (II)	Dunkel et al., 1988 Schechtman et al., 1987
Anthracene 120-12-7	+w (1) – (2) +/– (3)	+ (1) + (2*) + (3) +/– (4)		– (2)		? (2)	–	Dunkel et al., 1988
Benzo(e)pyrene 192-97-2	+ (1) + (2) +/– (3)	+ (1) + (2*) +/– (3)	– (2^{10})				–/+	Dunkel et al., 1988
Benzoin 119-53-9	–/+w (1) +/– (3)	+ (1) – (2*) +/– (3)	+/– (3)	– (1)	– (1)	– (1)	–	Dunkel et al., 1988
Caffeine 58-08-2	– (1) – (2) – (3)	? (2*)	– (2^{10})	+ (2)	? (2)	+ (2)	+	Chan and Little, 1982
Caprolactam 105-60-2	– (1) – (3)	– (1) – (2*) – (3) – (4)	– (2^{9}) – (3)	– (1)	– (1)	– (1) – (4)	– –	Nesnow et al., 1985 Lawrence and McGregor, 1985
Cytosine arabinoside 147-94-4	– (3)	+ (2*) + (3)	+ (2^{10}) +/– (3)	+ (2)		+ (2)	+	Heidelberger et al., 1983
Diazanon 333-41-5	– (1) – (2) – (3)	+ (1) ? (2*)		– (1)			–/+ (II)	Schechtman et al., 1987
2,7-dichlorodibenzo-p-dioxin 33857-26-0	– (1) – (5)						–/– (P)	Abernethy and Boreiko, 1987
N,N'-Dicyclohexylthiourea 1212-29-9	– (1) – (3)	+/– (1) # (2*) +/– (3)		– (1)			–/–	Dunkel et al., 1988

Table 9: Non-carcinogenic and inconclusive for carcinogenicity organic chemicals tested with the C3H10Ht1/2 cell transformation assay

Non-Carcinogen CAS number	Point mutations			Cytogenetics (rodent and human cells)			C3H/10T1/2 cell transformation	References C3H/10T1/2 cell transformation assays
	Ames	Mammalian cells		*in vitro*	*in vivo*			
		Mouse Lymphoma TK	HPRT		Chrom. Ab.	Micro nucleus		
Lithocholic acid 434-13-9	– (1) – (3)	+/– (1) ? (2*) +/– (3)		– (1)			+/– –/+ (P) –/+ (P)	Dunkel et al., 1988 Kaibara et al., 1984 Kawasumi et al., 1988
6-Mercaptopurine 50-44-2	+/– (3)	+ (2*)	+ (3)	+ (1)	+ (2)	+ (2)	– –	Benedict et al., 1977 Heidelberger et al., 1983
Methotrexate 59-05-2	? (2) – (3)	+ (2*) + (3)	– (3)	+ (2)	+ (2)	+ (2)	+ –/– (init)	Heidelberger et al., 1983 Frazelle et al., 1984
Methoxychlor 72-43-5	– (1) – (2)	+ (1) # (2*) +/– (3)	– (3)	– (1)			–/– (II) –/–	Schechtman et al., 1987 Dunkel et al., 1988
Methyl parathion 298-00-0	+ (1) ? (2) +/– (3)	# (2*)		– (1)		+ (2)	–	Breau et al, 1985
4-Nitro-o-phenylenediamine 99-56-9	+ (1) + (2) +/– (3)	+ (1) eq (2*) + (3)	– (3)	– (1) + (2)	– (1)	eq (1) ? (2)	+ –/?	Heidelberger et al., 1983 Dunkel et al., 1988
6-Nitrobenzo[a]pyrene 63041-90-7	+ (2) +/– (3) +/– (5)		? (2^{9}) – (3) + (5)				–	Sala et al., 1987
3-Nitropropionic acid 504-88-1	+ (1) +/– (3) +/– (5)	+ (1) # (2*) + (3) + (5)	– (3) – (5)	+w (1)			+/?	Dunkel et al., 1988
Phenanthrene 85-01-8	+w (1) – (2) +/– (3)			– (2)		? (2)	–/?	Dunkel et al., 1988
p-Phenylenediamine dihydrochloride 624-18-0	+ (1)	+ (1) eq (2*) + (3)	– (3)	+ (1)			–	Dunkel et al., 1988
Pyrene 129-00-0	+/– (1) – (2) +/– (3)	+ (1) + (2*) + (3)	– (2^{10}) – (3)	– (1) – (2)	– (1)	? (2)	+ –	Dunkel et al., 1988 Lubet et al., 1990
Saccharin 81-07-2	– (1)	? (2)					– –	Saxholm et al., 1979 Heidelberger et al., 1983

Table 9: Non-carcinogenic and inconclusive for carcinogenicity organic chemicals tested with the C3H10Ht1/2 cell transformation assay

Non-Carcinogen CAS number	Point mutations			Cytogenetics (rodent and human cells)			C3H/10T1/2 cell transformation	References C3H/10T1/2 cell transformation assays
	Ames	Mammalian cells		*in vitro*	*in vivo*			
		Mouse Lymphoma TK	HPRT		Chrom. Ab.	Micro nucleus		
							+(P)	Mondal et al., 1978

NTP database
GENETOX database
CCRIS database
IUCLID database
Genetic Activity Profile
RTECS
(2*) GENETOX Phase III: review (Mitchell *et al.,* 1997)
(2^9) GENETOX Phase III: HPRT/CHO (Li *et al.,* 1988)
(210) in GENETOX : HPRT/V79 (Bradley *et al.,* 1981)

(P) tested as a promoter of transformation (+(P); -(P): positive or negative results, respectively)
\+ Positive result
+/+ Positive standard transformation in independent assays
– Negative result
–/– Negative standard transformation result in independent assays
+w Weakly positive result
? Inconclusive result
+/– Diverging results inside a database or between independent transformation assays within a single report
\# not testable
eq equivocal
init tested as an initiator of transformation
(II) Level 2 replating assay (+(II) or –(II) positive or negative results, respectively)

Table 10: Inorganic compounds tested with the C3H10T1/2 cell transformation assay and classification for carcinogenicity

Inorganic compound CAS number	Point mutations			Cytogenetics (rodent and human cells)			Carcinog. Class	C3H10T1/2	References C3H10HT1/2 cell transformation assay
	Ames	Mammalian cells		*In vitro*	*In vivo*				
			HPRT		Chrom. Ab.	Micro nucleus			
Ammonium metavanadate 7803-55-6	+ (3)		+ (3)				ND	+ +(P)	Parfett and Pilon, 1995
Amosite 12172-73-5	– (6)		+w (6)	+ (6)			SE (8)	+(P) +(P)	Brown et al, 1983b Hei et al., 1984
Beryllium sulfate 13510-49-1	– (1) – (9)			+/– (9)		– (9)	SE (8)	?/ –	Dunkel et al, 1988
Calcium chromate 13765-19-0	+ (1) + (2) + (5)	+ (1) + (2*) + (3)	+ (3) + (5)	– (1) + (5)		– (5)	SE (8)	+ –	Dunkel et al, 1988 Patierno et al, 1988
Carbon Black 1333-86-4	+/– (5)	– (5)						–	Kirwin et al., 1981
Chrysotile 12001-29-5	– (6)		+w (6)	+ (6)			SE (8)	+(P)	Hei et al, 1984
Crocidolite 12001-28-4	– (3)		+/– (3)	+ (6)			SE (8)	+(P) +(P) +(P)	Brown et al, 1983 Hei et al, 1984 Parfett et al., 1996
Hydrogen peroxide 7722-84-1	? (2) + (9)	+ (2*) + (9)	+ (3)	+ (9)	+ (9)		+ (10)	+	Nassi-Calò et al., 1989
Lead acetate 301-04-2	– (1)	– (1) # (2*) – (3)	+ (3)	– (2)	– (2)	– (1) +/?(2)	SE (8)	–/–	Dunkel et al., 1988
Lead chromate 7758-97-6	–/+ (if solubilized) (5)		– (3)	+ (5)		+ (5)	SE (8)	+	Patierno et al., 1988
Nickel chloride 7718-54-9	– (3)	+ (2*)	+ (3)	+ (5)	+ (5)	– (5)	SE (8)	– –	Miura et al, 1989 Landolph, 1994
Nickel monoxide 1313-99-1	– (1)		+ (3)			– (1)	SE (8)	+	Miura et al., 1989
Nickel subsulfide 12035-72-2	? (1) – (3)		+ (3)			– (1)	SE (8)	+ +	Saxholm et al., 1981 Miura et al., 1989
Nickel sulfate 7786-81-4	– (5)		+ (5)	+ (5)	– (5)		SE (8)	– –	Miura et al, 1989 Landolph, 1994

Table 10: Inorganic compounds tested with the C3H10T1/2 cell transformation assay and classification for carcinogenicity

Inorganic compound CAS number	Point mutations			Cytogenetics (rodent and human cells)			Carcinog. Class	C3H10T1/2	References C3H10HT1/2 cell transformation assay
	Ames	Mammalian cells		*In vitro*	*In vivo*				
			HPRT		Chrom. Ab.	Micro nucleus			
Nickel sulfide(crystalline) 16812-54-7			+ (5)	+ (5)			SE (8)	+	Miura et al., 1989
Ozone 10028-15-6	+ (1) +/– (3)				– (2)		+ (10)	+/+(P)	Borek et al., 1989
Potassium dichromate 7778-50-9	+ (1)	+ (2*) + (5)	+ (2^9) + (5)	+ (2) + (5)	+ (5)	+ (2) + (5)	SE (8)	–	Patierno et al, 1988
Sodium arsenate 7778-43-0		+ (2*)					SE (8)	– –	Landolph, 1989 Landolph et al, 1996
Sodium arsenite 7784-46-5	– (3)	+ (2*) + (3)	? (2^9)			+ (2)	SE (8)	+w	Landolph, 1989

(1) NTP database
(2) GENETOX database
(2*) in GENETOX : MLA (Mitchell *et al.,* 1997)
(2^9) in GENETOX : HPRT/ CHO (Li *et al.,* 1988)
(3) CCRIS database
(4) IUCLID database
(5) IARC monographs on the evaluation of carcinogenic risks to humans. Vol 49: Chromium, Nickel and Welding, 1990, IARC, Lyon, France
(6) Kane AB, Boffetta P., Saracci R., Wilbourn J.D. (eds), Mechanisms of Fibre Carcinogenesis. IARC Sc Publ. N°140, 1996
(7) Oberly *et al.,* J. Toxicol Environ Health, 1982, 9, 367-376
(8) IARC database
(9) Genetic Activity Profiles
(10) Carcinogenic Potency Database
(P) Tested as a promoter of transformation
* ND No IARC summary specific to ammonium metavanadate, but the related pentavalent vanadium oxide (Vanadic acid anhydride) is classified SE (8); CCRIS lists ammonium metavanadate as a tumor inhibitor in drinking water

+	Positive result	#	not testable
–	Negative result	?	Inconclusive result
+w	Weakly positive result	eq	equivocal

+/– Diverging results inside a database

IV. PERFORMANCES

Performances of the 3 CTA

95. The CTA results obtained for the same chemicals are presented in three tables for organic carcinogens (table 11), organic non-carcinogens (table 12) and inorganic chemicals (carcinogens in table 13-1 part A and non-carcinogens in table 13-2 part B). Human and animal carcinogenicity according to the IARC classification is presented in parallel. Results of the CPDB data base is given to confirm rodent carcinogenicity of chemicals in class 3 or chemicals not documented by IARC. When no data were available in CPDB, the CCRIS and NTP, databases and cancer literature were consulted.

96. Tables 11-2, 12-2 and 13-2 list the chemicals by their CAS registry number. The list of carcinogens includes chemicals classified as tumor promoters (identified in italic in table 11-1).

97. It appears that the three CTA results agree when conducted on the same chemicals in most cases.

98. Regarding tumour promotion, a number of laboratories have modified the SHE, BALB/c 3T3, and C3H10T1/2 assays so that they could be used for identification of tumor promoters. The results from tests with these promoter assays are included in the data tables for the three tests, but there were insufficient chemicals tested with each of the modified assays to allow an evaluation of their performance.

99. Regarding human carcinogenicity, the three assays detect 90% of carcinogens in class 1 and 95% in class 2. The exceptions, negative and ambiguous chemicals for cell transformation, have already been cited in section III.

Table 11-1: Rodent carcinogenic organic chemicals tested with the SHE, BALB/c 3T3 and C3H/10T1/2 cell transformation assays and IARC classification*

CAS Registry Number	Chemical name	SHE 7 day pH 6.7	SHE 7 day pH ≥7.0	BALB/c 3T3	C3H/ 10/T1/ 2	IARC CLASS** Human	IARC CLASS** Animal	Gand Z	CCRIS	Ref
75-07-0	Acetaldehyde				–	2B	SE			
60-35-5	Acetamide		+			2B	SE			
103-90-2	Acetaminophen [paracetamol]				+	3	IE	+		
53-96-3	2-Acetylaminofluorene [2-Acetaminofluorene] [2,Fluorenyl acetamide (2-FAA)]	D2	+	+	D2			+		
79-06-1	Acrylamide	+	+	+	+	2A	SE			
107-13-1	Acrylonitrile	+	+	D1	+	2B	SE			
50-76-0	Actinomycin D		+	+	+	3	LE	+		
1162-65-8	Aflatoxin B1		+	+	+	1	SE			
2835-39-4	Allyl isovalerate			–		3	LE	+		
57-06-7	Allyl isothiocyanate			+		3	LE	+		
97-56-3	2-Amino-5-azotoluene [o-Aminoazotoluene]		+			2B	SE			
92-67-1	4-Amino-1,1'-biphenyl [4-Aminobiphenyl]		+		–	1	SE			
68808-54-8	3-Amino-1,4-dimethyl-5*H*-pyrido[4,3-*b*]indole (Trp-P-1)			+		2B	SE			
72254-58-1	3-Amino-1-methyl-5*H*-pyrido[4,3-*b*]indole (Trp-P-2)			+				+		
76180-96-6	Amino-3-methylimidazo [4,5-f] 2-quinoline (IQ)			+	+	2A				
99-57-0	2-Amino-4-nitrophenol					3	LE	+		
613-13-8	2-Aminoanthracene		+	–	D2				+	
60-09-3	*p*-Aminoazobenzene		+			2B	SE			
61-82-5	Amitrole [3-Aminotriazole]		+			3	SE	+		
51264-14-3	Amsacrine				+	2B	SE			
62-53-3	Aniline		–	–	D2	3				
142-04-1	Aniline HCl [Benzenamine HCl]							+		
90-04-0	*o*-Anisidine		+w			2B	SE			
134-29-2	*o*-Anisidine HCl	D2				2B	SE	+		
1143-38-0	Anthralin [Dithranol]			+(P) NC	–/+(P)	3			TP	
84-65-1	Anthraquinone	–								+[NTP TR494]
11097-69-1	Aroclor 1254		–					+		
11096-82-5	Aroclor 1260		+ [1]					+	+	
2465-27-2	Auramine		?					+		
320-67-2	5-Azacytidine		+	+	+	2A	SE			
17804-35-2	Benomyl	–								+[8]
56-55-3	Benz(*a*)anthracene		+	D2	+	2A	SE			
71-43-2	Benzene		+	D1	D2	1	LE			
92-87-5	Benzidine		+	+	D1	1	SE			
531-85-1	Benzidine dihydrochloride			+						
50-32-8	Benzo(*a*)pyrene	+	+	+	+	2A	SE			
105-11-3	1,4-Benzoquinone dioxime [p-Quinonedioxime]				D2	3	LE	+		
94-36-0	Benzoyl peroxide		+(P) NC						+ TP	
100-44-7	Benzyl chloride		+		–	2A	SE			

Table 11-1: Rodent carcinogenic organic chemicals tested with the SHE, BALB/c 3T3 and C3H/10T1/2 cell transformation assays and IARC classification*

CAS Registry Number	Chemical name	SHE 7 day pH 6.7	SHE 7 day pH ≥7.0	BALB/c 3T3	C3H/ 10/T1/ 2	IARC CLASS** Human	IARC CLASS** Animal	Gand Z	CCRIS	Ref
11056-06-7	Bleomycin				+	2B				
2784-94-3	HC Blue 1		+	?		2B				
59-14-3	5-Bromo-2'-deoxyuridine		+		–				+	
25013-16-5	Butylated hydroxyanisol (BHA)			+(P) NC		2B	SE			
128-37-0	Butylated hydroxytoluene (BHT)		+(P) NC	+(P) NC	+ (P) NC	3	LE	+		
85-68-7	Butylbenzylphthalate	+		–		3	IE	+		
120-80-9	Catechol		+	+(P) NC		2B	SE			
302-17-0	Chloral hydrate	+				3	LE	+		
57-74-9	Chlordane Analytical		+(P) NC			2B	SE			
77439-76-0	3-Chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone (MX)				+ (P) NC			+		
112309-61-2	3-Chloro-4-methyl-5-hydroxy-2(5H)-furanone (MCF)				–			+		
3165-93-3	4-Chloro-o-toluidine hydrochloride			+		2A	SE			
106-47-8	*p*-Chloroaniline		+		+	2B	SE			
75-00-3	Chloroethane			–		3	LE	+		
6959-48-4	3-(Chloromethyl)-pyridine HCl			D2	–			+		
1897-45-6	Chlorothalonil		+			2B	SE			
69-09-0	Chlorpromazine	NT								+ TP[9]
81-25-4	Cholic acid			+(P) NC	+(P) NC					+ TP[10]
218-01-9	Chrysene Benzophenanthrene		+	–		3	LE		+	
87-29-6	Cinnamyl anthranilate	–	+	D2	D2	3	LE	+		
637-07-0	Clofibrate	+	+	–	+ (P) NC	3	LE	+		
27208-37-3	Cyclopenta[cd]-pyrene				+	3	LE		+	
50-18-0	Cyclophosphamide		+	D2	+	1	SE			
6055-19-2	Cyclophosphamide H_2O	NT								
59865-13-3	Cyclosporin A	NT				1	SE			
1596-84-5	Daminozide				–			+		
1163-19-5	Decabromo diphenyloxide	–						+		
95-80-7	2,4-Diaminotoluene	+	+	+		2B	SE			
53-70-3	Dibenz(*a,h*)anthracene		+	+	D2	2A	SE			
106-93-4	1,2-Dibromoethane			+		2A	SE			
72-55-9	Dichlorodiphenyldichloroethylene (DDE)				+	2B	SE			
72-54-8	p-p'-Dichlorodiphenyldichloroethane (DDD, TDE)				+	2B	SE			
50-29-3	Dichlorodiphenyl trichloroethane (DDT)		–	+	+	2B	SE			
107-06-2	1,2-Dichloroethane			–		2B	SE			
609-20-1	2,6-Dichloro-*p*-phenylenediamine		+			3	LE	+		
62-73-7	Dichlorvos		+	D1		2B	SE			
60-57-1	Dieldrin	NT				3	LE	+		
1464-53-5	Diepoxybutane, 1,2 :3,4		+	+	+	2B				
111-42-2	Diethanolamine	+				3	LE		+	

Table 11-1: Rodent carcinogenic organic chemicals tested with the SHE, BALB/c 3T3 and C3H/10T1/2 cell transformation assays and IARC classification*

CAS Registry Number	Chemical name	SHE 7 day pH 6.7	SHE 7 day pH ≥7.0	BALB/c 3T3	C3H/ 10/T1/ 2	IARC CLASS** Human	IARC CLASS** Animal	Gand Z	CCRIS	Ref
117-81-7	di (2-Ethylhexyl) phthalate (DEHP)	+	+	–	D2	3	SE	+		
56-53-1	Diethylstilbestrol (DES)	–	+	+	+ (P) D2	1	SE			
105-55-5	*N*,*N'*-Diethylthiourea	+				3	LE	+		
60-11-7	*N*,*N'*-Dimethyl-4-aminoazobenzene [4-Dimethylamino-azobenzene]		+	–	+	2B	SE	+		
57-97-6	7,12-Dimethyl benzanthracene		+	+	+			+		
540-73-8	1,2-Dimethylhydrazine		+			2A	SE			
513-37-1	Dimethylvinyl chloride			+		2B	SE			
121-14-2	2,4-Dinitrotoluene	+	–	–		2B	SE			
606-20-2	2,6-Dinitrotoluene	+				2B	SE			
123-91-1	1,4-Dioxane			+		2B	SE			
57-41-0	Diphenylhydantoin		+			2B	SE			
106-89-8	Epichlorhydrin	+		+	+	2A	SE			
106-88-7	1,2-Epoxybutane		+	–		2B	LE			
50-28-2	Estradiol	NT	+	+	+	1				
57-63-6	Ethinylestradiol		–	–		1	SE			
13073-35-3	l-Ethionine		+	–				+		
140-88-5	Ethyl acrylate			+		2B	SE			
64-17-5	Ethyl alcohol [Ethanol]	–	–	+	+	1	IE			
100-41-4	Ethyl benzene	+				2B	SE	+		
62-50-0	Ethyl methanesulfonate		+	+	+	2B				
111-76-2	Ethylene glycol butyl ether [2-Butoxyethanol]	NT							+	
75-21-8	Ethylene oxide				+	1	SE			
96-45-7	Ethylene thiourea		?	D1		3	SE	+		
51-21-8	5-Fluorouracil				+	3	IE	+		
50-00-0	Formaldehyde	+			+, +(P)	2A				
98-00-0	Furfuryl alcohol	–							+	
3688-53-7	Furylfuramide (AF-2)		+	+		2B				
446-72-0	Genistein	–								+[11]
765-34-4	Glycidaldehyde		+	+		2B	SE			
126-07-8	Griseofulvin	–				2B	SE			
57653-85-7	1,2,3,6,7,8-HCDD				+(P)[2]	3	LE	+		
19408-74-3	1,2,3,7,8,9-HCDD				NC	TP				
87-68-3	Hexachloro-1,3-butadiene		+			3	LE	+		
67-72-1	Hexachloroethane			–		2B	SE			
680-31-9	Hexamethyl phosphoramide		+	+	D2	2B	SE			
10034-93-2	Hydrazine sulfate		?					+	+	
123-31-9	Hydroquinone		+			3	LE	+		
53-95-2	*N*-Hydroxy-2-acetylaminofluorene		+	+	D2			+		
78-59-1	Isophorone			+				+	+, eq	
5989-27-5	*d*-Limonene	–	–	–		3				
148-82-3	Melphalan [Sarkolysin] [Phenyl alanine mustard]	NT	+	+	+	1	SE			
5874-97-5	Metaproterenol hemisulfate	–								+[12]
135-23-9	Methapyrilene HCl	+	+	–				+		
3544-23-8	3-Methoxy-4-aminoazobenzene		+					+		

Table 11-1: Rodent carcinogenic organic chemicals tested with the SHE, BALB/c 3T3 and C3H/10T1/2 cell transformation assays and IARC classification*

CAS Registry Number	Chemical name	SHE 7 day pH 6.7	SHE 7 day pH ≥7.0	BALB/c 3T3	C3H/ 10/T1/ 2	IARC CLASS** Human	IARC CLASS** Animal	Gand Z	CCRIS	Ref
298-81-7	8-Methoxypsoralen + UVA				+	1	SE			
55-80-1	3-Methyl-4'-(dimethyl amino)azobenzene		?					+		
93-15-2	Methyl eugenol	+							+	
74-88-4	Methyl iodide		+		–		LE		+	
66-27-3	Methyl methane sulfonate (MMS)	NT	+	+	+	2A	SE			
592-62-1	Methylazoxymethanol acetate		+	+	D1	2B				
598-55-0	Methylcarbamate (Methyl urethane)	–	–	+		3	IE	+		
56-49-5	3-Methylcholanthrene (MCA)	+	+	+	+			+		
21340-68-1	Methylclofenapate		+					+		
101-61-1	4,4'-Methylene bis-(*N*,*N*'-dimethylaniline) [Bis(p-dimethylamino)diphenylmethane]		+	+	+	3	LE	+		
64091-91-4	4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK)				D2	2B	SE	+		
23564-05-8	Methylthiophanate			+						+[13]
443-48-1	Metronidazole				+	2B	SE			
34807-41-5	Mezerein		+(P) NC	+	+ (P) NC				TP	
90-94-8	Michler's ketone, [4,4'Bis-(dimethylamino)benzophenone]				+			+		
50-07-7	Mitomycin C		+	+	+	2B	SE			
150-68-5	Monuron		+	–		3	LE	+		
389-08-2	Nalidixic acid			–			CE			+[14]
91-59-8	2-Naphthylamine	+	+	D2	D1	1	SE			
139-13-9	Nitrilotriacetic Acid	–	–	+	+	2B	SE			
24554-26-5	N-(4-(5-Nitro-furanyl)-2-thiazolyl)formamide		+					+		
5307-14-2	2-Nitro-p-phenylenediamine [2-Nitro-1,4-phenylenediamine]		+	+	+	3		+		
99-55-8	5-Nitro-*o*-toluidine [2-Amino-4-nitrotoluene]	NT	–		+	3	LE	+		
51-75-2	Nitrogen mustard				+	2A	SE			
79-46-9	2-Nitropropane		+			2B	SE			
5522-43-0	1-Nitropyrene			+		2B	SE			
789-07-1	2-Nitropyrene			+		3	IE		+	
56-57-5	4-Nitroquinoline-1-oxide [4-Nitroquinoline-N-oxide]		+	+	+				+	
612-64-6	*N*-Nitroso-*N*-ethylaniline		+	D2					n d	
759-73-9	*N*-Nitroso-*N*-ethylurea [N-Ethyl-N-nitrosourea (ENU)] [1-Ethyl-1-nitrosoruea]		+	+	+	2A	SE			
70-25-7	*N*-Nitroso-*N*-methylnitroguanidine [N-Methyl-N'-nitro-N-nitrosoguanidinen (MNNG)]	+	+	+	+	2A	SE			
684-93-5	*N*-Nitroso-*N*-methylurea [N-Methyl-N-nitrosourea (MNU)]	NT		+	+	2A	SE			

Table 11-1: Rodent carcinogenic organic chemicals tested with the SHE, BALB/c 3T3 and C3H/10T1/2 cell transformation assays and IARC classification*

CAS Registry Number	Chemical name	SHE 7 day pH 6.7	SHE 7 day pH ≥7.0	BALB/c 3T3	C3H/ 10/T1/ 2	IARC CLASS** Human	IARC CLASS** Animal	Gand Z	CCRIS	Ref
55-18-5	*N*-Nitrosodiethylamine [Diethyl nitrosamine]		+	D1	D2	2A	SE			
62-75-9	N-Nitrosodimethylamine [Dimethyl nitrosamine]		?	+	D2	2A	SE			
86-30-6	*N*-Nitrosodiphenylamine [Diphenyl nitrosamine]		+	D2	D2			+		
78111-17-8	*Okadaic acid*		+(P) D	+(P) D	–				TP	
101-80-4	4,4'-Oxydianiline		+	+		2B	SE			
434-07-1	Oxymetholone	+							+	
62-44-2	Phenacetin	–			+	2A	SE			
50-06-6 57-30-7	Phenobarbital sodium Phenobarbital	+ 	D2 +	D2	–	2B	SE			
77-09-8	Phenolpthalein	NT	+			2B	SE			
24928-17-4	Phorbol 12,13-didecanoate (PDD)		+(P)[3] NC	+(P)[4,5] NC	+(P)[6] NC				TP	
67774-32-7	Polybrominated biphenyls [Firemaster FF1]	+						+		
1336-36-3	Polychlorobiphenyl (PCB)			+		2A				
366-70-1	Procarbazine HCl [Natulan]		+	–	–					
57-83-0	Progesterone		D2	D2		2B	SE			
1120-71-4	1,3-Propane sultone		?		+	2B	SE			
57-57-8	β-Propionolactone		+	+	+	2B	SE			
75-56-9	Propylene oxide	+			+	2B	SE			
75-55-8	1,2-Propyleneimine Propyleneimine		+	+	–	2B	SE			
110-86-1	Pyridine	–				3	LE		+	
59-33-6	Pyrilamine maleate			–				+		
117-39-5	Quercetin			+	+	3	LE	+		
50-55-5	Reserpine	+	+	D2		3	LE	+		
569-61-9	*p*-Rosaniline (CI Basic Red 9) [Magenta]		+	D2	–	2B	SE			
94-59-7	Safrole		+	+	D1	2B	SE			
128-44-9	Sodium saccharin			+		3	SE	+		
100-42-5	Styrene				–	2B	LE	+		
96-09-3	Styrene oxide				–,+(P)	2A	SE			
723-46-6	Sulfamethoxazole	NT				3	LE		+	
58-22-0	Testosterone		+			2A	SE			
1746-01-6	2,3,7,8-Tetrachlorodibenzodioxin (TCDD)				+(P)	1				
630-20-6	1,1,1,2-Tetrachloroethane			–		3	LE	+		
79-34-5	1,1,2,2-Tetrachloroethane			D2		3	LE	+		
127-18-4	Tetrachlorethylene			D2		2A	SE			
16561-29-8	12-O- Tetradecanoyl phobol-13-acetate (TPA)	+	+	+(P) NC	+(P)				+ TP	
109-99-9	Tetrahydrofuran	–						+		
62-55-5	Thioacetamide		+		–	2B	SE			
52-24-4	Thio-TEPA				+	1	SE			
62-56-6	Thiourea		+			3	L(I)E	+		
95-53-4	*o*-Toluidine		+	+	D1	2A	SE			

Table 11-1: Rodent carcinogenic organic chemicals tested with the SHE, BALB/c 3T3 and C3H/10T1/2 cell transformation assays and IARC classification*

CAS Registry Number	Chemical name	SHE 7 day pH 6.7	SHE 7 day pH ≥7.0	BALB/c 3T3	C3H/ 10/T1/ 2	IARC CLASS** Human	IARC CLASS** Animal	Gand Z	CCRIS	Ref
636-21-5	*o*-Toluidine HCl	+								
79-00-5	1,1,2-Trichloroethane			+		3	LE	+		
79-01-6	Trichloroethylene			+		2A	SE			
88-06-2	2,4,6-Trichlorophenol		+			2B	LE			
126-72-7	Tris (2,3-dibromopropyl phosphate)				D2	2A	SE			
115-96-8	Tris (2-chloroethyl) phosphate				D1	3	IE	+		
78-42-2	Tris(2-ethylhexyl)phosphate (TEHP)	–		–				+		
51-79-6	Urethane [Ethyl carbamate]	+	+			2B	SE			
50471-44-8	Vinclozolin			+[7]						+ [15]
75-01-4	Vinyl chloride			+		1	SE			
50892-23-4	Wyeth-14,643	+	+					+		

*The results of the Carcinogenic Potency Data Base according to Gold and Zeiger (1997) (GandZ), the Chemical Carcinogenesis Research Information System (CCRIS) and the National Toxicology Program (NTP) are given for chemicals not classified 1, 2A or 2B by IARC.

**IARC CLASS:

[Carcinogen risks to Humans] 1 (Known human carcinogen), 2A (Probable human carcinogen), 2B (Possible human carcinogen), 3 (Not classifiable for human carcinogen)

[Evidence for animal carcinogen] SE, LE, IE : sufficient, limited, inadequate evidence for animal carcinogenicity

***Chemicals in italic are tumor promoters included in Table 11-1, but not considered in the evaluation of CTA performances for predicting carcinogenicity reported in table 14.

Nd no data on carcinogenicity in CCRIS
Neg negative response for carcinogenicity
CE clear evidence for animal carcinogenicity
(P) *in vitro* tumor promoter
(ST) sequential treatment (initiation-promotion)
+ (P) positive as a tumor promoter
TP *in vivo* tumor promoter
TI *in vivo* tumor inhibitor
+ Positive result
+w Weekly positive result
– Negative result
IC Inconclusive for carcinogenicity
Eq equivocal
? inconclusive result
D divergent results within (D1) or among (D2) laboratories
NC no call
NT Not tested in 7 day pH 6.7 SHE assay
[] Synonym

References for chemicals not included in tables 2, 5, 8 because no genotoxicity data was available

Rivedal *et al.,* 2000
Abernethy and Boreiko, 1987
Rivedal and Sanner, 1982
Frixen and Yamazaki, 1987
Tsuchiya *et al.,* 2000
Mondal *et al.,* 1976
Perocco *et al.,* 1993
http://www.parpharm.com/pdf/product/metaproterenol_po.pdf
Thiophanate-methyl | Pesticides | US EPA http://www.epa.gov/oppsrrd1/reregistration/tm/index.htm
NTP Technical Report (TR) 368
Vinclozolin | Pesticides | US EPA http://www.epa.gov/oppsrrd1/reregistration/vinclozolin/index.htm

References for carcinogenicity data

McCarroll *et al.,* 2002
McClain *et al.,* 2001
Pereira *et al.,* 2004
Newbold *et al.,* 2001

Table 11-2: Index of Rodent carcinogenic organic chemicals tested for their transformation potency by Chemical Abstract Service (CAS) Registry Number

CAS Registry Number	Chemical Name	CAS Registry Number	Chemical Name
50-00-0	Formaldehyde	60-11-7	*N*,*N'*-Dimethyl-4-aminoazobenzene 4-Dimethylamino-azobenzene
50-06-6	Phenobarbital	60-35-5	Acetamide
50-07-7	Mitomycin C	60-57-1	Dieldrin
50-18-0	Cyclophosphamide (anhydrous form)	61-82-5	Amitrole (3-Aminotriazole)
50-28-2	Estradiol	62-44-2	Phenacetin
50-29-3	Dichlorodiphenyl trichloroethane (DDT)	62-50-0	Ethyl methanesulfonate (EMS)
50-32-8	Benzo(*a*)pyrene	62-53-3	Aniline
50-55-5	Reserpine	62-55-5	Thioacetamide
50-76-0	Actinomycin D	62-56-6	Thiourea
51-21-8	5-Fluorouracil	62-73-7	Dichlorvos
51-75-2	Nitrogen mustard	62-75-9	N-Nitrosodimethylamine Dimethyl nitrosamine
51-79-6	Urethane (ethyl carbamate)	66-27-3	Methyl methane sulfonate (MMS)
		67-72-1	Hexachloroethane
52-24-4	Thio-TEPA	69-09-0	Chlorpromazine
53-70-3	Dibenz(*a*,*h*)anthracene	70-25-7	Nitroso-*N*-methylnitroguanidine (MNNG) N-Methyl-*N'*-nitro-*N*-nitrosoguanidine
53-95-2	*N*-Hydroxy-2-acetylaminofluorene	71-43-2	Benzene
53-96-3	2-Acetylaminofluorene 2-Acetaminofluorene 2,Fluorenyl acetamide (2-FAA)	72-54-8	p-p'-Dichlorodiphenyldichloroethane (DDD, TDE)
55-18-5	*N*-Nitrosodiethylamine Diethylnitrosamine	72-55-9	Dichlorodiphenyldichloroethylene (DDE)
55-80-1	3-Methyl-4'-(dimethyl amino)azobenzene	74-88-4	Methyl iodide
		75-00-3	Chloroethane
56-49-5	3-Methylcholanthrene (MCA)	75-01-4	Vinyl chloride
56-53-1	Diethylstilbestrol (DES)	75-07-0	Acetaldehyde
56-55-3	Benz(*a*)anthracene	75-21-8	Ethylene oxide
56-57-5	4-Nitroquinoline-1-oxide 4-Nitroquinoline-*N*-oxide	75-55-8	Propyleneimine 1,2-Propyleneimine
57-06-7	Allyl isothiocyanate	75-56-9	Propylene oxide
57-30-7	Phenobarbital sodium	77-09-8	Phenolphtaleine
57-41-0	Diphenylhydantoin Phenytoin	78-42-2	Tris(2-ethylhexyl)phosphate (TEHP)
		78-59-1	Isophorone
57-57-8	β-Propionolactone	79-00-5	1,1,2-Trichloroethane
57-63-6	Ethinylestradiol	79-01-6	Trichlorethylene
57-74-9	Chlordane Analytical	79-06-1	Acrylamide
57-83-0	Progesterone	79-34-5	1,1,2,2-Tetrachloroethane
57-97-6	7,12-Dimethyl benzanthracene	79-46-9	2-Nitropropane
58-22-0	Testosterone	81-25-4	Cholic acid
59-14-3	5-Bromo-2´-deoxyuridine	84-65-1	Anthraquinone
59-33-6	Pyrilamine maleate	85-68-7	Butylbenzylphtalate
60-09-3	*p*-Aminoazobenzene		
86-30-6	*N*-Nitrosodiphenylamine Diphenyl nitrosamine		
87-68-3	Hexachloro-1,3-butadiene	111-76-2	Ethylene glycol butyl ether Butyl glycol
88-06-2	2,4,6-Trichlorophenol		
90-04-0	*o*-Anisidine	115-96-8	Tris (2-chloroethyl) phosphate
90-94-8	Michler's ketone,	117-39-5	Quercetin

Table 11-2: Index of Rodent carcinogenic organic chemicals tested for their transformation potency by Chemical Abstract Service (CAS) Registry Number

CAS Registry Number	Chemical Name	CAS Registry Number	Chemical Name
	4,4'Bis-(dimethylamino) benzophenone	117-81-7	Di (2-ethylhexyl) phthalate (DEHP)
91-59-8	2-Naphthylamine		
92-67-1	4-Amino-1,1'-biphenyl 4-Aminobiphenyl	120-80-9	Catechol
		121-14-2	2,4-Dinitrotoluene
92-87-5	Benzidine	121-66-4	2-Amino-5-nitrothiazole
93-15-2	Mehyl eugenol	123-31-9	Hydroquinone
94-36-0	Benzoyl peroxide	123-91-1	1,4-Dioxane
94-59-7	Safrole	126-07-8	Griseofulvin
95-53-4	*o*-Toluidine	126-72-7	Tris (2,3-dibromopropyl phosphate)
95-80-7	2,4-Diaminotoluene	127-18-4	Tetrachlorethylene
96-09-3	Styrene oxide	128-37-0	Butylated hydroxytoluene (BHT)
96-45-7	Ethylene thiourea	128-44-9	Sodium saccharin
97-56-3	*2*-Amino-5-azotoluene *o*-Aminoazotoluene	134-29-2	*o*-Anisidine HCl
		135-23-9	Methapyrilene HCl
98-00-0	Furfuryl alcohol	139-13-9	Nitrilotriacetic Acid
99-55-8	2-Amino-4-nitrotoluene 5-Nitro-*o*-toluidine	140-88-5	Ethyl acrylate
		142-04-1	Aniline, HCl (Benzenamine, HCl)
99-57-0	4-Amino-2-nitrophenol	148-82-3	Melphalan Sarkolysin, Phenyl alanine mustard
100-41-4	Ethylbenzene		
100-42-5	Styrene	218-01-9	Chrysene Benzophenanthrene
100-44-7	Benzyl chloride		
101-61-1	Bis(p-dimethylamino)diphenylmethane 4,4'-Methylene bis-(*N*,*N*'-dimethylaniline)	298-81-7	8-Methoxypsoralen + UVA
		302-17-0	Chloral hydrate
		320-67-2	5-Azacytidine
101-80-4	4,4'-Oxydianiline	366-70-1	Procarbazine HCl, Natulan
103-90-2	Acetaminophen (paracetamol)	389-08-2	Nalidixic acid
		434-07-1	Oxymetholone
105-11-3	1,4-Benzoquinone dioxime *p*-Quinonedioxime	443-48-1	Metronidazole
		446-72-0	Genistein
105-55-5	*N*,*N'*-Diethylthiourea	504-88-1	3-Nitropropionic acid
106-47-8	*p*-Chloroaniline	513-37-1	Dimethylvinyl chloride
106-88-7	1,2-Epoxybutane	531-85-1	Benzidine dihydrochloride
106-89-8	Epichlorhydrin	540-73-8	1,2-Dimethylhydrazine
106-93-4	1,2-Dibromoethane	569-61-9	*p*-Rosaniline (CI Basic Red 9) Magenta
107-06-2	1,2-Dichloroethane		
107-13-1	Acrylonitrile	592-62-1	Methylazoxymethanol acetate
109-99-9	Tetrahydrofuran	598-55-0	Methylcarbamate Methyl urethane
110-86-1	Pyridine		
111-42-2	Diethanolamine	606-20-2	2,6-Dinitrotoluene
		609-20-1	2,6-Dichloro-*p*-phenylenediamine
		612-64-6	*N*-Nitroso-*N*-ethylaniline
		613-13-8	2-Aminoanthracene
630-20-6	1,1,1,2-Tetrachloroethane	10034-93-2	Hydrazine sulfate
636-21-5	*o*-Toluidine HCl	11056-06-7	Bleomycin
637-07-0	Clofibrate	11096-82-5	Aroclor 1260
680-31-9	Hexamethyl phosphoramide	11097-69-1	Aroclor 1254
684-93-5	*N*-Nitroso-*N*-methylurea (MNU) N-Methyl-N-nitrosourea Methyl nitroso urea	13073-35-3	l-Ethionine
		15663-27-1	Cisplatine Cis-Diammine dichloroplatinum
723-46-6	Sulfamethoxazole	16561-29-8	12-*O*- Tetradecanoyl phobol 13-acetate (TPA)
759-73-9	*N*-Nitroso-*N*-ethylurea (ENU)		

Table 11-2: Index of Rodent carcinogenic organic chemicals tested for their transformation potency by Chemical Abstract Service (CAS) Registry Number

CAS Registry Number	Chemical Name	CAS Registry Number	Chemical Name
	N-Ethyl-*N*-nitrosourea 1-Ethyl-1-nitrosoruea	17804-35-2	Benomyl
		19408-74-3	1,2,3,7,8,9-HCDD
765-34-4	Glycidaldehyde	21340-68-1	Methylclofenapate
789-07-1	2-Nitropyrene	23564-05-8	Methylthiophanate
1120-71-4	1,3-Propane sultone	24554-26-5	N-(4-(5-Nitro-furanyl)-2-thiazolyl)formamide
1143-38-0	Anthralin Dithranol		
		24928-17-4	Phorbol 12,13-didecanoate (PDD)
1162-65-8	Aflatoxin B1	25013-16-5	Butylated hydroxyanisol (BHA)
1163-19-5	Decabromo diphenyloxide	27208-37-3	Cyclopenta[c,d]pyrene
1336-36-3	Polychlorobiphenyl (PCB)	34807-41-5	Mezerein
1464-53-5	Diepoxybutane, 1,2 :3,4	50471-44-8	Vinclozoline
1596-84-5	Daminozide	50892-23-4	Wyeth-14,643
1746-01-6	2,3,7,8-Tetrachlorodibenzodioxin (TCDD)	51264-14-3	Amsacrine
		57653-85-7	1,2,3,6,7,8-HCDD
1897-45-6	Chlorothalonil	59865-13-3	Cyclosporin A
2465-27-2	Auramine	64091-91-4	4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK)
2784-94-3	HC blue 1		
2835-39-4	Allyl isovalerate	67774-32-7	Polybrominated biphenyls Firemaster FF1
3165-93-3	4-Chloro-o-toluidine hydrochloride		
3544-23-8	3-Methoxy-4-aminoazobenzene	68808-54-8	3-Amino-1,4-dimethyl-5*H*-pyrido[4,3-*b*]indole (Trp-P-1)
3688-53-7	Furylfuramide (AF-2)		
5307-14-2	2-Nitro-1,4-phenylenediamine 2-Nitro-*p*-phenylenediamine	72254-58-1	3-Amino-1-methyl-5*H*-pyrido[4,3-*b*]indole (Trp-P-2)
5522-43-0	1-Nitropyrene	76180-96-6	Amino-3-methylimidazo [4,5-f] 2-quinoline (IQ)
5874-97-5	Metaproterenol hemisulfate		
5989-27-5	*d*-Limonene	77439-76-0	3-Chloro-4-(dichloromethyl)-5-hydroxy-2(5*H*)-furanone (MX)
6055-19-2	Cyclophosphamide		
6959-48-4	3(Chloromethyl)-pyridine HCL	78111-17-8	Okadaic acid
		112309-61-2	3-Chloro-4-methyl-5-hydroxy-2(5H)-furanone (MCF)

Table 12-1: Summary of cell transformation assay results for non-carcinogenic or inconclusive organic chemicals(IC)*

CAS registry Number	Chemical Name	SHE 7 day pH 6.7	SHE 7 day pH ≥7.0	BALB/c 3T3	C3H/10T1/2	IARC Class**		GandZ	CCRIS	NTP
						Human	Animal			
67-64-1	Acetone		–	+			neg			
28322-02-3	4-Acetylaminofluorene	–	–	D2			neg	neg		
3567-69-9	Acid red 14 (IC)	–		+		3		neg		TR[220]
79-10-7	Acrylic Acid		–			3		neg		
3544-24-9	3-Aminobenzamide			+(P) NC	+(P) NC				TI	
2642-98-0	6-Aminochrysene				D1				neg	
7177-48-2	Ampicillin	–				3	LE	neg		TR[318]
101-05-3	Anilazine	–		?	D2			neg		TR[104]
104-94-9	p-Anisidine	+			–		EE		neg	TR[116]
120-12-7	Anthracene	–	–	–	–	3	NE			
118-92-3	Anthranilic acid			–		3	IE	neg		TR[36]
50-81-7	l-Ascorbic acid	–	–	–				neg		TR[247]
192-97-2	Benzo(e)pyrene		–	–	D2	3	IE		neg	
119-53-9	Benzoin		–	–	–			neg		TR[204]
100-51-6	Benzyl alcohol			+				neg		TR[343]
80-05-7	Bisphenol A	–	D2	–				neg		TR[215]
33229-34-4	HC Blue 2	–	–	–		3	IE	neg		TR[293]
141-32-2	n-Butyl acrylate		–			3	IE		neg	
1948-33-0	*2-tert*-Butyl-1,4-hydroquinone	–		–			TI	neg		TR[459]
58-08-2	Caffeine		–		+	3	IE	neg		
105-60-2	Caprolactam	–	+	–	–	4	neg	neg		TR[214]
7235-40-7	β-Carotene			+(P) NC				neg	TI	
56-75-7	Chloramphenicol		–			2A	IE	neg		
95-51-2	o-Chloroaniline		–	–						neg[TOX43]
107-07-3	2-Chloroethanol [Ethylene chlorohydrin]			D2					neg	TR[275]
477-30-5	Colcemid (IC)	+	+					neg		
21725-46-2	Cyanazine			+						neg[TOX36]
147-94-4	Cytosine arabinoside Cytarabine		D2	+	+				neg	
50-02-2	Dexamethasone		–[1]		–[2]			neg	TI	
823-40-5	2,6-Diaminotoluene	–		+				neg		TR[200]
15481-70-6	2,6-Diaminotoluene-2HCl									
439-14-5	Diazepam	–				3	IE	neg		
333-41-5	Diazinon				D1			neg		TR[137]
33857-26-0	2,7-Dichlorodibenzo-*p*-dioxine (DCDD)				–	3	IE	neg		
75-34-3	1,1-Dichloroethane			–				neg		TR[66]
120-83-2	2,4-Dichlorophenol		+			2B	neg	neg		TR[353]
94-75-7	2,4-Dichlorophenoxyacetic acid		–			2B	IE	neg		
1212-29-9	N,N'-Dicyclohexylthiourea				–			neg		
54150-69-5	2,4-Dimethoxyaniline HCl	–		+				neg		
68-12-2	Dimethylformamide		–			3	neg	neg		
134-72-5	Ephedrine sulfate			–				neg		TR[307]
150-38-9 60-00-4	EDTA, trisodium salt trihydrate Ethylenediaminetetracetic acid	–	–	–				neg		
97-53-0	Eugenol	–	+	?		3	LE	neg		
105-87-3	Geranyl acetate		+	–				neg		TR[252]

Table 12-1: Summary of cell transformation assay results for non-carcinogenic or inconclusive organic chemicals(IC)*

CAS registry Number	Chemical Name	SHE 7 day pH 6.7	SHE 7 day pH ≥7.0	BALB/c 3T3	C3H/10T1/2	IARC Class** Human	IARC Class** Animal	GandZ	CCRIS	NTP
50-23-7	Hydrocortisone			–				neg	TI	
148-24-3	8-Hydroxyquinoline		–	D2		3	IE	neg		TR[276]
78-84-2	Isobutyraldehyde	–							neg	TR[472]
434-13-9	Lithocholic acid		+	+(P) NC	+(P) NC			neg		TR[175]
69-65-8	*d*-Mannitol	–		D2				neg		TR[236]
15356-70-4	*dl*-Menthol			–				neg		TR[98]
50-44-2	6-Mercaptopurine		–		–	3	IE	neg		
59-05-2	Methotrexate		–		D2	3	IE	neg		
72-43-5	Methoxychlor	+	–	D2	–	3	IE	neg		TR[98]
298-00-0	Methyl parathion				–	3	neg		neg	TR[157]
134-32-7	1-Naphthylamine	–	–	+		3	IE		neg	
551-06-4	Naphthyl isothiocyanate		–	+						TI[3]
99-56-9	4-Nitro-*o*-phenylenediamine 4-Nitro-1,2-benzenediamine	–	+	D2	D2	3	IE	neg		TR[180]
63041-90-7	6-Nitrobenzo[a]pyrene		+		–	3	LE		neg	
108-03-2	1-Nitropropane						NE	neg		
504-88-1	3-Nitropropionic acid	–		+	+			neg		
2058-46-0	Oxytetracycline-HCl			–				neg		TR[315]
132-98-9	Penicillin VK			+				neg		TR[336]
6493-05-6	Pentoxifylline	–							neg	
85-01-8	Phenanthrene		–	–	–	3	IE		neg	
108-95-2	Phenol		+P NC	+		3 TP	IE	neg		
624-18-0	p-Phenylenediamine 2HCl 4-Aminoaniline dihydrochloride	–		+	–	3	IE	neg		
85-44-9	Phthalic anhydride	–	–	–				neg		TR[159]
121-79-9	Propyl gallate			+					neg	TR[240]
58-14-0	Pyremethamine (IC)	NT				3	LE		neg	TR[77]
129-00-0	Pyrene		–	–	D2	3	NE		neg	
2871-01-4	HC Red 3		–	+		3	IE	neg[l]		TR[281]
108-46-3	Resorcinol	–	+w			3	IE	neg		TR[403]
83-79-4	Rotenone	+		–				neg		TR[320]
81-07-2	Saccharin (acid form)		–	–	–,+(P)	3	IE	neg		
1300-72-7	Sodium xylenesulfonate	–							neg	TR[464]
110-44-1	Sorbic acid		–					neg		
108-30-5	Succinic anhydride		+			3	IE	neg		TR[373]
127-69-5	Sulfisoxazole	–				3	IE	neg		TR[138]
64-75-5	Tetracycline hydrochloride		–	–				neg		TR[344]
97-77-8	Tetraethylthiuramdisulfide Disulfiram	+		+		3	IE	neg		TR[166]
148-79-8	Thiabendazole	–						neg		
71-55-6	1,1,1-Trichloroethane			+		3	IE	neg		
93-76-5	2,4,5-Trichlorophenoxyacetic acid		–			2B	IE	neg		
76-87-9	Triphenyltin hydroxide	–		?				neg		TR[139]
2068-78-2	Vincristine sulfate (IC)		+			3	IE			
2783-94-0	FD and C Yellow n°6		–			3	NE	neg		

*The results of the Carcinogenic Potency Data Base according to Gold and Zeiger (1997) (GandZ), the Chemical Carcinogenesis Research Information System (CCRIS) and the National Toxicology Program (NTP) are given in addition to the IARC classification for human and animal carcinogenicity.
**IARC CLASS:
[Carcinogen risks to Humans] 1 (Known human carcinogen), 2A (Probable human carcinogen), 2B (Possible human carcinogen), 3 (Not classifiable for human carcinogen)
[Evidence for animal carcinogen] SE, LE, IE : sufficient, limited, inadequate evidence for animal carcinogenicity

neg negative results
neg (IARC conclusions) : results suggest lack of animal carcinogenicity
neg[I] negative in a study considered as inadequate by NTP
+ positive results
+w weakly positive
* positive in BALB/c 3T3
? inconclusive result
D divergent results within (D1) or among (D2) laboratories
nc no call
nd no data available
NT Not tested in 7 day pH 6.7 SHE assay
P Promoter
TI Tumor inhibitor in animal experiments
TR NTP Technical report (number indicated)

References for dexamethasone not included in tables 3, 6(because no genotoxicity data was available)
(1) Bessi *et al.,* 1995; Rivedal, 1982
(2) Kuroki and Sasaki, 1985

Other reference
(3) Sugie *et al.,* 2005 (THIS REF DOES NOT RELATE TO DEXAMETHASONE, BUT TO 551-06-4 NAPHTHYL ISOTHIOCYANATE, column NTP)

Table 12-2: Index of organic chemicals not classified as rodent carcinogens tested for their transformation potency by Chemical Abstract Service (CAS) Registry Number

CAS Registry Number	Chemical Name	CAS Registry Number	Chemical Name
50-02-2	Dexamethasone	120-83-2	2,4-Dichlorophenol
50-23-7	Hydrocortisone	121-79-9	Propyl gallate
50-44-2	6-Mercaptopurine	127-69-5	Sulfisoxazole
50-81-7	l-Ascorbic acid	129-00-0	Pyrene
56-75-7	Chloramphenicol	132-98-9	Penicillin VK+
58-08-2	Caffeine	134-32-7	1-Naphthylamine
58-14-0	Pyremethamine (IC)	134-72-5	Ephedrine sulfate
59-05-2	Methotrexate	141-32-2	n-Butyl acrylate
60-00-4	Ethylenediaminetetracetic acid	147-94-4	Cytosine arabinoside, Cytarabine
64-75-5	Tetracycline Hydrochloride	148-24-3	8-Hydroxyquinoline
67-64-1	Aceton	148-79-8	Thiabendazole
68-12-2	Dimethylformamide	150-38-9	EDTA, trisodium salt trihydrate
69-65-8	*d*-Mannitol	192-97-2	Benzo(e)pyrene
71-55-6	1,1,1-Trichloroethane	298-00-0	Methyl parathion
72-43-5	Methoxychlor	333-41-5	Diazinon
75-34-3	1,1-Dichloroethane	434-13-9	Lithocholic acid
		439-14-5	Diazepam
76-87-9	Triphenyltin hydroxide	477-30-5	Colcemid (IC)
78-84-2	Isobutyraldehyde	504-88-1	3-Nitropropionic acid
79-10-7	Acrylic Acid	551-06-4	Naphthyl isothiocyanate
80-05-7	Bisphenol A	624-18-0	p-Phenylenediamine dihydrochloride 4-Aminoaniline dihydrochloride
81-07-2	Saccharin (acid form)		
83-79-4	Rotenone		
85-01-8	Phenanthrene	823-40-5	2,6-Diaminotoluene
85-44-9	Phthalic anhydride	1212-29-9	N,N'-Dicyclohexylthiourea
93-76-5	2,4,5-Trichlorophenoxyacetic acid	1300-72-7	Sodium xylene sulfonate
94-75-7	2,4-Dichlorophenoxyacetic acid	1948-33-0	2-tert-Butyl-1,4-hydroquinone
95-51-2	o-Chloroaniline	2058-46-0	Oxytetracycline-HCl
97-53-0	Eugenol	2068-78-2	Vincristine sulfate (IC)
97-77-8	Tetraethylthiuramdisulfide Disulfiram	2642-98-0	6-Aminochrysene
		2783-94-0	FD and C Yellow n°6
99-56-9	4-Nitro-*o*-phenylenediamine 4-Nitro-1,2-benzenediamine	2871-01-4	HC Red 3
		3544-24-9	3-Aminobenzamide
100-51-6	Benzyl alcohol	3567-69-9	Acid red 14 (IC)
101-05-3	Anilazine	6493-05-6	Pentoxifylline
104-94-9	p-Anisidine	7177-48-2	Ampicillin
105-60-2	Caprolactam	7235-40-7	β-Carotene
105-87-3	Geranyl acetate	15356-70-4	*dl*-Menthol
107-07-3	2-Chloroethanol Ethylene chlorohydrin	15481-70-6	2,6-Toluenediamine-2HCl
		21725-46-2	Cyanazine
108-03-2	1-Nitropropane	28322-02-3	4-Acetylaminofluorene
108-30-5	Succinic anhydride	33229-34-4	HC Blue 2
108-46-3	Resorcinol	33857-26-0	2,7-Dichlorodibenzo-*p*-dioxine DCDD
108-95-2	Phenol		
110-44-1	Sorbic acid	54150-69-5	2,4-Dimethoxyaniline HCl
118-92-3	Anthranilic acid	63041-90-7	6-Nitrobenzo(a)pyrene
119-53-9	Benzoin		
120-12-7	Anthracene		

Table 13-1: Inorganic chemicals – Rodent carcinogens (A) and non-carcinogens (B) – tested with the SHE, BALB/c 3T3 and C3H/10T1/2 cell transformation assays

CAS Registry Number	Chemical Name	SHE 7 day pH 6.7	SHE 7 day pH ≥7.0	BALB/c 3T3	C3H/10T1/2	IARC Class Human	IARC Class Animal + NTP^{TR}	GandZ	CCRIS
A /	CARCINOGENS								
12172-73-5	Amosite		+		+(P)	1	+		
17068-78-9	Anthophyllite		+[1]			1	+		
10294-40-3	Barium chromate		+	+		1	+		
1344-38-3	Basic lead silicochromate [c]		+			1	+		
13510-49-1	Beryllium sulfate		+	+	–	1	+		
7787-56-6	Beryllium sulfate tetrahydrate		+			1	+		
1302-27-8	Biotite		+[2]				+		
543-90-8	Cadmium acetate		+			1	+		
10108-64-2	Cadmium chloride	+	+			1	+		
13765-19-0	Calcium chromate		+	+	D2	1	+		
1333-86-4	Carbon Black				–	2B			
1333-82-0	Chromium trioxide		+			3	LE		+
12001-29-5	Chrysotile	+	+	+	+(P)	1	+		
15663-27-1	cis-Platinium diammine dichloride		+			2A	+		
10026-24-1	Cobalt sulfate heptahydrate	NT	+				+^{TR471}		+
14464-46-1	Cristobalite		+			1	+		
12001-28-4	Crocidolite			+	+(P)	1	+		
12510-42-8	Erionite				+[3]		+		
1303-00-0	Gallium arsenide	NT					+^{TR492}		+
No CAS Nb	Glass fibre 100		+			2B			
No CAS Nb	Glass fibre 110		D2			2B			
7722-84-1	Hydrogen peroxide	+	+		+	3		+	+
301-04-2	Lead acetate	+	+	D2	–	2B	+		
7758-97-6	Lead chromate		+		+	1	+		
12709-98-7	Lead molybdenum chromates [a]		+			1	+		
7752-97-6	Lead sulphate chromates [b]		+			1	+		
1309-38-2	Magnetite		+[2]				+[(5)]		neg
1313-27-5	Molybdenum trioxide	NT					+^{TR462}		+
1317-43-7	Nemalite Brucite		+[2]				+		+
7440-02-0	Nickel (metallic)		+			2B	+		
7718-54-9	Nickel chloride		+		–	1	+		
1313-99-1	Nickel monoxide		+		+	1	+		
12035-72-2	Nickel subsulfide		+		+	1	+		
7786-81-4	Nickel sulfate		+		–	1	+		
10101-98-1	Nickel sulfate heptahydrate	+				1	+		
10101-97-0	Nickel sulfate hexahydrate		+			1	+		
16812-54-7	Nickel sulfide(crystalline)		+		+	1	+		
10028-15-6	Ozone				+		+^{TR440}	+	+
7789-00-6	Potassium chromate		+	+		1	+		
7778-50-9	Potassium dichromate		+		–	1	+		
14808-60-7	Quartz Min-U-Sil5 Silica Quartz		+	+		1	+		
No CAS Nb	Refractory ceramic fibre-1,2,3,4		+			2B	+		
7446-34-6	Selenium sulfide			+			+^{TR194}	+	+
7778-43-0	Sodium arsenate		+	+	–	1	+		
7784-46-5	Sodium arsenite		+	+	+	1	+		
10034-82-9	Sodium chromate tetrahydrate		+	+		1	+		
10588-01-9	Sodium dichromate		+			1	+		
7632-00-0	Sodium nitrite	NT	+	+				+	
7789-06-2	Strontium chromate		+			1	+		

Table 13-1: Inorganic chemicals – Rodent carcinogens (A) and non-carcinogens (B) – tested with the SHE, BALB/c 3T3 and C3H/10T1/2 cell transformation assays

CAS Registry Number	Chemical Name	SHE 7 day pH 6.7	SHE 7 day pH ≥7.0	BALB/c 3T3	C3H/10T1/2	IARC Class Human	IARC Class Animal + NTP^{TR}	GandZ	CCRIS
13463-67-7	Titanium dioxide	–	–	–		3	LE	neg	+
14567-73-8	Tremolite		+			1	+		
1314-62-1	Vanadium (V) pentoxide	+	+	+		2B	+		
7646-85-7	Zinc chloride	+	D2						+TP
13530-65-9	Zinc chromate		+			1	+		
15930-94-6	Zinc chromate hydroxides[d]					1	+		
1314-13-2	Zinc oxide		+						+TP
11103-86-9	Zinc potassium chromates[d]		+[4]			1	+		
B /	NON RODENT CARCINOGENS								
7803-55-6	Ammonium metavanadate				+				neg TI
10326-27-9	Barium chloride dihydrate			+			–		neg
10025-73-7	Chromium (III) chloride		–	+		3	neg		
1308-38-9	Chromium (III) oxide		+			3	neg		
7440-47-3	Chromium (metallic)		+			3	neg		
68855-54-9	Diatomaceous earths		+				IE		
1309-37-1	Ferric oxide		–			3	neg		
7647-14-5	Sodium chloride			?				neg	
7681-49-4	Sodium fluoride	–	+	+				neg	
1271-19-8	Titanocene dichloride		+	+				neg	

a : industrial " molybdate red" or "molybdate orange" pigments ; b : industrial "chromium yellow" pigments ; c : industrial "chromium orange" pigments ; d : industrial "zinc yellow" pigments

- * IARC classification and evidence for animal carcinogenicity are indicated. The data from the technical reports (TR) of National Toxicology Program (NTP), the Carcinogenic Potency Data Base according to Gold and Zeiger (1997) (GandZ) and the Chemical Carcinogenesis Research Information System (CCRIS) are given for chemicals not classified 1, 2A or 2B by IARC.

**IARC CLASS:
[Carcinogen risks to Humans] 1 (Known human carcinogen), 2A (Probable human carcinogen), 2B (Possible human carcinogen), 3 (Not classifiable for human carcinogen)
[Evidence for animal carcinogen] SE, LE, IE : sufficient, limited, inadequate evidence for animal carcinogenicity
\+ Positive result
– Negative result
D Divergent results within (D1) or among (D2) laboratories
NT Not Tested in 7 day pH6.7 SHE assay
+w Weakly positive result
? Inconclusive result
neg negative response for carcinogenicity
(P) *in vivo* tumor promoter
TP *in vivo* tumor promoter
TI *in vivo* tumor inhibitor
TR : NTP (National Toxicology Program)Technical Report

References for chemicals not included in tables 4, 10 (no genotoxicity data available)

1. Mikalsen *et al.,* 1988 ; DiPaolo *et al.,* 1983
2. Elias *et al.,* 1995
3. Poole *et al.*, 1983
4. Elias *et al.*, 1989

Other reference

5. *Pott et al.,* 1994

Table 13-2: Index of inorganic chemicals tested for their transformation potency by Chemical Abstract Service (CAS) Registry Number

CAS Registry Number	Chemical Name	CAS Registry Number	Chemical Name
No CAS Nb.	Glass fibre 100	7789-06-2	Strontium chromate
No CAS Nb.	Glass fibre 110	7803-55-6	Ammonium metavanadate
No CAS Nb.	Refractory ceramic fibre-1,2,3,4	10025-73-7	Chromium [III] chloride
		10026-24-1	Cobalt sulfate heptahydrate
301-04-2	Lead acetate	10028-15-6	Ozone
543-90-8	Cadmium acetate	10034-82-9	Sodium chromate tetrahydrate
1271-19-8	Titanocene dichloride	10101-97-0	Nickel sulfate hexahydrate
1302-27-8	Biotite	10101-98-1	Nickel sulfate heptahydrate
1303-00-0	Gallium arsenide	10108-64-2	Cadmium chloride
1308-38-9	Chromium [III] oxide	10294-40-3	Barium chromate
1309-37-1	Ferric oxide	10326-27-9	Barium chloride dihydrate
1309-38-2	Magnetite	10588-01-9	Sodium dichromate
		11103-86-9	Zinc potassium chromates
1313-27-5	Molybdenum trioxide	12001-28-4	Crocidolite
1313-99-1	Nickel monoxide	12001-29-5	Chrysotile
1314-13-2	Zinc oxide	12035-72-2	Nickel subsulfide
1314-62-1	Vanadium (V) pentoxide	12172-73-5	Amosite
1317-43-7	Nemalite Brucite	12510-42-8	Erionite
		12709-98-7	Lead molybdenum chromates
1333-86-4	Carbon black	13463-67-7	Titanium dioxide
1333-82-0	Chromium trioxide	13510-49-1	Beryllium sulfate
1344-38-3	Basic lead silicochromate	13530-65-9	Zinc chromate
7440-02-0	Nickel (metallic)	13721-39-6	Sodium o-vanadate
7440-47-3	Chromium (metallic)	13765-19-0	Calcium chromate
7446-34-6	Selenium sulfide	14464-46-1	Cristobalite
7632-00-0	Sodium nitrite	14567-73-8	Tremolite
7646-85-7	Zinc chloride	14808-60-7	Quartz Min-U-Sil5 Silica (Quartz)
7647-14-5	Sodium chloride		
7681-49-4	Sodium fluoride	15663-27-1	cis-Platinium diammine dichloride
7718-54-9	Nickel chloride		
7722-84-1	Hydrogen peroxide	15930-94-6	Zinc chromate hydroxides[d]
7752-97-6	Lead sulphate chromates	16812-54-7	Nickel sulfide
7758-97-6	Lead chromate	17068-78-9	Anthophyllite
7778-43-0	Sodium arsenate	68855-54-9	Diatomaceous earths
7778-50-9	Potassium dichromate		
7784-46-5	Sodium arsenite		
7786-81-4	Nickel sulfate		
7787-56-6	Beryllium sulfate tetrahydrate		
7789-00-6	Potassium chromate		

Comparison of CTAs

100. The performances of the individual cell transformation assays for predicting rodent carcinogenicity were evaluated individually. The SHE assay was evaluated as two separate assays (pH 6.7 and pH ≥ 7.0). The results from this evaluation are presented in Table 14. The performance of the genotoxicity assays in predicting rodent carcinogenicity of the set of chemicals considered for cell transformation was also evaluated. The results are summarized in Annex IV.

101. All analyses were performed using the results in the summary tables (Tables 11-13). In these data summaries, chemicals and their salts (*e.g.,* o-anisidine and o-anisidine HCl; benzidine and benzidine HCl) that had been tested independently and reported as such in the data tables have been combined so that all salts of a single organic moiety are considered as a single substance.

102. All analyses are based on the CTA results that were summarized as + or – following the rules described in Section I. The proportions of chemicals not included in the analyses because the results were not clearly positive or negative are included in the tables. Also not included in the analyses were the chemicals that were tested only for tumor promotion.

Analyses performed

103. The performances of the assays (+ or -) for predicting rodent carcinogenicity were determined using 2 x 2 tables (see paragraph 23) based on the results in tables 11-13. Calculations performed included concordance, sensitivity, specificity, positive predictivity, negative predictivity, false negatives, false positives, and prevalence (proportions of carcinogens in the database evaluated). The results of these analyses are presented in Table 14.

Table 14. Performance characteristics of the cell transformation assays for the prediction of rodent carcinogenicity

	SHE pH 6.7	SHE pH ≥ 7.0	BALB/c 3T3	C3H10T1/2
Total chemicals	88	204	149	96
Prevalence of carcinogens	54/88 = .61	142/192 = .74	100/147 = .68	81/96 = .84
Concordance	65/88 = .74	164/192 = .85	100/147 = .68	70/96 = .73
Sensitivity	36/54 = .66	131/142 = .92	75/100 = .75	58/81 = .72
Specificity	29/34 = .85	33/50 = .66	25/47 = .53	12/15 = .80
+ Predictivity	36/41 = .88	131/148 = .88	75/97 = .77	58/61 = .95
– Predictivity	29/47 = .62	33/44 = .75	25/50 = .50	12/35 = .34
False +	5/34 = .15	17/50 = .34	22/47 = .47	3/15 = .20
False –	18/54 = .33	11/142 = .08	25/100 = .25	23/81 = .28
Chemicals not incl.	2	12	28	30

104. The proportions of carcinogens in the various chemicals were relatively high, ranging from 61% for the pH 6.7 SHE assay to 85% for the C3H10T1/2 assay. As a result, some assays were evaluated using relatively many carcinogens; this is the opposite of the use they get when evaluating products in an industry situation where the majority of screened substances are anticipated to be negative. These figures are relevant because the sensitivity and specificity of a test are highly dependent on the proportions of true positives (*i.e.,* carcinogens) in the database.

Results of the analyses

105. As previously noted, the SHE assay was evaluated as two separate protocols performed at different pH (Table 14). Both protocols had equivalent performance for the prediction of rodent carcinogens. The pH 6.7 procedure had a concordance of 74%, sensitivity and specificity of 66 and 85%,

respectively, positive predictivity of 88%, and false positive and negative rates of 15 and 33%, respectively. The proportion of carcinogens in the database of 88 chemicals was 61%.

106. For comparison, the pH ≥ 7.0 protocol had a concordance of 85%, sensitivity and specificity of 92 and 66%, respectively, positive predictivity of 88%, and false positive and negative rates of 34 and 8%, respectively. The proportion of carcinogens in the database of 204 chemicals was 74%.

107. The two datasets obtained with pH 6.7 and pH ≥ 7.0 protocols do not completely overlap and the relatively small differences between the two pH's may be a consequence of the specific chemicals tested, or the smaller numbers of chemicals tested at pH 6.7, the higher proportion of carcinogens in the database for pH ≥ 7.0. Although there are some differences among the performance characteristics between the two procedures, the overall performances is basically equivalent.

108. The BALB/c 3T3 assay showed a concordance of 68%, sensitivity and specificity of 75 and 53%, respectively, positive predictivity of 77%, and false positive and negative rates of 47 and 25%, respectively. The proportion of carcinogens in the database of 149 chemicals was 68%. The results summarized for this assay were derived from a number of different protocol variations, including one that used a two-stage, initiation-promotion protocol designed to increase the sensitivity of the assay. Data from the different assay variations were insufficient to enable separate analyses.

109. Regarding the C3H10T1/2 assay, the assay had a concordance of 73%, sensitivity and specificity of 72 and 80%, respectively, positive predictivity of 95%, and false positive and negative rates of 20 and 28%, respectively. The proportion of carcinogens in the database was 84%. Of the 96 chemicals in this database, only 15 were negative. This value may be considered too low to permit an accurate evaluation of the performance of this assay in the identification of noncarcinogens.

110. The issue of reproducibility of results of *in vitro* CTA experiments is of central importance and should be addressed using both inter-, and intra-laboratory investigations. Each type of investigation assessing reproducibility should use the same test protocol and the same set of chemicals (preferable coded and from the same chemical source).

Reproducibility

111. The data ensambled in this DRP enables an assessment of some measure of reproducibility beyond that suggested by reports in the scientific literature referenced in this document. Excluding chemicals with only one result, consistency between laboratories is 87.7% for the SHE assay (57/65 chemicals), 68.4% for the BALB/c 3T3 assay (39/57 chemicals), and 54.3% for the C3H10T1/2 assay (38/70 chemicals). The apparent lower reproducibility for the latter two assays may be attributable to substantial protocol differences.

112. The results of inter-laboratory variability comparisons of the three CTAs are described in section II of the DRP. Regarding the intra-laboratory variability a large investigation that was funded by the NIEHS was conducted using the BALB/c 3T3 CTA (Matthews *et al.,* 1993a, b). Almost 200 different chemicals were tested in two or more experiments and the results showed that the BALB/c 3T3 CTA has a high consistency in positive or negative results for replicate experiments for more than 95% of the chemicals tested. These experiments were conducted over a several year period using the same experimental protocol, but utilized different technicians and many different lots of serum and other tissue culture reagents. The results clearly show the robustness of the BALB/c 3T3 CTA and the high level of intra-laboratory reproducibility.

113. The intra-laboratory reproducibility of the C3H10T1/2 and SHE CTAs has also been documented by several different laboratories for smaller numbers of test chemicals. The intra-laboratory reproducibility

for the C3H10T1/2 CTA has been assessed most thoroughly by Dunkel *et al.,* (1988) for 3-methylcholanthrene (MCA). Multiple experiments with this transforming agent (Oshira *et al.,* 1982; Oshira and Balwierz, 1982; Frazelle *et al.,* 1983; Sanchez *et al.,* 1986) and with benzo[*a*]pyrene (Nesnow *et al.,* 1985) have been performed. These PAH's consistently gave highly reproducible results in this assay. In a limited study, repeated assays with MNNG appeared to give somewhat less reproducible results, possibly due to the lower frequencies of transformants produced by this direct alkylating agent (Sanchez *et al.,* 1986).

114. EC-ECVAM is presently engaged in validation studies of the SHE and BALB/c 3T3 assays.

Other performances

115. With regard to the sensitivity and specificity values for the cell transformation assays using immortal tissue culture cell lines (BALB/c 3T3 and C3H10T1/2), it is important to note that these CTAs have been employed in *in vitro* carcinogenicity investigations for over three decades. These assays have been used to examine different activation systems, initiation and promotion protocols, and detection of anti-carcinogenesis.

116. Data from multiple-protocol assays contributes to the lower sensitivity and specificity exhibited by these assays compared to the SHE CTA. For example, the explanation for the lesser sensitivity of the BALB/c 3T3 assay is known: the early BALB/c 3T3 CTA studies by Sivak and Tu (1980) used a 1:5 dilution protocol to pick treatment doses for the initiation assay and did not select doses that were moderately toxic to the cell cultures. More recent studies by Matthews (1985, 1993a, b) showed the importance of picking doses producing up to 50% cell toxicity.

117. Explanations of the relatively high rate of false positive responses in the BALB/c 3T3 assay are also known, and can be attributed to the CTA protocol used. Protocols designed to detect promoting activity, rather than initiation, have detected non-carcinogenic chemicals. Furthermore, the use of very high treatment doses (>5 mg/ml) cause BALB/c 3T3 transformation and are clearly tissue culture artefacts. False positives can also be obtained when test chemicals are excessively toxic (10% survival), or when test chemicals destroy necessary tissue culture medium nutrients.

118. All of these artifacts are known and can be controlled in a standardized protocol. The true predictive performance values for the BALB/c 3T3 assay would more likely reflect the values reported by Matthews (1985, 1993a, b) compared to the values presented in the DRP, if the improved protocol were implemented. It was a basic understanding by the experts who reviewed the BALB/c 3T3 and C3H10T1/2 data for the DRP that a standardized protocol could easily be written for detection of initiating activity for both assays. Japanese studies has demonstrated that these assays produce highly reproducible results (Umeda, 2004).

V. CONCLUSIONS AND RECOMMENDATIONS

The SHE cell transformation assay

119. Based on the general performance of the SHE CTA, the *ECM in Washington DC* in October 2006 recommended that the SHE CTA should be developed into an official OECD Test Guideline. Although there is insufficient information on mechanism of action and usage for pharmaceuticals at this stage, *the Washington ECM* were of the opinion that the SHE assay has the potential of being used as a screen for pharmaceutical testing as a part of a tiered testing strategy. In addition to its ability to identify potential rodent carcinogens, it has shown promise for identifying carcinogens that are not genotoxic. Because of the ability to identify potential rodent carcinogens, it has been proposed for use as a second level *in vitro* screening test for carcinogenic potential. Another proposed use of the assay is for the identification of potential carcinogens that have no evidence of genetic toxicity in the currently used assays. The assay could also be a replacement for the *in vitro* mammalian cell mutagenicity assays with similar or lower predictive capacity.

120. The *Washington ECM* agreed that the available information as presented in the DRP31 on the concordance, sensitivity, specificity, positive and negative predictive capacity, false positive and negative rates and evidence for intra-, and inter-laboratory reproducibility (even though slightly limited) of the test does supports its performance and justifies the recommendation that the SHE CTA should be developed into an OECD Test Guideline.

121. The ongoing prevalidation study coordinated by EC-ECVAM will hopefully provide the necessary information on the intra-, and inter- laboratory reproducibility and the first data from the prevalidation phase is expected to be available in the 2nd quarter of 2007.

122. The *Washington ECM* recommended that the EC-ECVAM protocols (as used in the ECVAM prevalidation study - acidic and standard pH - 7 day protocol) should be considered in the development of the Test Guideline.

123. The *Washington ECM* further emphasised the need to include pharmaceutical compounds in the final validation study. It is also suggested that the 24h protocol in addition to the 7 day protocol be considered in a further step and a performance evaluation of the 24 hr protocol has been added as Annex V.

The BALB/c 3T3 and C3H10T1/2 cell transformation assays

124. The transformation process in the BALB/c 3T3 and C3H/10T1/2 cell lines parallel the induction and progression of tumours *in vivo*. The cell lines also show promise for the identification of tumour promoters. In addition to their ability to identify potential rodent carcinogens, they have also shown promise for identifying carcinogens that are not genotoxic. Because of their ability to identify potential rodent carcinogens they have been proposed for use as a second level *in vitro* screening test for carcinogenic potential. Another proposed use of the assays is for the identification of potential carcinogens that have no evidence of genetic toxicity in the currently used assays. Another possible use for the assays would be as replacements for the *in vitro* mammalian cell mutagenicity assays with similar or lower predictive capacity. Although the test can be applicable for detecting genotoxic compounds, there is no clear advantage to using it as a replacement for the *in vitro* mammalian cell mutagenicity assays.

125. The *Washington ECM* recommended that based on the available information presented to the meeting on the concordance, sensitivity, specificity, positive and negative predictive capacity and false positive and negative rates, an OECD Test Guideline for the BALB/c 3T3 CTA should be developed. The

BALB/c 3T3 assay has been subjected to a number of inter-laboratory studies and the 1-stage protocol is currently undergoing a prevalidation study coordinated by EC-ECVAM. After a first phase of standardisation and harmonisation of the protocols, the prevalidation phase will be completed by 2nd quarter of 2007. An ongoing prevalidation study in Japan is evaluating the effectiveness of a 2-stage protocol. Given the pending results of the two ongoing prevalidation studies and the established use of the two protocols, the Washington ECM recommended that a single protocol should be developed incorporating both the 1-, and 2-stage procedures. The details of the Test Guideline proposal will be dependent on the results of the final validation study.

126. With regard to the C3H/10T1/2 assay, there are no available studies that evaluates the intra-, or inter-laboratory reproducibility using a standardized protocol. A number of chemicals have been tested in more than one laboratory, but differences in protocols and evaluation criteria, and changes in the protocols over time, preclude any evaluation of reproducibility for this assay. One study evaluated the inter-laboratory reproducibility of the assay between two laboratories. The reproducibility of the assay was approximately 62%, but there was no indication of how the protocol differences between the labs may have affected the results. The *Washington ECM* concluded that based on the limited data available it was not possible to make a recommendation for Test Guideline development of the C3H/10T1/2 CTA at this time.

Human Cell Transformation

127. Cell transformation assays in human cell lines have not been discussed thus far in the DRP. Although cellular transformation assays using rodent cells can clearly make a positive contribution to carcinogenicity testing, the development of human cell lines holds promise of enhancing the predictability of the test with regard to human carcinogenicity even further. This premise is supported, for example, by the proposed development of human cell lines for the murine-cell based Embryonic Stem Cell Test (Pellizer *et al*., 2005), and in general by the continued development of a comprehensive and varied range of human cell lines that are commercially available.

128. Work in this area was progressing at an impressive pace in the 1990s and into the early part of this decade. Combes *et al*., (1999) summarised the development of two human cell lines specifically for use in CTAs, namely the HaCaT keratinocyte model (Fusenig and Boukamp 1994) and the MSU-1.1 cell line system (McCormick and Maher, 1989, 1994) based on the immortalized human fibroblast cell line. Evidence of the potential of these cell lines is more recently described in Mueller *et al*., (2001) and Boley *et al*., (2000).

129. With increasing numbers of human cell lines potentially applicable to the CTA, and the resources that would be devoted to the development of the assay following Test Guideline development and increased adoption and utilisation in the near future, development and validation of human cell-based assays should be encouraged to improve the relevance, reliability, and predictably due to the human context.

The Washington ECM recommendations for Test Guideline Development

130. Based on the available data, the *Washington ECM* considered that the performances of the SHE and the BALB/c 3T3 CTAs are adequate for recommending that they be developed into official OECD Test Guidelines. The C3H/10T1/2 was not considered to fulfill the data need at this time.

131. In order to understand the predictability of the various CTAs, it is important to separate the carcinogenicity results into two groups: chemicals that are positive in only one species/gender, and those that are positive in more than one species/gender. The *Washington ECM* recommend that an evaluation of such classification should be considered before Test Guidelines are developed.

132. The results of the EC-ECVAM final validation studies of the SHE and BALB/c3T3 CTAs should be considered before Test Guidelines are developed.

WNT19 Discussion

133. Whilst appreciating the Washington ECM recommendations, some member countries expressed reservations and considered that Test Guideline development may be premature. These countries considered that the major difficulty with cell transformation assays is that the underlying mechanisms and their relationship to tumour development *in vivo* are not understood and that the regulatory value of these assays is still unclear.

134. The WNT will reconsider the value of Test Guideline development when information from ongoing prevalidation and validation studies are available.

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ANNEX I: PROTOCOL OF THE SHE CTA

Introduction

1. The Syrian Hamster Embryo *in vitro* cell transformation assay provides a system to detect carcinogenic chemicals that act via genotoxic and non-genotoxic mechanisms. SHE cells are primary and normal diploid cells, which derive from mid-gestation embryos (13 days gestation). The cells are genetically stable, metabolically competent and have a finite life span. SHE cells show a high proliferation rate, good plating efficiency (20 - 40 %) and a low spontaneous transformation frequency. Exposure to carcinogenic agents results in an increase in the percentage of morphological transformed (MT) colonies compared to controls. MT colonies are characterized by a random growth pattern, which expresses a loss of growth inhibition and cell-cell orientation at confluency

Principle

2. The principle of the test consists in seeding target SHE cells at clonal density onto a feeder layer of X-irradiated SHE cells. Twenty four hours after seeding feeder cells, the target cells are seeded onto the feeder layer at a density appropriate to obtain 25-45 colonies per plate (60 mm diameter) and are treated 24 hours later. After 7 days necessary for clonal expansion, cells are washed, fixed and stained with Giemsa. Dishes are coded and colonies are scored for their morphological phenotype under a stereomicroscope. Cytotoxicity is evaluated by inhibition of cloning efficiency. The number of MT colonies reported versus the total number of scorable colonies is calculated for each concentration tested. A statistically higher percentage of MT colonies at two concentration levels compared to control vehicle will wield a conclusion of a positive response.

Materials

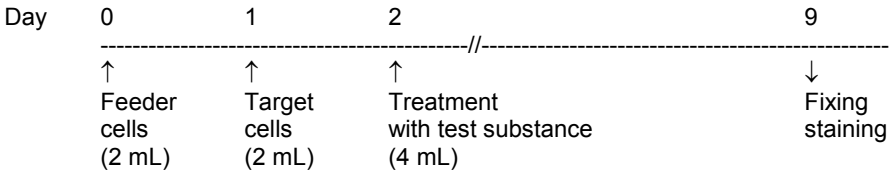
Preparation of the cells

3. SHE cells are obtained from primary culture of individual Syrian hamster embryos at 13 days of gestation. After enzymic tissue digestion, cells are collected and stored in liquid nitrogen. One part of cryopreserved SHE cells will be used as feeder cells, the other part as target cells. These 'feeder cells' will be X-ray irradiated (5000 rads) to be no longer capable of replication and seeded as nutrient base and support for metabolic activity.

Medium and culture

4. The test medium is the Dulbecco's modified Eagle's medium (DMEM) containing 1g/L glucose, 4 mM glutamine and 110 mg/L sodium pyruvate, without phenol red. The pH is adjusted to either 6.7 with 0.75g/L $NaHCO_3$ or to 7.0 with 1.5 g/L $NaHCO_3$ depending on the method used (acidic or neutral pH, respectively). The medium is sterilized by membrane filtration (0.1 µm porosity). The culture medium is stored at 4° C during a period not exceeding 2 weeks. The complete culture medium is prepared with addition of 20% (at acidic pH) or 15 % (at neutral pH) of fetal bovine serum. The pH of the culture medium is checked so as to be 6.7 or 7.0 after incubation at 37 ± 0.1°C with 10 ± 0.5 % CO_2 in a humidified incubator for at least 4 hours.

Experimental design



Feeder cells

5. On day 0 (feeder cells day), the irradiated SHE cells are counted for viability using the Trypan Blue dye exclusion test. The cell concentration is adjusted so as to obtain a concentration comprised between 20,000 and 30,000 cells/mL. Two mL of the cell suspension are seeded into each 60mm culture dish. The culture dishes are incubated at 37±0.1°C and 10± 0.5% CO_2 in humidified air for 24 hours before adding target cells.

6. For each test, at least 5 dishes filled with feeder cells only will be used concurrently to check their inability to replicate and to form colonies. No colonies are allowed to be formed in these dishes.

Target cells

7. Cryopreserved SHE cells are thawed and seeded for growth in culture flasks. After an incubation period of 5 to 24 hours, the target cells will be detached, counted with a hemocytometer and adjusted with the complete growth medium to a concentration allowing us to obtain approximately 25 - 45 colonies/dish at the end of the test. Two mL of the target cell suspension will be added into each culture dish containing a feeder layer of 40,000-60,000/dish. Dishes will be incubated in a humidified incubator (37 ± 0.1°C; 10 ± 0.5 % CO_2) for 24 hours prior to treatment.

Test chemicals, positive and negative controls

8. The test substance will be weighed and the solutions prepared on the day of treatment. A series of solutions of the test substance (500 x the final concentration) in the appropriate vehicle will be prepared so as to obtain the final desired concentration in the test medium. Four mL of the chemical solutions will be added into each culture dish, 24h after the seeding of target cells.

9. In case the test substance is not water soluble, the vehicle control selected will be DMSO used at a concentration that will not exceed 0.2%. The final concentration of DMSO will be the same in all vehicle control and treated dishes : 0.2 %. The positive control is benzo[a]pyrene (B[a]P) dissolved in DMSO and tested at a concentration of 1 or 5 µg/mL.

Cytotoxicity Assay / Dose range finding study

10. A cytotoxicity test is carried out prior to the cell transformation assay in order to determine the appropriate range of concentrations to be tested for transformation. The maximum dose of the test substance will be determined taking into account the solubility and any relevant cytotoxicity information available for the test substance. The highest dose level tested for soluble test substance will be 5 mg/mL or 10 mM. A range of at least 10 concentrations to achieve a wide toxicity range will be tested in parallel to the vehicle, with 10 dishes per group. The conditions of testing (test medium, incubation conditions and time) are the same as the ones described for the main cell transformation experiment. The cultures will be incubated for a period of 7 days to allow colony development. Solubility of the test chemical, osmolality and pH of the test cultures should be controlled during the exposure period.

11. The relative cytotoxicity of each treatment group will be measured by the reduction in plating efficiency and/or colony density and size of the treated SHE cells compared to the vehicle control group.

Test chemical concentrations

12. Five concentrations will be selected from the cytotoxicity test results to be tested for cell transformation. The range of tested concentrations will include (i) a high dose causing an approximate 50% decrease in relative plating efficiency and/or ≥ 50% reduction in relative colonies density/size (by visual appearance), (ii) a low dose having no effect on plating efficiency, and (iii) 3 intermediary doses more or less evenly spaced between these two extremes.

13. If the test substance is essentially non-toxic, then at least five concentrations will be selected up to a maximum of 5 mg/mL or 10mM. For non-toxic and insoluble test substances, the highest dose level tested will be within 2-times the visible solubility limit in complete medium. For toxic and insoluble test substance, the highest dose level tested should cause an approximate 50% decrease in relative plating efficiency or relative colony density, regardless of the number of insoluble dose levels.

Cell Transformation Assay

14. As outlined in paragraphs 5 and 7, a sufficient number of target cells (around 100-150 cells/dish) to produce an average of 25 - 45 colonies will be dispensed in 2 mL of complete medium per 60 mm culture dish, each of which was seeded approximately 24 hours earlier with feeder cells (4×10^4 - 4×10^6) in 2 mL of complete medium. In case of cytotoxicity, the cell number should be adjusted to obtain the number of colonies required. The assay should include at least 5 scorable concentrations of the test compound and the appropriate vehicle and positive controls. The dishes will be incubated for a period of 7 days in a humidified incubator ($37 \pm 0.1°C$; 10 ± 0.5 % CO_2) following treatment initiation. The culture dishes will be labelled with a code /assay number, trial number and dose level.

15. After the 7 day exposure period, the medium is discarded, the dishes are rinsed with phosphate saline buffer. Cells are fixed with ethanol (10 min), stained with a 10% Giemsa solution in pure water (20 min), and plates are rinsed under tap water before cells are air-dried.

Evaluation

Morphological Cell Transformation

16. The stained colonies are blindly scored under stereomicroscope for plating efficiency (PE) and morphological transformation (MT). Morphologically-transformed colonies are characterized by a multi-layered, criss-cross pattern of growth throughout the colony and by a piling up of cells.

17. Sparse colonies are not scored for MT: However they will be included in the total number of colonies for plating efficiency determination. A colony containing less than 50 cells is not counted or recorded. Colonies at the edge of the plates can be scored for MT if clearly morphologically transformed. Generally, for each test group ≥ 1000 colonies will be evaluated for morphological cell transformation.

18. The morphological transformation frequency (MTF) will be calculated for each test group : MTF = (number of transformed colonies/ total number of scorable colonies) x 100

Cytotoxicity

19. The average number of colonies per dish, the plating efficiency (PE) and the relative plating efficiency (RCE) will be determined to evaluate cytotoxicity in each test group.

20. The plating efficiency (% PE) is defined as : (total number of colonies per dish/ total number of target cells seeded per dish) x 100.

The relative plating efficiency (%RPE) is defined as : ($PE_{\text{dose group}}$ / $PE_{\text{vehicle control group}}$) x 100.

Acceptance criteria

21. The acceptance criteria for this study are as follows.

- 1000 colonies per treatment group should be available for morphological transformation (less than 1000 colonies is acceptable in case of significant increase in morphological transformation rate). The average number of colonies per plate should not be less than 25.

- An average of 25-45 colonies per dish for each treatment group (a colony number beyond these limits is acceptable in case of negative results with < 25 colonies, or positive results with > 45 colonies per dish).

- Cloning efficiency of the negative/vehicle control should be ≥ 20%.

- No colony formation should be observed in the feeder cell control dishes. Feeder cells must be visible in the chemical treatment groups unless they are affected selectively by the compound.

- Transformation frequency in the negative controls (untreated and vehicle) are within historical controls.

- The positive control substance must lead to a statistically-significant increase of morphological cell transformation.

- There should be at least 5 scorable concentrations.

Statistics

22. The data of one or several trials are pooled for each treatment group. Results are analysed using the one-sided Fisher's exact test (Armitage, 1955) to determine if an increase in morphological transformation occurred compared to vehicule control.

23. The Cochran-Armitage trend test for a positive dose-related response is performed when only one chemical concentration shows a statistically significant response.

ANNEX II: PROTOCOL OF THE BALB/C 3T3 CTA

Standard method

Introduction

1. The BALB/c 3T3 cell transformation assay aims to determine the carcinogenic potential of genotoxic and non-genotoxic chemicals. Clone A31-1-1 is derived from BALB/c 3T3 cell line originated from BALB/c mouse embryo cultures. It is used as a sensitive and stable cell line for the focus formation assay. Genotoxic chemicals produce foci in the cultures subjected to a standard protocol or emphasized by post-treatment with tumor promoter TPA: non-genotoxic chemicals are evaluated for promoting activity of carcinogenesis in a two-stage method where cultures are first treated with a known carcinogen and then with a test chemical.

Materials

Preparation of cells

2. Cells should be obtained from a reliable source and expanded by two passages being maintained less than subconfluence in culture plates to avoid the predominant proliferation of transformed variants after showing cell-to-cell contact inhibition. Health Science Research Resource Bank (Osaka, Japan) supplies frozen cultures of BALB/c 3T3 cells clone A31-1-1 that may be used for this assay. Expanded cells are cryo-preserved in liquid nitrogen in an aliquot until required for assays. The cryo-preserved cells must be confirmed for their low background of spontaneous transformants and the ability to form appropriate numbers of transformed foci before using them for an assay. The transformation assay must be started using cryo-preserved stock cells. Conventionally expanded cells can be used for cytotoxicity tests to determine the optimal concentration range of a chemical for performing a transformation assay.

Medium and culture

3. Cells are cultured in Eagle's minimal essential medium (MEM), supplemented with 10% heat-inactivated fetal bovine serum (FBS) and antibiotics (60 μg/ml kanamycin). Another medium and other antibiotics are occasionally used. FBS must be prescreened for plating efficiency (about 50%) , low saturation density, low spontaneous transformation frequency and a sensitivity to carcinogens to form appropriate numbers of foci per dish when treated with 1 to 5 μg/ml 3-methylcholanthrene (MCA). Cultures are maintained in a humidified incubator with an atmosphere of 5% CO_2 in air at 37ºC.

Test chemicals and positive and negative controls

4. Chemicals should be weighed and diluted immediately prior to use. Distilled water, dimethylsulfoxide, acetone, and ethanol can be used to dissolve chemicals, and final solvent concentrations in the medium should not exceed 5%, 0.2%, 0.5%, and 0.1 %, respectively. Positive controls are usually 1 to 5 μg/ml MCA and negative controls are the solvents used for dissolving the test chemical. In a two-stage protocol, MCA can be used as an initiator at 0.1 to 0.2 μg/ml, and 12-*o*-tetradecanoylphorbol-13-acetate (TPA) as a tumour promoter at a concentration of 0.1 to 0.2 μg/mL.

Experimental design

Cytotoxicity test

5. A cytotoxicity test is carried out prior to the standard and the two-stage transformation assays to select the optimal range of test chemical concentrations for the transformation assays, and parallel to the transformation assays to evaluate the cytotoxicity of each treatment. Cytotoxicity is usually determined by the colony-forming efficiency. Cells are seeded at a density 100 to 200 cells/60-mm dish (3 or 4 dishes for each treatment) and are treated with a test chemical in the same manner as in the transformation assay. The cultures are fixed and stained with Giemsa's solution 7 to 10 days after seeding, and the colonies are counted. Cell survival of a treatment is expressed relative to that of the vehicle control (relative colony forming efficiency). Alternatively, a crystal violet staining method is used as a cytotoxicity test, where the inoculum cell density is the same as in the transformation assays.

Test chemical concentration

6. Five concentrations will be selected from the cytotoxicity test results to be tested for cell transformation. The range of tested concentrations will include (i) a high dose causing an approximate80-90% decrease in relative plating efficiency or relative cell density, (ii) a low dose having no effect on plating efficiency, and (iii) 3 intermediary doses more or less evenly spaced between these two extremes.

7. If the test substance is essentially non-toxic, then at least five concentrations will be selected up to a maximum of 5 mg/mL or 10mM. For non-toxic and insoluble test substances, the highest dose level tested will be within 2-times the visible solubility limit in complete medium. For toxic and insoluble test substance, the highest dose level tested should cause an approximate 80-90% decrease in relative plating efficiency or relative cell density, regardless of the number of insoluble dose levels.

Standard transformation assay

8. The frozen stock cells are rapidly thawed and cultured. Actively growing cells are seeded at a density of 10^4 cells/60-mm dish (10 to 20 dishes for each treatment) in 4 ml of culture medium. After 24 hr incubation subsequent to plating cells, culture medium is removed from each plate and replaced with medium containing a test chemical, or a chemical dissolved in a small volume of vehicle is added to each plate without replacement of medium. Cells are exposed to the test chemical for 72 hr. The medium is replaced with fresh medium and changed twice a week during 3½ weeks and once a week during the following 2 weeks. At the end of incubation the medium is removed and the cells are rinsed with saline, fixed in methanol, and stained with 5 to 10% aqueous Giemsa for scoring focus formation.

Two-stage transformation assay

9. The treatment of cells with the initiating agent in the first stage of the two-stage assay is conducted as described above for the standard transformation assay. Initiating agents most often employed are 3-methylcholanthrene (MCA) or *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG). Concentrations of the initiators are chosen that give minimal increases above spontaneous background values for transformation in standard assays. Typical concentrations are 0.1 μg/ml for MCA, and 0.5 μg/ml for MNNG. Following removal of the medium containing the initiating agent, cells are refilled with fresh normal medium for 3 days. The medium is removed and replaced with medium containing the promoting agent for 2 weeks. The timing of promoter addition can have variable effects on the frequency of promoted focus formation. Investigation of the timing of promoter addition may be required to determine an optimal response (Sakai and Sato, 1989).

10. Medium with promoter is replaced at least twice per week for the duration of promoter treatment. Thereafter medium is changed with normal medium twice a week during the first week and once a week during the following 2 weeks. Cells are fixed and stained as described for the standard transformation assay.

Evaluation of the results

Scoring morphological transformation

11. Transformed foci (type III) are scored for morphological transformation under a stereomicroscope. Transformed foci (type III) are characterized by bizarre shapes, dense multilayer structures, predominance of spindle-shaped cells, basophilic staining, random orientation of cells at the periphery and invasion into the surrounding contact-inhibited monolayer.

12. Scores are reported as a number of dishes with focus(i) among total dishes and the average number of transformed foci per dish in a treatment group.

Statistical analysis

13. The standard transformation and two-stage transformation assay results are analysed using the one-sided Fisher's exact test to determine if an increase in morphological transformation has occurred relative to the solvent control.

Modified Method

14. In order to increase the transformation frequency, the standard method may be modified as follows: (1) use of serum-reduced medium (DMEM/F12 medium supplemented with 2% FBS and 2 μg/ml insulin) during the stationary phase of cell growth; (2) use of 90 mm dishes instead of 60 mm dishes; and, (3) culture for 3½ weeks but with a medium change once a week during the last 2 weeks. These modifications make the assay more sensitive and more economical.

ANNEX III: PROTOCOL OF THE C3H10T1/2 CTA

Introduction

1. The development and use of the C3H/10T1/2, clone 8, focus formation assay was reviewed in detail by an IARC working group in 1985, which compiled a series of recommended procedures for conducting the assay (IARC, 1985). Several methodological and technical weaknesses have been identified, especially for issues of fetal bovine serum variability, effects of subjectivity on quantisation in scoring transformed foci, and validation of the major assay modifications mentioned above (Landolph, 2006). The level II assay has been the most substantial development of the assay since that time, but since it has not received substantial validation, it will not be described here.

2. The following brief description of the basic assay protocol covers the main features important to conducting and scoring both single- and two-stage focus formation assays, with mention of minor variations that have been reported.

Materials

Preparation of cells

3. Low-passage frozen stock cultures should be obtained from a reliable source and expanded by one or two passages, then cryopreserved in liquid nitrogen until required for assays. Passages 8 to 15 have been generally available and are recommended for their low-backgrounds of spontaneous transformants. The American Type Culture Collection currently supplies frozen cultures of clone 8 at passage 10. Subsequent culture passages should be performed prior to cells reaching confluence, which minimizes the appearance of transformed variants in the stock cultures. Seeding 2.5 x 10^4 cells per 100 mm dish or 75 cm^2 flask would start typical stock cultures, with passage at 7 days incubation.

Medium and culture conditions

4. Culture medium is most frequently Earle's basal medium with Earle's salts (EBME) containing 10% heat inactivated foetal bovine serum. Cultures often are supplemented with 25 μg/ml gentamycin, although stock cultures maintained in antibiotic free medium will allow the presence of bacterial or fungal contamination to be detected prior to distributing cells into the transformation assay vessels. Tests for mycoplasma contamination should be made. Serum lots must be pre-screened for plating efficiency (20 to 30%), low saturation density (less than 10^6 cells/60 mm dish), low spontaneous transformation frequency (less than 0.15 focus per dish) and a response of 1 to 2 foci per dish when treated with 1 to 2.5 μg/ml 3-methylcholanthrene.

5. Standard culture conditions are employed: humidified incubation at 37ºC, in an atmosphere of 5% CO2 and 95 % air (preferably HEPA filtered to maintain sterility).

Test chemical and positive and negative controls

6. Test chemicals should be weighed and diluted in aseptic vehicle immediately prior to use. Distilled water, acetone, dimethylsulfoxide and ethanol are solvents compatible with the C3H/10T1/2 cell transformation assay. Final solvent concentration in the medium should be non-toxic and not exceed 0.1 % (v/v). It is preferable to use medium to dissolve water-soluble compounds, if they are not highly chemically reactive. The positive control in one-stage assays is 3-methylcholanthrene (MCA) and negative controls are medium alone and medium with vehicle.

7. The positive control for promotion of initiated cells in two-stage assays is 12-*O*-tetradecanoyl phobol 13-acetate (TPA) and the negative control is medium with vehicle. Two-stage assays also require a series of three control cultures consisting of uninitiated cells (a vehicle control) that are treated during the promotion phase with vehicle alone, TPA alone and with the test promoter alone.

Experimental Design

Cytotoxicity test

8. Dishes for cytotoxicity measurements are seeded with 100 to 250 cells and treated concurrently with the dishes used for the first-stage assay. After removal of the medium containing test agent, 5 ml of complete medium is returned to the dish, and incubation continued for 8 to 14 days. Surviving colonies are fixed and stained for counting.

9. For cytotoxicity measurement relevant to testing promoting agents in the two-stage assay, it is necessary to measure effects on cellular proliferation prior to setting up a two-stage assay. In the two-stage assay, the promoter must be added during exponential growth period of the culture (beginning one to two days after removal of the initiating agent) and continued during the confluent (plateau) phase, for up to six weeks, therefore it is necessary that the promoter not inhibit growth to the extent that confluence cannot be attained, or be toxic to the continued viability of the confluent monolayer. Determination of cell growth curves, in the continuous presence of test agent, by cell counts or dye-redution assays (tetrazoof test agents to be assessed. C3H/10T1/2 cells may be seeded into the wells of multi-well tissue culture plates and grown to achieve confluence for one or two days. Test agents should be added to the wells for 18 to 48 hours. At the end of the incubation period, a multiplate reader may be used for automated recording of dye reduction activity.

Test chemical concentration

10. Concentrations of test agents used for cell transformation in the one-stage assay should reduce plating efficiency to as little as 10% of vehicle control values, but should cover a range(over 4 to 5 dilutions) up to 90% of control values so that sufficient colony-forming cells remain to form a confluent monolayer. MCA (1 to 2.5 μg/ml) serves as the positive control. Negative controls are usually the vehicle alone (water, acetone, dimethylsulfoxide and ethanol up to 0.1%).

11. In the two-stage assay, a concentration of an initiator is chosen that gives minimal increases above spontaneous background values for transformation in single-stage assays. Typical concentrations for initiation are 0.1 μg/ml for MCA, and 0.5 μg/ml for MNNG. These concentrations may vary somewhat with serum batch and should be known prior to beginning the two-stage assay. Concentrations of second-stage test agent sufficient to achieve suppression of culture growth rates or of dye reduction in confluent monolayers to between 90% and 50% of controls are suggested. Enhancements of proliferation or dye reduction may be observed following treatments with certain non-genotoxic chemicals.TPA (0.1 to 1 μg/ml) is most commonly used as the second-stage positive control, or as the promoter of test initiating agents.

Standard Transformation Assay (one-stage)

12. Cells for dispersal into assay plates are harvested from low-passage stock cultures in a logarithmically growing state and washed in buffered saline with low-speed centrifugation to remove trypsin and cellular debris, then resuspended in complete medium while cell counts are obtained. Both the single and two-stage assays are timed from the point at which 1000 to 2000 cells (up to 300 to 600 surviving colonies) are seeded into dishes.

13. Vessels for assays most often used are individual 50 mm diameter plastic dishes (nominally 60 mm with lids), 12 to 50 dishes per treatment condition, each containing 5 ml of complete medium. Multiwell dishes are best avoided due to potential cross-contamination of wells if fungal contamination occurs in any single well. 25 cm^2 flasks may be advantageous when testing volatile chemicals or during exposures in radiation-emitting devices situated outside of tissue culture incubators. Closed vessels are, however, somewhat disadvantageous for executing the weekly/biweekly medium changes for the multiple replicates and treatment groups during the 6 week incubation period following exposures.

14. After 24 to 48 hr of incubation subsequent to plating cells, culture medium is removed from each plate and replaced with medium containing dissolved test agent, or added directly in a small volume of medium. Highly reactive compounds may be added to the plates in serum-free medium for short durations (4 to 6 hr).

15. The C3H/10T1/2 cell transformation assay may be made sensitive to promutagens and procarcinogens that require metabolic activation by mixed function oxidases before they are significantly biologically reactive, toxic or carcinogenic. Metabolic conversion reactions are achieved by addition of an exogenous metabolic activation system consisting of a subcellular S9 fraction from induced liver homogenate. The metabolic capabilities, protein concentration, and inherent cytotoxicity of the activation system should be characterized beforehand. For treatment, medium is completely removed from the dishes followed by addition of 1 ml of an activation mixture, along with the test compound. Activation mixtures may be composed of an NADPH regenerating system such as NADH, NADP, NADPH and glucose-6-phosphate, and 1 to 20 mg/ml of S9 protein (from frozen stocks) diluted in serum-free or low-serum supplemented medium. Incubation is continued for up to 4 hr. Cytotoxicity tests with and without agent and S9 activation mix must be conducted in the same manner as the treatment for cell transformation.

16. Culture medium with test agent (or with agent plus S9 activation mix) is completely removed and 5 ml of fresh complete medium is then returned immediately to the plates. Eagle's minimal essential medium (MEM) has been used in transformation assays and focus-formation frequencies were indistinguishable from those in EBME (Dunkel et al., 1988). Dulbecco's modification of MEM has also been reported to support transformation (Parfett and Pilon, 1995). Antibiotics (gentamycin, 25 μg/ml or penicillin 100 U/ml and streptomycin 50 μg/ml) may be added to cultures to suppress contamination during the 6 week incubation subsequent to treatment with the test agent. Fungizone should be avoided as it substantially reduces MCA-induced transformation frequency (Resznikoff et al., 1973).

17. Medium is changed at least once per week for the duration of the assay. After 6 weeks incubation, the medium is removed, the cells are rinsed with saline, fixed in methanol, and stained with 10% aqueous Giemsa for scoring focus formation. Reducing the concentration of heat inactivated FBS from 10 % to 5 % after cells reach confluence (roughly at day 10), has been reported to elevate the frequency of transformed colonies in positive control dishes (Bertram, 1977).

Two-stage transformation assay

18. The treatment of cells with the initiating agent in the first stage of the two-stage assay is conducted as described above for the single-stage assay. Initiating agents most often employed have been 3-methylcholanthrene (MCA), or N-methyl-N'-nitro-N-nitrosoguanidine (MNNG), but unknown test agents may also be used. Following removal of the medium with initiating agent, complete medium is returned to the dishes for 24 to 48 hrs. The medium is removed and replaced with medium containing the promoting agent. The timing of promoter addition can have variable effects on the frequency of promoted focus formation. Investigation of the period of 1 to 8 days after initiator removal may be required to determine an optimal response (Mondal, et al., 1976; Parfett and Pilon, 1995).

19. Medium with promoter is replaced at least once per week for the duration of the assay incubation which is from 6 to 8 weeks in for the two-stage assay. Cell monolayers with foci are fixed and stained as described for the first stage assay.

Evaluation

Scoring morphological transformation

20. Three types of foci have been distinguished (I, II, III), although it is likely that a continuum of focal phenotypes exists. Type I foci, which are more tightly packed than the normal monolayer of C3H/10T1/2 cells and only slightly basophilic, are not scored since they do not give rise to neoplastic growths upon injection into irradiated C3H mice, while the latter two foci produce sarcomas. Type II foci display massive piling up into virtually opaque multilayers, the cells are moderately polar and criss-crossing is not pronounced. Type III foci are highly polar, fibroblastic, multilayered, criss-crossed arrays of densely stained cells and may display cording throughout the body of the focus. Invasive misoriented cellular projections radiating into the surrounding density-inhibited confluent monolayer of nontransformed cells are sometimes seen in Type III foci. Type III foci tend to produce greater numbers of sarcomas than Type II foci, upon injection into irradiated C3H mice. It is best practice to score foci blindly, with a dissecting stereomicroscope. Foci that are judged to be intermediate between two, types are scored conservatively by classifying them in the lower Type category. Scoring by multiple trained observers is also an advantage.

21. Scores are reported as the fraction of dishes in a treatment group with one or several foci and as the number of Type II and Type III foci per the total number of dishes counted in each group. Separate tallies are recorded for total, Type II and Type III foci.

Statistical analysis

22. The single-stage morphological transformation assay results are analysed using the one-sided Fisher's exact test to determine if an increase in morphological transformation has occurred relative to the solvent control. The two-stage morphological transformation results are analysed similarly, but the foci from the initiated second-stage treatment groups are compared to sum of the foci in initiator-only plus promoter-only controls. Promotion is characterized as a synergistic increase in foci above the effects of initiator-only and promoter-only treatments.

ANNEX IV: PERFORMANCES OF THE GENOTOXICITY ASSAYS TO PREDICT RODENT CARCINOGENICITY OF THE CHEMICALS EVALUATED FOR THEIR TRANSFORMING POTENTIAL

1. The performances of the genotoxicity assays to predict rodent carcinogenicity of the whole set of chemicals evaluated for their transformation potency in this document were evaluated. Tumor promoters were not included in the analysis. The results are presented in the table A.

Table A : Performances of *in vitro* and *in vivo* genotoxicity assays to predict rodent carcinogenicity established from the whole data sets of chemicals (not including tumor promoters)

	Ames	ML	HPRT	CA *In vitro*	CA *In vivo*	MN *In vivo*
Concordance (%)	52	72	78	62	60	58
Sensitivity (%)	39.5	86	80	65	57	58
Specificity (%)	79	26	70	59	68	55
+ Predictivity (%)	81	79	91	76.5	81	83
- Predictivity (%)	37	36	48.5	45	39.5	27
False negative (%)	60.5	14	20	35	43	42
False positive (%)	21	74	30	41	32	45
Chem. not incl. (%)	26	29	18	15	22	31
Total No. of chemicals	315	215	135	238	110	198
Non carcinogens (%)	29	30	22	30	29	25

2. Appropriate comparisons of genotoxicity and CTA assays would require that the same chemical sets are used to calculate the performances. Therefore, performances were evaluated also on the data sets restricted to the common compounds (non-carcinogens and carcinogens) tested in a CTA and a genotoxicity assay. Results are presented in tables B1, B2 and B3 for chemicals analyzed with the SHE, BALB/c 3T3, and C3H10T1/2 assays.

Table B1: Performances of *in vitro* and *in vivo* genotoxicity assays and the SHE assay to predict rodent carcinogenicity of chemicals. Performances were established from the sets of common chemicals evaluated with each genotoxicity assay and the SHE assay.

SHE set of chemicals	SHE	Ames	SHE	ML	SHE	HPRT	SHE	CA In vitro	SHE	CA In vivo	SHE	MN In vivo
Concordance (%)	86	48	86	75	92	78	86	62	86	66	83	56
Sensitivity (%)	90.5	36	88	86	96	79	89	63	91	62.5	87	57
Specificity (%)	75	78	80	35	74	71	77	59	75	71	71	54
+ Predictivity (%)	90.5	80	92	82	94	91	91	77	91	81	90	81
- Predictivity (%)	75	33	72	41	82	50	72	43	75	50	63	27
False negative (%)	10	64	12	14	4	21	11	37	9	37.5	13	43
False positive (%)	25	22	20	65	26	29	23	41	25	29	29	46
Chem. not incl. (%)	10	21	10	28	10	15	12	15	6	22	10	32
Total Number of chemicals	242		166		111		180		78		154	
Non carcinogens (%)	29		28		21		29		29		26	

Table B2: Performances of the *in vitro* and *in vivo* genotoxicity assays and the BALB/c 3T3 assay to predict rodent carcinogenicity of chemicals. Performances were established from the sets of common chemicals evaluated with each genotoxicity assay and the BALB/c 3T3 assay.

BALB/c 3T3 set of chemicals	BALB	Ames	BALB	MLI	BALB	HPRT	BALB	CA In vitro	BALB	CA In vivo	BALB	MN In vivo
Concordance (%)	70	51	70	71	76	79	68	61	75	65	71	64
Sensitivity (%)	78	40	78	89	84	76	79	62	84	57	78	61.5
Specificity (%)	52	73	53	23	43	92	46	60	53	83	52	72
+ Predictivity (%)	78.5	75	77	76	86	98	74	72	82	87.5	82	89
- Predictivity (%)	51	38	54	43	40	48	53	48	56	48	47	34
False negative (%)	22	60	22	11	16	24	21	38	16	43	22	38
False positive (%)	48	27	47	77	57	8	54	40	47	17	48	28
Chem. not incl. (%)	13	25	17	29	13	18	15	15	19	24	15	30
Total Number of chemicals	179		139		82		143		75		119	
Non carcinogens (%)	31		34		21		35		32		28	

Table B3: Performances of *in vitro* and *in vivo* genotoxicity assays and the C3H10T1/2 assay to predict rodent carcinogenicity of chemicals. Performances were established from the sets of common chemicals evaluated with each genotoxicity assay and the C3H10T1/2 assay.

C3H10T1/2 set of chemicals	C3H	Ames	C3H	MLI	C3H	HPRT	C3H	CA In vitro	C3H	CA In vivo	C3H	MN In vivo
Concordance (%)	79	47	76	86	80	83	92	76	86	71	81	67
Sensitivity (%)	81	42	78	92	83	83	83	79	87	71	81	71
Specificity (%)	67	71	69	29	64	82	77	65	75	67	75	29
+ Predictivity (%)	93	88	93	93	93	96	95	90	98	93	96	89
- Predictivity (%)	40	20	37.5	25	41	47	43	44	33	29	33	11
False negative (%)	19	58	22	8	17	17	17	21	13	29	19	29
False positive (%)	33	29	31	71	36	18	23	35	25	33	25	71
Chem. not incl. (%)	18	38	21	23	20	21	20	24	18	30.5	23	29
Total Number of chemicals	137		101		89		111		62		93	
Non carcinogens (%)	17		18		17		16		11		12	

ANNEX V: PERFORMANCES OF THE SHE ASSAY AT PH ≥ 7.0, ON CHEMICALS TESTED USING THE STANDARD PROTOCOL

Exposure period of 7 days

1. SHE results of chemicals tested using a standard protocol and/or a sequential treatment (ST) during 7 days of exposure.

In the 4th version of the DRP (december 2006), the performances of the SHE assay (pH ≥ 7.0) was calculated on a set of chemicals whose 14 chemicals had been tested using a standard treatment and/or a sequential treatment:

- 12 rodent carcinogens
- 2 rodent non carcinogens (table A).

The sequential treatment improved the results for 4 chemicals: benzoyl peroxide, butyl hydroxytoluene, chlordane and testosterone, which lead to the evaluation indicated in table A (left column). The evaluation will change for these chemicals if results of the sequential treatment are not considered, which gives 3 chemicals evaluated "D" (benzoyl peroxide, chlordane and testosterone) and 1 chemical "negative" (butyl hydroxytoluene) as indicated in table A (right column).

Two chemicals (2-nitropropane as rodent carcinogen, and 1-nitropropane as rodent non carcinogen) which had been tested using a sequential treatment only, will be withdrawn from the set of chemicals, because there is no data using a standard treatment.

Table A: Results of the SHE assay at pH ≥ 7.0 of chemicals tested using a standard protocol and/or a sequential treatment (ST) during 7 days of exposure.

Chemical			Prior evaluation (dec 2006)	Results of sequential treatment	Results of standard treatment	Evaluation excluding data of sequential treatment
Rodent carcinogens						
Benzo(a)pyrene	50-32-8		+	+	+	+
Benzoyl peroxide	94-36-0	P	+ (P)NC	+	+/-,-	D2*
Butyl hydroxytoluene	128-37-0		+ (P) NC	+		-
Chlordane	57-74-9		+ (P) NC	+	+/-	D1
Clofibrate	637-07-0		+	+	+	+
Diethylstilbestrol	56-53-1		+	+	+	+
Methyl clofenapate	21340-68-1		+	+	+	+
2-nitropropane	79-46-9		+	+		
Testosterone	58-22-0		+	+	+, -	D2
12-O-Tetradecanoyl phorbol- 13-acetate.	16561-29-8	P	+	+	+	+*
Nickel sulphate	7786-81-4		+	+	+	+
Potassium chromate	7789-00-6		+	+	+	+
Rodent non carcinogens						
1-nitropropane	108-03-2			+		
Phenol	108-95-2		+ (P)	+	+	+

* Tumor promoters were not included in the evaluation of performances

2. Performances calculated from chemicals tested using standard protocol

The performances of the SHE assay calculated on data obtained using a standard protocol only and excluding data of sequential treatment are indicated in table B.

As tumor promoters were not included in the analysis in the 4th version of the DRP, benzoyl peroxide and TPA are not taken into account in the present calculation ; butyl hydroxytoluene, chlordane and phenol, which were classified as tumor promoters, will now be accounted on in the present analysis.

The performances of the SHE assay calculated on data obtained using a standard protocol indicate minor change from the precedent calculation.

Table B : Performance characteristics of the SHE cell transformation assay (pH ≥7.0) based on data of standard protocol for the prediction of rodent carcinogenicity.*

	Standard treatment
Total chemicals	206
Prevalence of Carcinogens	155/206=.75
Concordance	162/ 190=.85
Sensitivity	129/140=.92
Specificity	33/50=.66
+ Predictivity	129/146=.88
– Predictivity	33/44=.750
False +	17/50=.34
False –	11/140=.08
Chemicals not incl.	16