Regulatory Toxicology and Pharmacology 55 (2009) 52–68

Contents lists available at ScienceDirect

Regulatory Toxicology and Pharmacology

journal homepage: www.elsevier.com/locate/yrtph

Practical issues on the application of the GHS classification criteria for germ cell mutagens

Takeshi Morita [a,*], Makoto Hayashi [b], Madoka Nakajima [b], Noriho Tanaka [c], David J. Tweats [d], Kaoru Morikawa [a], Toshio Sofuni [e]

[a] National Institute of Health Sciences, 1-18-1, Kamiyoga, Setagaya-ku, Tokyo 158-8501, Japan
[b] Biosafety Research Center, Foods, Drugs and Pesticides, 582-2, Shioshinden, Iwata, Shizuoka 437-1213, Japan
[c] Hatano Research Institute, Food and Drug Safety Center, 729-5, Ochiai, Hadano, Kanagawa 257-8523, Japan
[d] The School of Medicine, University of Swansea, Swansea, SA2 8PP, UK
[e] Formerly National Institute of Health Sciences, 1-18-1, Kamiyoga, Setagaya-ku, Tokyo 158-8501, Japan

ARTICLE INFO

Article history:
Received 12 February 2009
Available online 6 June 2009

Keywords:
GHS
Germ cell mutagens
Hazard classification
Expert judgment
Weight of evidence
GHS implementation

ABSTRACT

The Globally Harmonized System of Classification and Labeling of Chemicals (GHS) requires classification of chemicals on germ cell mutagenicity. The Japanese government has conducted GHS classification on about 1400 chemicals in a 2-year project (J-GHS) for implementing GHS domestically. Prior to the classification work, the technical guidance for classification of germ cell mutagens was prepared. This guidance introduces the concept of heritable mutagenicity, and presents detailed criteria for germ cell mutagens, test data to be used, and a practical decision tree for classification. These practical guidance and supporting explanations are useful for non-expert Classifiers (scientists applying the classification criteria). Several issues, however, were identified during the course of J-GHS and in re-evaluating the classification results. These include: (1) the information sources when available data are limited; (2) lack of understanding GHS classification criteria or insufficient review of the information by Classifiers; (3) varying opinions of experts on data quality and weight of evidence, and; (4) decision tree approaches, e.g., inadequacy for use in overall evaluation in some cases. Ideally, classification should be performed by Classifiers with high expertise using high quality information sources. Genetic toxicologists as experts should consider data quality and reliability, and give a critical review of all available information for support of classification. A weight of evidence approach is also required to assess mutagenic potential of chemicals. Critical points for suitable classification for GHS are discussed.

1. Introduction

Communicating the hazards of dangerous chemicals to workers and the public is a key foundation for protecting human health and the environment. As a major break-through in this area, the Globally Harmonized System of Classification and Labeling of Chemicals (GHS) was adopted by the United Nations Economic and Social Council Subcommittee (UN ECOSOC) of Experts on the GHS in December 2002 and endorsed by ECOSOC in July 2003 (UN, 2003, 2005, 2007). The GHS has the ultimate goal of ensuring that information on chemical hazards (such as on labels and safety data sheets) is made available to workers and consumers in a harmonized and comprehensible format in all countries around the world. The GHS has become the major international tool for effective chemical classification and hazard communication. It represents an important step in harmonizing national chemical hazard communication systems worldwide and has a great potential to improve chemical safety across all relevant sectors. The GHS is a consistent and coherent approach to identifying the hazards of chemicals, and providing information on these hazards and associated protective measures to users or those who may be exposed. The system is structured so that appropriate elements for classification and communication, which consider the target population, can be selected. Those who then use chemicals can take the proper steps to protect themselves and the environment. Target populations include employers, workers (including those involved in transport), consumers, and emergency responders. Others who provide services to these people will also find the information useful (e.g., doctors, toxicologists, nurses, safety engineers and occupational hygienists) (UNITAR, 2007). The GHS covers all hazardous chemical substances, dilute solutions and mixtures. It also addresses how labels and safety data sheets should be used to convey information about their hazards, and how to protect people from these effects. However, pharmaceuticals, food additives, cosmetics, and pesticide residues in food will not necessarily be

* Corresponding author. Fax: +81 3 3700 1483.
E-mail address: morita-tk@nihs.go.jp (T. Morita).

0273-2300/$ - see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.yrtph.2009.06.001

covered by the GHS in terms of labeling at the point of intentional intake.

In response to the recommendation made by the United Nations and the agreement endorsed at an Asia–Pacific Economic Cooperation (APEC) meeting, the Japanese Government has started a series of national projects for the implementation of the GHS. One of the projects is classification of approximately 1400 chemicals which are regulated under current legislation. In Japan, material safety data sheets (MSDS) are required for approximately 1400 chemicals that are regulated by the Industrial Safety and Health Law, the Poisonous and Deleterious Substances Control Law or the Pollutant Release and Transfer Register (PRTR). The Japanese government decided to implement classification of these chemicals as 2-year project (Japanese GHS Classification Project, J-GHS) in 2005 and 2006, aiming to help industries issuing MSDS and to develop infrastructure for GHS implementation. The classification work was performed by experts from laboratories and industries and the results were checked by the members of the Inter-ministerial Committee if deemed necessary. The classification results are not compulsory and allow industries to use their own data and classify chemicals on the basis of their own judgment. The results are available in the websites of National Institute of Technology and Evaluation (NITE, http://www.safe.nite.go.jp/english/ghs_index.html#results).

Prior to the classification work, a "GHS Classification Manual" and a "Technical Guidance Document on the GHS Classification" were developed to facilitate the classification of chemicals within the limited time schedule, and to eliminate any conflicting results amongst experts (both are available in English from the website http://www.safe.nite.go.jp/english/ghs_index.html) (NITE, 2005a, 2005b). The Manual provides the main rules and reliable data sources for classification on physical, health and environmental hazards, e.g., peer-reviewed documents prepared by international authorities or governments. In general original scientific literature was not used. This enabled industries to avoid checking the peer-review of the international organizations in their voluntary classification. The technical guidance provides precise rules for GHS classification in J-GHS on each item of health hazards.

Germ cell mutagenicity is included as one of health hazards in the GHS. Definitions of mutagenicity and genotoxicity, classification criteria for substances or mixtures, and decision logic are provided in the GHS text (UN, 2007). However, these may still create some difficulties for non-experts to understand the definitions and explanations. Therefore, precise descriptions were provided on the Guidance for the classification of germ cell mutagens (GCM). It introduces the concept of heritable mutagenicity, and presents detailed criteria of GCM, mutagenicity or genotoxicity test data to be used, and a flow chart for classification.

GHS classifications were performed in accordance with the Manual and the Guidance in J-GHS. Despite these rules, some inappropriate classifications were found. Several issues for a practical approach to the GCM classification were identified during the review process of the project. In this paper, practical approaches of the classification of GCM and examples are presented and its usefulness is discussed. The issues identified in the classifications are also discussed.

2. GHS classification systems for germ cell mutagens

Category 1 is used for chemicals known to induce heritable mutations (Category 1A) or known to be regarded as if they induce heritable mutations (Category 1B) in germ cells of humans. Category 2 is used for chemicals which cause concern for humans owing to the possibility that they may induce heritable mutations in the germ cells of humans. In the case of no concern of induction of heritable mutations in the germ cells of humans or no sufficient evidence of inclusion in Category 1 or 2, the substances are regarded as "not classified". If there are not enough data to be evaluated to determine the mutagenicity of the substance, it is regarded as "classification not possible" (Fig. 1) (UN, 2007). Hazard categories and the criteria for germ cell mutagens in GHS are summarized in Table 1.

The basic concept of classification criteria in GHS is hazard identification and not risk based evaluation (UN, 2007). The germ cell mutagens should be classified by considering the weight of evidence. For classification, test results are considered from experiments determining mutagenic and/or genotoxic effects in germ and/or somatic cells of exposed animals. Mutagenic and/or genotoxic effects determined in *in vitro* tests may also be considered on a case-by-case basis. The tests should be well conducted and sufficiently validated, preferably as described in OECD Test Guidelines. Evaluation of the test results should be done using expert judgment and all the available evidence should be weighed for

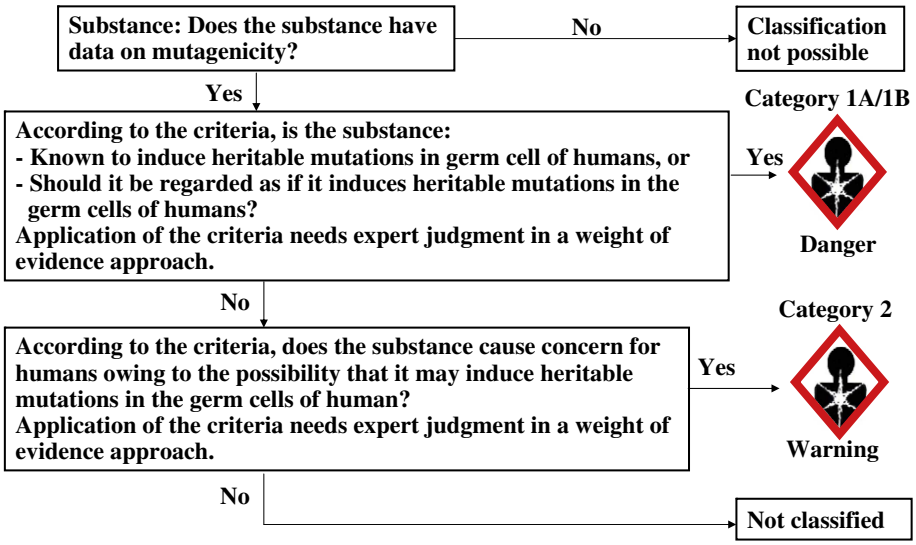

Fig. 1. Decision logic for germ cell mutagenicity for substances in the GHS. The mixture will be classified as a mutagen when at least one ingredient has been classified as a Category 1 or Category 2 mutagen and is present at or above the cut-off value/concentration limit (i.e., 0.1% for Category 1 mutagen, 1.0% for Category 2 mutagen) for Category 1 or 2, respectively.

Table 1
GHS classification on germ cell mutagens.

Category	Classification	Criteria
Category 1	Chemicals known to induce heritable mutations (Category 1A) or to be regarded as if they induce heritable mutations in the germ cells of humans (Category 1B)	
Category 1A	Chemicals known to induce heritable mutations in germ cells of humans	Positive evidence from human epidemiological studies.
Category 1B	Chemicals which should be regarded as if they induce heritable mutations in the germ cells of humans	– Positive result(s) from *in vivo* heritable germ cell mutagenicity tests in mammals; or – Positive result(s) from *in vivo* somatic cell mutagenicity tests in mammals, in combination with some evidence that the substance has potential to cause mutations to germ cells. This supporting evidence may, for example, be derived from mutagenicity/genotoxic tests in germ cells *in vivo*, or by demonstrating the ability of the substance or its metabolite(s) to interact with the genetic material of germ cells; or – Positive results from tests showing mutagenic effects in the germ cells of humans, without demonstration of transmission to progeny; for example, an increase in the frequency of aneuploidy in sperm cells of exposed people. Examples of *in vivo* heritable germ cell mutagenicity tests are: – Rodent dominant lethal mutation test – Mouse heritable translocation assay – Mouse specific locus test Examples of *in vivo* somatic cell mutagenicity test are: – Mammalian bone marrow chromosome aberration test – Mouse spot test – Mammalian erythrocyte micronucleus test Examples of mutagenicity/genotoxicity tests in germ cells are: (a) Mutagenicity tests: – Mammalian spermatogonial chromosome aberration test – Spermatid micronucleus assay (b) Genotoxicity tests: – Sister chromatid exchange analysis in spermatogonia – Unscheduled DNA synthesis test (UDS) in testicular cells
Category 2	Chemicals which cause concern for humans owing to the possibility that they may induce heritable mutations in the germ cells of humans	– Positive evidence obtained from experiments in mammals and/or in some cases from *in vitro* experiments, obtained from: – Somatic cell mutagenicity tests *in vivo*, in mammals; or – Other *in vivo* somatic cell genotoxicity tests which are supported by positive results from *in vitro* mutagenicity assays. Examples of genotoxicity tests in somatic cells are: – Liver UDS *in vivo* – Mammalian bone marrow sister chromatid exchanges Examples of *in vitro* mutagenicity tests are: – *In vitro* mammalian chromosome aberration test – *In vitro* mammalian cell gene mutation test – Bacterial reverse mutation tests

Note. Chemicals which are positive in *in vitro* mammalian mutagenicity assays, and which also show chemical structure activity relationship to known germ cell mutagens, should be considered for classification as Category 2 mutagens.

classification. A single well-conducted test can be used for classification, if it provides a clear and unambiguously positive result. If new and well validated tests are developed, these may also be used in the total weight of evidence. The relevance of the route of exposure used in the *in vivo* study should also be taken into account the comparison with the route of human exposure.

3. Information sources used in J-GHS

It is not only inefficient but also not practical for general industrial Classifiers to collect original papers or relevant unpublished documents and to review them for GHS classification. Therefore, in general, international or national review documents or databases should be used as data sources for mutagenicity test results for the classification (NITE, 2005a). The major 21 information sources used in J-GHS are from International Program on Chemical Safety (IPCS), European Union (EU), OECD, US, Germany, Canada, Australia and Japan. These information sources with their abbreviated names or web addresses are shown in Table 2. Most of them can be accessed easily on the internet free of charge. Some original papers were also used for the review of the classification results, if necessary.

The availability of suitably independent, commercial, in confidence, data and documents from industry sources (a major source of genotoxicity data) is also a problem.

4. Development of supportive guides for GHS classification

The Guidance for practical classification of GCM has been prepared by J-GHS. It consists of precise explanations of GCM for GHS classification, additional examples of mutagenicity or genotoxicity tests, and a practical decision tree for classification of GCM. Definition of criteria of germ cell mutagens in GHS are also proposed here. These practical approaches will be of help to Classifiers.

4.1. Additional examples of mutagenicity or genotoxicity tests for classification

In the GHS (UN, 2007), the term "mutation" applies both to heritable genetic changes that may be manifested at the phenotypic level and to the underlying DNA modifications when known (including, for example, specific base pair changes and chromosomal translocations). The terms "mutagenic" and "mutagen" are used for chemicals giving rise to an increased occurrence of mutations in populations of cells and/or organisms (UN, 2007). The more general terms "genotoxic" and "genotoxicity" apply to chemicals or processes which alter the structure, information content, or segregation of DNA, including those which cause DNA damage by interfering with normal replication processes, or which in a non-physiological manner (temporarily) alter its replication. GHS pro-

Table 2
Major information sources used for GHS classification for germ cell mutagens in the Japanese GHS Classification Project (J-GHS).

Abbreviated name	Information source	Access or note
ACGIH	Documentation of the threshold limit values for chemical substances by American Conference of Governmental Industrial Hygienists	7th edition (2001 or later) Issued by ACGIH (http://www.acgih.org/home.htm)
ATSDR	Toxicological Profile by US Agency for Toxic Substances and Disease Registry	http://www.atsdr.cdc.gov/toxpro2.html
CERI	Chemical hazard data sheet by Chemicals Evaluation and Research Institute	In Japanese http://www.cerij.or.jp/db/date_sheet_list/list_sideindex_cot.html
CERI-NITE	CERI-NITE hazard assessment report by Chemicals Evaluation and Research Institute (CERI) and National Institute of Technology and Evaluation (NITE)	In Japanese http://www.safe.nite.go.jp/data/sougou/pk_list.html?table_name=hyoka_risk
CICAD	Concise International Chemical Assessment Document by International Program on Chemical Safety (IPCS)	http://www.inchem.org/pages/cicads.html
DFGOT	Occupational Toxicants: Critical Data Evaluation for MAK Values and Classification of Carcinogens by Deutsche Forschungsgemeinschaft (DFG, German Research Foundation)	http://www.dfg.de/en/ Issued by WILEY-VCH (The MAK-Collection, http://www.wiley-vch.de/books/info/mak/collection.php)
ECETOC	Technical Report by European Center of Ecotoxicology and Toxicology of Chemicals	http://www.ecetoc.org/publications
EHC	Environmental Health Criteria by IPCS	http://www.inchem.org/pages/ehc.html
EURAR	European Union Risk Assessment Report by European Chemical Bureau (ECB)	http://ecb.jrc.it/home.php?CONTENU=/DOCUMENTS/Existing-Chemicals/RISK_ASSESSMENT/REPORT/
HSDB	Hazardous Substance Data Bank by US National Library of Medicine, Toxicology Data Network	http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB
IARC	Monographs on the Evaluation of Carcinogenic Risk to Humans by International Agency for Research on Cancer	http://monographs.iarc.fr/ENG/Monographs/allmonos90.php and Printed versions
IRIS	Integrated Risk Information System by US Environmental Protection Agency	http://cfpub.epa.gov/ncea/iris/index.cfm
IUCLID	International Uniform Chemical Information Database in European chemical Substances Information System by ECB	http://ecb.jrc.it/esis/esis.php?PGM=hpv&DEPUIS=autre
JECDB	Japan Existing Chemical Data Base by Ministry of Health, Labor and Welfare in Japan, Toxicity Testing Report for Environmental Chemicals	In Japanese http://dra4.nihs.go.jp/mhlw_data/jsp/SearchPageENG.jsp
NITE	Preliminary risk evaluation report of chemicals by National Institute of Technology and Evaluation	In Japanese http://www.safe.nite.go.jp/risk/riskhykdl01.html
NTP DB	Testing Information Data Base by US National Toxicology Program	http://ntp.niehs.nih.gov:8080/index.html?col=010stat
PATTY	Patty's Toxicology by Bingham, E., Cohrssen, B., Powell, C.H., (eds.)	5th edition (2001) Issued by John Wiley and Sons (http://www3.interscience.wiley.com/cgi-bin/mrwhome/104554795/HOME?CRETRY=1&SRETRY=0)
PECAR	Priority Existing Chemical Assessment Reports in National Industrial Chemical Notification and Assessment Scheme by Australia	http://www.nicnas.gov.au/publications/car/PEC.asp
PSAR	Priority Substance Assessment Reports by Environment Canada	http://www.hc-sc.gc.ca/ewh-semt/contaminants/existsub/psap-pesip_e.html Full set by CD-ROM
RTECS	Registry of Toxic Effects of Chemical Substances by US National Institute for Occupational Safety and Health	http://www.cdc.gov/niosh/npg/npgdrtec.html or other commercial providers
SIDS	OECD Screening Information Data Set by United Nations Environmental Program	http://www.chem.unep.ch/irptc/sids/OECDSIDS/sidspub.html OR http://www.inchem.org/pages/sids.html

vides hazard categories and their criteria for germ cell mutagens including the examples of mutagenicity/genotoxicity tests to be used for classification (see Table 1). However, the examples presented in the GHS text are not sufficient for the classification. There are many kinds of mutagenicity or genotoxicity tests developed, some of which are validated or are being validated on the detection of mutagenic or genotoxic effects of chemicals. These include gene mutation tests with transgenic animal models, assays of (covalent) binding or adduct formation to DNA *in vivo*, assays of DNA damage *in vivo* including comet assay and *in vitro* chromosome damage assays such as the mammalian cell micronucleus test. Some of these tests are used in the EU (European Communities, 2001; Pratt and Barron, 2003), German Maximale Arbeitsplatz-Konzentration (MAK) Commission (Adler et al., 2000; DFG, 2007) or US EPA scientists (Dearfield et al., 2002) for the classification of mutagens (Morita et al., 2006). Human monitoring or epidemiological data (e.g., chromosome analysis of peripheral lymphocytes, comet assays in lymphocytes or sperm) will be also available and useful for certain chemicals, though these data might be insufficient for drawing general conclusions. Additional examples of mutagenicity/genotoxicity tests for practical GHS classification of GCM are shown in Table 3. On the other hand, several tests are considered not to be used for classification in general. These tests include a number of *in vitro* genotoxicity tests, e.g., the comet assay or UDS test using mammalian cultured cells, host-mediated assays using bacterial gene mutation systems, DNA-repair test (Rec-assay) in bacteria, umu test or SOS test using bacteria, gene conversion test or aneuploidy test using yeast. The sperm abnormality test using rodents and several *Drosophila* tests (sex-linked recessive lethal test, wing spot test, etc.) also are not used for classification in principle. This is because sperm abnormalities might be due sometimes to the effects on targets other than genetic material, and the ADME (absorption, distribution, metabolism, excretion) profile and reproductive and developmental processes in insects differ from those in mammals. However, these tests may be considered on a case-by-case basis with expert judgment.

4.2. Practical decision tree for classification of germ cell mutagens and proposed definition of them for GHS

GHS provides the decision logic for germ cell mutagenicity. Simple decision trees have been devised, because expert judgment on a weight of evidence approach is always needed for application of the GHS criteria (see Fig. 1). However, it is difficult for Classifiers who are not expert in this field to classify the chemicals without guidance if they use it in combination with criteria of hazard categories in Table 1. Therefore, a practical decision tree for classification of GCM in GHS has been developed (Fig. 2). The judgment in the practical decision tree flows from upstream (i.e., Category 1A) to downstream (i.e., Category 2 or Not yet classified as genotoxic [as Not classified]) as well as the original tree in GHS. The practical tree uses all tests to be used for classification shown in Table 3. Each number in the box in Fig. 2 corresponds to the test examples in Table 3. The basic concept of this tree is that positive results outweigh negative results in each test because, when conflicting results were obtained, negative results sometimes arise from

Table 3
Examples of mutagenicity or genotoxicity tests for practical GHS classification of germ cell mutagens.

#1	*In vivo* heritable germ cell mutagenicity tests in mammals
	1.1 Mouse specific locus test
	1.2 Mouse heritable translocation test
	1.3 Rodent dominant lethal test
#2	*In vivo* germ cell mutagenicity tests in mammals
	2.1 Chromosomal aberration test in spermatogonia
	2.2 Micronucleus test in spermatid cells
	2.3 Gene mutation test in germ cells of transgenic rodents*
	2.4 Analysis of aneuploidy in sperm cells of exposed people*
#3	*In vivo* somatic cell mutagenicity tests in mammals
	3.1 Chromosome aberration test in bone marrow cells or peripheral lymphocytes
	3.2 Mouse spot test
	3.3 Micronucleus test in hematopoietic cells
	3.4 Gene mutation test in somatic cells of transgenic rodents*
	3.5 Metaphase or micronucleus formation analysis of peripheral lymphocytes of exposed people*
#4	*In vivo* germ cell genotoxicity tests in mammals
	4.1 Sister chromatid exchange (SCE) test in spermatogonia
	4.2 Unscheduled DNA synthesis (UDS) test in testicular cells
	4.3 Assay of covalent binding or adduct formation to germ cell DNA*
	4.4 Assay of DNA damage in germ cells (comet assay, alkaline elution assay, etc.,)*
#5	*In vivo* somatic cell genotoxicity tests in mammals
	5.1 UDS test in liver
	5.2 SCE test in bone marrow cells or peripheral lymphocytes
	5.3 Assay of covalent binding or adduct formation to somatic cell DNA*
	5.4 Assay of DNA damage in somatic cells (comet assay, alkaline elution assay, etc.,)*
	5.5 SCE analysis of peripheral lymphocytes of exposed people*
#6	*In vitro* mutagenicity tests
	6.1 Chromosomal aberration test in cultured mammalian cells
	6.2 Micronucleus test in cultured mammalian cells*
	6.3 Gene mutation test in cultured mammalian cells
	6.4 Reverse mutation test in bacteria (i.e., Ames test)

* Added to the examples in GHS.

inadequate experiments. Therefore, judgment of accuracy of the negative results, especially in table form, will be difficult for Classifiers using the GHS classification. While a single positive result can sometimes be pivotal to a decision about a classification, such findings should be considered on a case-by-case basis and are not necessarily definitive for classification.

Criteria of practical GHS classification of GCM used in the present J-GHS is presented in Table 4. This will be helpful to understand the practical classification scheme suggested. When human heritable germ cell mutagenicity is identified by human epidemiological studies, the substance will be classified as Category 1A, and can be regarded as a "human heritable germ cell mutagen" (Table 4). The existence of any such substance is not confirmed at present. However, exposures in the environment are far too many and far too complex, as are the potential genetic targets. Therefore, this does not mean that such substances do not exist.

When a positive result(s) from an *in vivo* mutagenicity test is available with suggestive data on mutagenicity in germ cells, the substance will be classified as Category 1B. Practically, the following examples will apply for this category: (1) positive results in heritable germ cell mutagenicity tests in mammals, e.g., dominant lethal test, heritable translocation test, or specific locus test; (2) positive results from *in vivo* germ cell mutagenicity tests in mammals, e.g., chromosomal aberration test in mammalian spermatogonia, micronucleus test in mammalian spermatid cells, or gene mutation test in germ cells of transgenic rodents (preferably, this will be supported by the positive result(s) from *in vivo* somatic cell mutagenicity tests in mammals); (3) positive findings of mutagenicity in human germ cells with no evidence of transmission to progeny, e.g., an increase in the frequency of aneuploidy in sperm cells of exposed people; (4) positive results from *in vivo* somatic cell mutagenicity test in mammals, e.g., chromosomal aberration test in mammalian bone marrow cells or peripheral lymphocytes, micronucleus test in mammalian hematopoietic cells, gene mutation test in somatic cells of transgenic rodents, or mouse spot test, with positive result(s) from *in vivo* germ cell genotoxicity tests in mammals, e.g., sister chromatid exchanges (SCE) test in mammalian spermatogonia, unscheduled DNA synthesis (UDS) test in mammalian testicular cells, assay of covalent biding or adduct formation to mammalian germ cell DNA, or assay of DNA damage (comet assay, alkaline elution assay, etc.) in mammalian germ cells, and; (5) positive results from *in vivo* somatic cell mutagenicity tests in mammals, with demonstration of exposure of the substance or its metabolite(s) to germ cells (preferably, with relevant route of exposure). A substance classified as Category 1B can be regarded as a "mammalian germ cell mutagen" (Table 4). Category 1B is similar to the categories of probable human germ cell mutagen and possible human germ cell mutagen in the proposed classification categories of Dearfield et al. (2002).

When positive result(s) from any *in vivo* somatic cell mutagenicity/genotoxicity test in mammals is available without supportive evidence of the mutagenicity in germ cell, the substance will be classified Category 2. The following cases will be normally classified in this category: (1) positive result(s) from *in vivo* somatic cell mutagenicity tests in mammals (see above); (2) positive finding(s) of mutagenicity in human somatic cells, e.g., an increase in the frequency of chromosomal aberrations or micronuclei in peripheral lymphocytes of exposed people; (3) positive result(s) in germ or somatic cell genotoxicity tests in mammals, e.g., SCE test in mammalian bone marrow cells or peripheral lymphocytes, UDS test in mammalian liver, assay of covalent biding or adduct formation to mammalian somatic cell DNA, or assay of DNA damage (comet assay, alkaline elution assay, etc.) in mammalian somatic cells, with positive result(s) from *in vitro* mutagenicity tests, i.e., chromosomal aberration test, micronucleus test, or gene mutation test in cultured mammalian cells, or reverse mutation test in bacteria; (4) positive finding(s) of genotoxicity in human somatic cells, e.g., an increase in the frequency of SCE in peripheral lymphocytes of exposed people, with positive result(s) from *in vitro* mutagenicity tests, and; (5) as a special case, (strong) positive results from *in vitro* mutagenicity tests with at least two different endpoints (without *in vivo* mutagenicity/genotoxicity test data), with a chemical structure activity relationship to known germ cell mutagens (Category 1 or 2 substances). In this case, expert judgment will be required. The "mutagen" covers the chemicals which induce the three levels of genetic damage, i.e., mutagenicity (gene mutation), clastogenicity (structural chromosomal aberration) and aneuploidy (numerical chromosomal aberration). The evidence that supports clastogenicity *in vivo* but not mutagenicity will lead to Category 1 or 2. However, if the only evidence of clastogenicity is from *in vitro* studies, this is insufficient for Category 1 or 2, without supportive data. In all categories, it is preferable for *in vivo* positive result(s) to be supported by the positive result(s) *in vitro*. This category can be regarded as describing "mammalian somatic cell mutagen" (Table 4).

When a negative result(s) in any *in vivo* germ or somatic cell mutagenicity tests is available, the substance will be classified "Not yet classified as genotoxic [as Not classified]". Substances with limited evidence (not sufficient evidence) in somatic cell mutagenicity/genotoxicity tests might be also considered as "Not yet classified as genotoxic". This situation might include negative result(s) from *in vivo* somatic and germ cell mutagenicity tests, but positive result(s) from *in vitro* mutagenicity tests. Expert judgment in a weight of evidence approach will be important to give a final call of classification in this example. This category can be regarded as "Not likely to be mammalian mutagen" (Table 4).

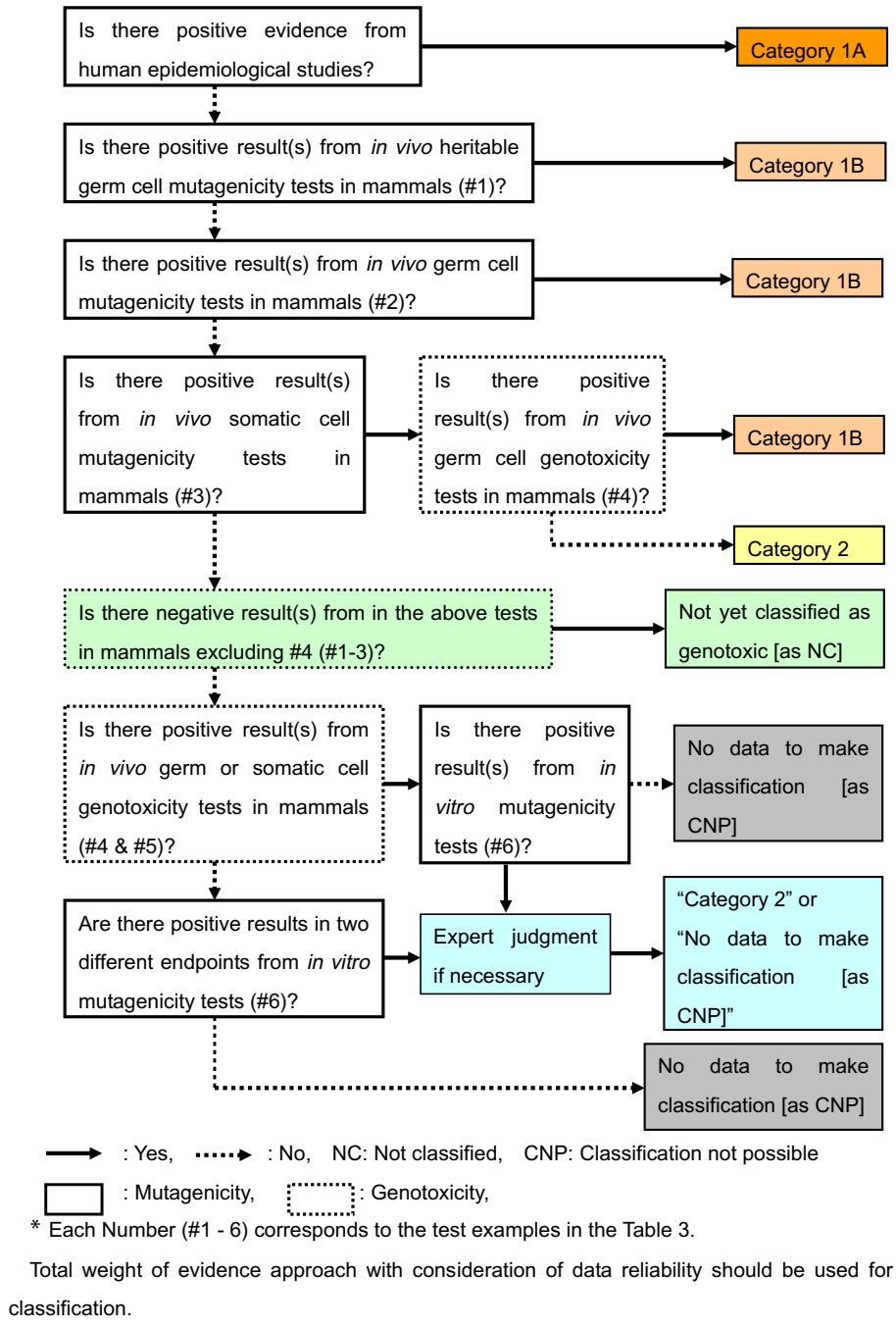

Fig. 2. Practical decision tree for classification of germ cell mutagens for GHS. Solid line is for Yes, dotted line is for No. Bold solid line box is for mutagenicity test set, dotted line box is for genotoxicity test set. Each number (#1–6) corresponds to the test examples in Table 3. Total weight of evidence approach with consideration of data reliability should be used for classification.

When no data from *in vivo* mutagenicity or genotoxicity tests in mammals are available, the substance will be classified "No data to make classification [as Classification not possible]". In addition, the case of no data from *in vivo* mutagenicity tests AND positive result(s) from *in vivo* somatic cell genotoxicity tests AND negative result(s) in any *in vitro* mutagenicity tests gives "No data to make classification". A recent analysis demonstrated that there is an extremely high false positive rate for *in vitro* mutagenicity/genotoxicity tests, when compared with carcinogenicity in rodents (Kirkland et al., 2005). It implies that reliable heritable genetic risk determination as well as cancer health risk cannot be made the basis of *in vitro* findings alone (Thybaud et al., 2007b). Exposure to germ cells should also be considered. Therefore, it is difficult to estimate human heritable germ cell mutagenicity from only the results of *in vitro* mutagenicity tests. When positive result(s) from only *in vitro* mutagenicity test data are available, the substance is also classified "No data to make classification" in principle. An exceptional case is described above (5) as a special case in Category 2.

5. Examples of results on the selected chemicals and re-evaluation

About 1400 chemicals regulated by Japanese laws were submitted for classification. Data for evaluation were obtained from selected documents or databases (see Section 3). Classification was performed by non-experts based on the classification guid-

Table 4
Criteria of practical GHS classification of germ cell mutagens.

GCM	GHS category	Practical classification [explanation]	Criteria [test*]
GCM	Category 1A	Human heritable germ cell mutagen [Human germ cell mutagen]	(1) Positive evidence from human epidemiological studies (no compound identified so far)
	Category 1B	Mammalian germ cell mutagen [Probable human germ cell mutagen]	(1) Positive result(s) from *in vivo* heritable germ cell mutagenicity tests in mammals [#1] (2) Positive result(s) from *in vivo* germ cell mutagenicity tests in mammals [#2] (preferably, this will be supported by the positive result(s) from *in vivo* somatic cell mutagenicity tests in mammals) (3) Positive findings from analysis showing mutagenic effects in the germ cells of humans, without demonstration of transmission to progeny [#2.4] (4) Positive result(s) from *in vivo* somatic cell mutagenicity tests in mammals AND positive result(s) from *in vivo* germ cell genotoxicity tests in mammals [#3 + #4] (5) Positive result(s) from *in vivo* somatic cell mutagenicity tests in mammals AND demonstration of exposure to germ cells by substance or active metabolite(s) [#3 + E] (preferably, in relevant route of exposure)
	Category 2	Mammalian somatic cell mutagen [Possible human germ cell mutagen]	(1) Positive result(s) from *in vivo* somatic cell mutagenicity tests in mammals [#3] (2) Positive results from tests showing mutagenic effects in the somatic cells of humans [#3.5] (3) Positive result(s) from *in vivo* germ or somatic cell genotoxicity tests in mammals AND positive result(s) from *in vitro* mutagenicity tests [(#4 or #5) + #6] (4) Positive finding(s) from analysis showing genotoxic effects in the somatic cells of humans AND positive result(s) from *in vitro* mutagenicity tests [#5.5 + #6] (5) (Strong) positive result(s) from *in vitro* mutagenicity test(s) with at least two different end points AND showing chemical structure activity relationship to known germ cell mutagens (i.e., Category 1or 2 substances) as special case [#6 + C]
Non-GCM	Not classified (Not yet classified as genotoxic)	Not likely to be mammalian mutagen [Probable non human germ cell mutagen]	(1) Negative result(s) in any *in vivo* germ or somatic cell mutagenicity tests in mammals (2) No sufficient evidence in somatic cell mutagenicity tests in mammals
–	Classification not possible (No data to make classification)	–	(1) No data on *in vivo* mutagenicity or genotoxicity tests in mammals (2) No data on *in vivoi* mutagenicity tests in mammals AND positive result(s) from *in vivo* genotoxicity tests in mammals AND negative result(s) in any *in vitroi* mutagenicity test(s) (3) Data available only *in vitro* mutagenicity test(s) except for special case (see criteria 5) in Category 2)

* Each number is corresponding to the test number in Table 3, "E" means exposure of substance or its metabolite(s) to germ cells, and "C" means chemical structure activity relationship to known germ cell mutagens.

ance and the practical decision tree. Expert review on the classification was limited to those chemicals that were considered to need the help of experts. Therefore, some of the results in this project will not be suitable for reliable classification: about 3% and 13% of chemicals were in Category 1B and Category 2, respectively. Nearly 30% of chemicals were in "Not Classified (NC)". More than 50% of chemicals were "Classification Not Possible (CNP)", due to no or insufficient data for classification. The high percentage of CNP's reflects the chemicals used in this project that are regulated by specific laws (e.g., the Industrial Safety and Health Law, the Poisonous and Deleterious Substances Control Law, and PRTR) in Japan.

The results of the classification on 20 chemicals are shown in Table 5. When questionable classification results were recognized by experts, re-evaluation was performed based on the new data search, evaluation of data reliability, and weight of evidence approach. The selected chemicals included ones that required re-evaluation by experts (acrylonitrile, phenol, nitrilotriacetic acid, ethanol, sodium chlorite and 1-chloro-2-nitrobenzene), or that were representative examples for classification using the practical decision tree (the other chemicals).

5.1. Acrylamide [79-06-1] (J-GHS ID 0001), Cat. 1B

Many positive results were found in variety of genotoxic endpoints including heritable mutagenicity (CERI-NITE, 2004b). Therefore, GHS Category 1B was applied. This is supported by EU Mutagenicity Category 2 in the EU Annex I (ECB, 2008) and MAK Germ Cell Mutagenicity (GCM) Category 2 in the MAK List (DFG, 2007).

5.2. Acrylic acid [79-10-7] (J-GHS ID 0002), NC

Negative results were obtained from a dominant lethal test and a chromosomal aberration test using rodents (ECB, 2002c), although positive results were obtained from an *in vitro* chromosomal aberration test and a gene mutation test with mammalian cells. Based on these findings, "Not Classified (NC)" was assigned according to the practical GHS decision tree. Acrylic acid is not categorized as a mutagen or GCM in EU Annex I or the MAK List, respectively.

5.3. Acrylonitrile [107-13-1] (J-GHS ID 0003), Changed to NC from Cat. 2

One positive finding was reported from *in vivo* mutagenicity test (rat splenic T-cell *hprt* mutation assay) together with one positive finding from *in vivo* genotoxicity test (liver UDS assay). In addition, *in vitro* mutagenicity tests in mammalian cells were also positive. On the other hand, negative results were obtained in rodent dominant lethal tests, mouse spermatogonial chromosomal aberration test and rat spermatocyte UDS tests (IPCS, 2002; CERI-NITE, 2003; ECB, 2004a). Following the practical decision tree,

Table 5
Results of GHS classification including re-evaluation on selected chemicals in the Japanese GHS Classification Project (J-GHS).

No.	Chemical Name [CAS] (J-GHS ID)	EU Mut cat.	MAK GCM cat.	GHS GCM cat.	#1 1.1	#1 1.2	#1 1.3	#2 2.1	#2 2.2	#2 2.3	#2 2.4	#3 3.1	#3 3.2	#3 3.3	#3 3.4	#3 3.5	#4 4.1	#4 4.2	#4 4.3	#4 4.4	#5 5.1	#5 5.2	#5 5.3	#5 5.4	#5 5.5	#6 6.1	#6 6.2	#6 6.3	#6 6.4
1	Acrylamide [79-06-1] (0001)	2	2	1B	+	+	+	+, –	+			+, –	+	+, –	+		+	–		+	+	+				+		+, –	–
2	Acrylic acid [79-10-7] (0002)			NC			–					–														+		+, –	–
3	Acrylonitrile [107-13-1] (0003)			2			–	–				–		–	+			–			+	–			–	+	+	+, –	–
	Re-evaluation for J-GHS			NC			–	–				–		–	(+)	–		–			(+), –	–	+, –		–	+	+	+, –	+, –
4	Aniline [62-53-3] (0007)	3		2			Inc							+								+	+	+		+		+	–
5	Antimony hydride (Stibine) [7803-52-3] (0010)	NL	3B*	CNP																									
6	Cadmium oxide [1306-19-0] (0015)	3	3A**	2										–		+, –									+, –				–
7	Vanadium(V) oxide [1314-62-1] (0026)	3	2***	1B			+							+, –						+		–		+, –		+	+	–	+, –
8	Phenol [108-95-2] (0061)	3		1B				+				+, –		+, –						–		+	–	–		–		+, –	–
	Re-evaluation for J-GHS			2				(+)				+, –		+, –						–		+	–	–		+	+	+, –	–
9	Formaldehyde [50-00-0] (0069)		5	2			Inc, –	–				+, –		+, –								+, –		+		+		+	+
10	*o*-Anisidine [90-04-0] (0083)	3		2										–	+						–		–			+		+	–
11	Glycidol [556-52-5] (0098)	3		2								+		+								+				+		+	+
12	Vinyl chloride [75-01-4] (0113)			2			–					+	–	+		+						+		+				+, –	+
13	1,4-Dioxane [123-91-1] (0125)			NC										+, –										+, –		–	–	–	–
14	4,4′-Methylenebis(2-chloroaniline) [101-14-4] (0130)			2										+								+	+			–		+	+
15	3,3′-Dichlorobenzidine [91-94-1] (0138)			2								+		+							+	+	+						+
16	Nitrilotriacetic acid [139-13-9] (0170)	NL	–$	1B			–	+				–		–								–				–	+	+, –	–
	Re-evaluation for J-GHS			NC			–	(+)				–		(+), –								–				+, –	+	+, –	–
17	1,3-Dibromopropane [109-64-8] (0539)	NL	NL	CNP																						+			+
18	Ethanol [64-17-5] (0662)		5$$	1B			+, –	+, –				–		+, –								+				–		–	–
	Re-evaluation for J-GHS			NC			(+), –	(+), –			(+)	–		(+), –			(+)					(+), –			(+)	–		–	–
19	Sodium chlorite [7758-19-2] (1109)	NL	NL	2								–		+, –												+			+, –
	Re-evaluation for J-GHS			NC								–		(+), –												+			+, –
20	1-Chloro-2-nitrobenzene [88-73-3] (1184)			2																				+		+, –		–	+, –
	Re-evaluation for J-GHS			CNP																				(+)		+, –		–	+, –

Abbreviation: EU Mut cat.: EU mutagen category by Annex I (ECB, 2008); MAK GCM cat.: MAK germ cell mutagen category by MAK List (DFG, 2007); GHS GCM cat.: GHS germ cell mutagen category; CNP: classification not possible; NC: not classified; NL: not listed; Test results: +: positive; (+): positive in special case, or questionable, non-relevant or non-conclusive positive, –: negative, Inc: inconclusive.
Mutagenicity/genotoxicity tests (some cases included modified methods).
#1 *In vivo* heritable germ cell mutagenicity tests in mammals.
1.1: Mouse specific locus test; 1.2: Mouse heritable translocation test; 1.3: Rodent dominant lethal test.
#2 *In vivo* germ cell mutagenicity tests in mammals.
2.1: Chromosomal aberration test in spermatogonia; 2.2: Micronucleus test in spermatid cells; 2.3: Gene mutation test in germ cells of transgenic rodents; 2.4: Analysis of aneuploidy in sperm cells of exposed people.
#3 *In vivo* somatic cell mutagenicity tests in mammals.
3.1: Chromosome aberration test in bone marrow cells or peripheral lymphocytes; 3.2: Mouse spot test; 3.3: Micronucleus test in hematopoietic cells; 3.4: Gene mutation test in somatic cells of transgenic rodents; 3.5: Metaphase or micronucleus formation analysis of peripheral lymphocytes of exposed people.
#4 *In vivo* germ cell genotoxicity tests in mammals.
4.1: Sister chromatid exchange (SCE) test in spermatogonia; 4.2: Unscheduled DNA synthesis (UDS) test in testicular cells; 4.3: Assays of covalent binding or adduct formation to germ cell DNA; 4.4: Assays of DNA damage in germ cells (comet assay, alkaline elution assay, etc.).
#5 *In vivo* somatic cell genotoxicity tests in mammals.
5.1: UDS test in liver; 5.2: SCE test in bone marrow cells or peripheral lymphocytes; 5.3: Assays of covalent binding or adduct formation to somatic cell DNA; 5.4: Assays of DNA damage in somatic cells (comet assay, alkaline elution assay, etc.); 5.5: SCE analysis of peripheral lymphocytes of exposed people.
#6 *In vitro* mutagenicity tests.
6.1: Chromosomal aberration test in cultured mammalian cells; 6.2: Micronucleus test in cultured mammalian cells; 6.3: Gene mutation test in cultured mammalian cells; 6.4: Reverse mutation test in bacteria (Ames test).
* As antimony [7440-36-0] and its inorganic compounds (except for stibine).
** As cadmium [7440-43-9] and its inorganic compounds (inhalable fraction).
*** As vanadium [7440-62-2] and its inorganic compounds.
$ Nitriloacetic acid and its sodium salt.
$$ Changed from Category 2.

GHS Category 2 was assigned for this chemical based on only one positive result in rat T-cell mutation assay (IPCS, 2002). Acrylonitrile is not classified as a mutagen or GCM in EU Annex I or the MAK List, respectively.

The T-cell mutation assay (column 3.4 in Table 4) is not a standard test and the reliability of the result is questionable, therefore, re-evaluation was performed on this chemical with data used initially and obtained additionally. The information came only from an abstract. Therefore, it was decided that this data should not be used for the classification.

Several data can be obtained from *in vivo* genotoxicity tests with somatic cells. A positive result in liver UDS test using liquid scintillation counting method (column 5.1) (IPCS, 2002; CERI-NITE, 2003; ECB, 2004a) was used initially. In the course of re-evaluation, a negative result was found in liver UDS test using the autoradiograph method (IPCS, 2002; ECB, 2004a). EU Risk Assessment Report (EURAR) discussed that "liquid scintillation counting method is not regarded as the most reliable means of establishing evidence of DNA-repair, preference being given to autoradiographical techniques (ECB, 2004a)." For DNA binding *in vivo* (column 5.3), Concise International Chemical Assessment Document (CICAD) reported inconsistent results (IPCS, 2002). With respect to *in vitro* mutagenicity tests (column 6), EURAR (ECB, 2004a) suggested that "Positive findings *in vitro* are not reliably reflected in the *in vivo* situation, because acrylonitrile or its active metabolites do not reach target tissues *in vivo*, possibly due to the detoxification of the epoxide metabolite cyanoethylene oxide via a glutathione conjugation pathway".

In the dominant lethal test in rats (column 1.3), one negative result is cited in hazard assessment report by Chemicals Evaluation and Research Institute (CERI) and National Institute of Technology and Evaluation (NITE) (CERI-NITE, 2003) and EURAR (ECB, 2004a). Two negative results in mice treated by i.p. or inhalation were found in addition (IARC, 1999a; IPCS, 2002). A negative result in the mouse spermatogonial chromosomal aberration test, two negative results in mouse bone marrow chromosomal aberration test were used initially (column 3.1), and two negative results in rodent chromosomal aberration test reported in IARC (1999a) or CICAD (IPCS, 2002) were cited additionally. In the rodent micronucleus test (column 3.3), two negative results in mice treated by i.p. were used. On the other hand, CICAD (IPCS, 2002) reported negative results in mice and inconclusive results in rats treated by multiple routes from a collaborative study by Morita et al. (1997). A negative result in rat spermatocyte UDS test was reported (IARC, 1999a; ECB, 2004a).

The following conclusion is made in the re-evaluation; acrylonitrile gave clear negative results in the rodent dominant lethal and micronucleus tests in spite of the mutagenic activities reported *in vitro*. Positive results in T-cell mutation cannot be evaluated, and the positive in rat liver UDS test is not regarded as reliable. Therefore, we propose that acrylonitrile should be assigned NC based on the practical decision tree.

Several issues were identified through classification of this chemical: (1) lack of understanding of reliability of test method: T-cell mutation assay is not a standard test; (2) insufficient review of documents: T-cell mutation study is abstract information only, and; (3) shortage of information collection: presence of negative result in rat liver UDS test and non-citation of IARC (1999a).

5.4. Aniline [62-53-3] (J-GHS ID 0007), Cat. 2

Negative results were obtained from bacterial mutagenicity tests. Findings in rodent dominant lethal tests were negative in general, but the final evaluation of the test result is considered to be inconclusive due to slight but statistically significant but slight, toxicologically significant effect at the highest dose (ECB, 2004b). A rodent erythrocyte micronucleus test gave positive results, as did several DNA endpoints in *in vivo* genotoxicity tests with somatic cells. These positive findings *in vivo* were supported by *in vitro* positives with mammalian cells (CERI-NITE, 2004c; ECB, 2004b). GHS Category 2 was applied. EU Annex I gives Category 3 for mutagenicity, but no classification for GCM in the MAK List.

It should be noted that the EU Risk Assessment Report provides a full discussion on the responses in micronucleus tests and the dominant lethal test. Weak positive effects were found in micronucleus tests, which were limited to high doses in the toxic range, and the result of the dominant lethal test is considered to be inconclusive in spite of general negative findings (ECB, 2004b). The conclusion of the report is that the available data of mutagenicity are not sufficient to classify aniline as a Category 2 mutagen in the EU classification, but as a Category 3 mutagen, due to the positive results from several *in vivo* and *in vitro* tests, especially in the bone marrow micronucleus test with rats. Aniline induces methaemoglobinanemia, which might lead erythrocyte degradation, resulting in the induction of micronuclei as a result of increases in cell division to replace lost erythrocytes (Tweats et al., 2007). If the involvement of increases in erythropoiesis after aniline treatment is resolved, the classification will be reconsidered.

5.5. Antimony hydride (Stibine) [7803-52-3] (J-GHS ID 0010), CNP

No data was found in the data source used. "Classification Not Possible (CNP)" was assigned. Antimony hydride (Stibine) is not listed in the EU Annex I. "Antimony and its inorganic compounds" were classified MAK GCM Category 3B, but stibine was excluded as an exception.

5.6. Cadmium oxide [1306-19-0] (J-GHS ID 0015), Cat. 2

A negative result was obtained in a rodent micronucleus test. Conflicting results were reported in cytogenetic and SCE evaluations of peripheral lymphocytes of exposed people (IARC, 1994; ECB, 2003; NTP, 2005a). Based on the positive findings in somatic cell mutagenicity in humans, GHS Category 2 was assigned. EU classification of cadmium oxide is mutagenicity Category 3 in Annex I, but this chemical was classified MAK GCM Category 3A as cadmium and its inorganic compounds (inhalable fraction) in the List.

5.7. Vanadium (V) oxide [1314-62-1] (J-GHS ID 0026), 1B

A rodent dominant lethal test was positive though conflicting results were obtained in rodent micronucleus tests. Positive results were obtained from *in vivo* germ and somatic cell genotoxicity tests and also *in vitro* mutagenicity tests (IPCS, 2001; NTP, 2005d). Based on the positive finding in the dominant lethal test, GHS Category 1B was given. EU classification gave mutagenicity Category 3 in Annex I. On the other hand, this chemical was classified MAK GCM Category 2 as vanadium and its inorganic compounds in the List.

5.8. Phenol [108-95-2] (J-GHS ID 0061), Changed to Cat. 2 from Cat. 1B

Positive results were obtained in cytogenetic analysis with mouse spermatogonia or spermatocytes. Negative and positive results were obtained in rodent chromosomal aberration or micronucleus tests with somatic cells. An assay of DNA damage in germ cells gave a negative result. *In vivo* genotoxicity tests with somatic cells and *in vitro* mutagenicity tests showed negative results generally (CERI-NITE, 2005a; NTP, 2005c). Based on the positive results in cytogenetic analysis in germ cells *in vivo*, GHS Category 1B was assigned. Phenol is classified as a mutagen, Cat-

egory 3 in the EU Annex I, but not classified as GCM in the MAK List.

Conflicting results obtained in rodent cytogenetic analysis with somatic cells raised questions about positive findings in chromosomal aberration tests with mouse germ cells. Therefore, re-evaluation was performed on this chemical. Positive findings in chromosomal aberration tests with mouse germ cells (Bulsiewicz, 1977) (column 2.1), cited from CERI-NITE (2005a), gave support to the classification of GCM. However, IARC (1999a) and EURAR (ECB, 2006b) have not cited these data. On the other hand, US EPA cited the data in the toxicological review document in support of summary information on the IRIS (EPA, 2002a), and stated a comment of "inconsistencies in reporting" to the data (EPA, 2002b), including that one of the inconsistencies is found in dosing concentration. The germ cell cytogenetic analysis used was not a standard test, and it had no statistical analysis.

Both positive and negative results were reported in rodent cytogenetic analysis with somatic cells (column 3.1 and 3.3) (IARC, 1999a; EPA, 2002b; CERI-NITE, 2005a; NTP, 2005c; ECB, 2006b). A mouse chromosomal aberration test gave a positive result up to an intraperitoneal injection (i.p.) dose of 300 mg/kg, but the test used a non-standard protocol (NTP, 2005c). In contrast, negative results were obtained in the rat at doses up to 180 mg/kg by i.p. and up to 510 mg/kg by oral administration (p.o.) (CERI-NITE, 2005a). Six mouse micronucleus tests showed positive results at doses up to 300 mg/kg by i.p. or p.o. dosing including three treatments, while three tests showed negative results at doses up to 250 mg/kg by i.p. or p.o. EURAR discussed that "Phenol should be regarded as a somatic cell mutagen, and that the high dose positive results in micronucleus tests might be due to phenol-induced hypothermia (ECB, 2006b)." It is also reported that a single i.p. dose of phenol to mice at 300 mg/kg produced a significant and prolonged hypothermia and a significant increase in micronuclei (Spencer et al., 2007).

A DNA damage test using alkaline elution in testicular cells was negative in rats treated by i.p. injection up to 79 mg/kg (column 4.4) (EPA, 2002b; CERI-NITE, 2005a; ECB, 2006b). With respect to *in vivo* genotoxicity tests with somatic cells, a positive result was reported in sister chromatid exchange analysis in mice treated by the i.p. route at doses up to 300 mg/kg (column 5.2,) (NTP, 2005c). However, it used non-standard protocol. Other endpoints including DNA adduct formation (column 5.3) or DNA damage induction (column 5.4) were negative in rats (p.o. dosing of 75 mg/kg/day for days) or mice (i.p. dosing of 75 mg/kg), respectively (IARC, 1999a; CERI-NITE, 2005a; ECB, 2006b). A negative result from an *in vitro* chromosomal aberration test (column 6.1) (CERI-NITE, 2005a) was cited initially, but phenol is recognized as positive in chromosomal aberration tests *in vitro* (EPA, 2002b; Kirkland et al., 2005; ECB, 2006b). In addition, several positive results from *in vitro* micronucleus tests were also reported (column 6.2) (IARC, 1999a; EPA, 2002b; Kirkland et al., 2005; ECB, 2006b).

The following conclusion is made in the re-evaluation. The positive result in the germ cell cytogenetic analysis is not considered as sufficient evidence due to this being a non-standard test without statistical analysis. Though phenol induces micronuclei in rodent somatic cells, recent published data demonstrated that the induction of micronuclei was exclusively associated with phenol-induced hypothermia (Spencer et al., 2007), suggesting that the increase in micronuclei may not be a result of any intrinsic direct genotoxic effects of phenol. On the other hand, Tweats et al. (2007) pointed out that the response is somewhat higher than with other compounds that induce hypothermia and it would be informative to ascertain if this response can be reversed by maintaining the core temperature of the treatment. The mechanism by which hypothermia induces micronuclei is not clearly established, but may involve disturbance of the mitotic spindle. Therefore, we propose that phenol should be classified as Category 2 in the practical decision tree in case of positive in somatic mutagenicity but negative in germ cell genotoxicity *in vivo*. This would need re-evaluation if further information becomes available.

Practical issues identified are (1) insufficient review; no critical review of a positive finding in mouse germ cells, and (2) shortage of information collection; non-citation of IARC (1999a) and EPA (2002b). It is not possible to cite recent publications of EURAR (ECB, 2006b), Spencer et al. (2007), and Tweats et al. (2007) at that time of the project operation.

5.9. Formaldehyde [50-00-0] (J-GHS ID 0069), Cat. 2

Negative or inconclusive results were obtained from rodent dominant lethal tests or a spermatogonial chromosomal aberration test. Cytogenetic evaluation as measured by the induction of micronuclei in bone marrow or peripheral blood cells showed negative results in mice treated by p.o. dosing or intravenous injection (i.v.) (Morita et al., 1997). On the other hand, positive findings were observed in a chromosome aberration test with pulmonary lavage cells and a micronucleus test with gastrointestinal or nasal cells *in vivo*. Positive results were also obtained from *in vivo* genotoxicity tests and *in vitro* mutagenicity tests (IPCS, 1989; OECD, 2004b; CERI-NITE, 2005c). Based on the positive findings in somatic cells *in vivo*, GHS Category 2 was assigned. Formaldehyde is not categorized as a mutagen in EU Annex I, but as Category 5 in MAK GCM classification in the list. MAK GCM category 5 is a relative new category (Adler et al., 2000; DFG, 2007) that is defined as "germ cell mutagens or suspected substances (according to the definition of Category 3A and 3B), the potency of which is considered to be so low that their contribution to genetic risk for man is expected not to be significant". At present, acetaldehyde, formaldehyde and ethanol are in Category 5 in the MAK List. A major limitation of many studies is the well established cytotoxic effects of formaldehyde.

It should be noticed that there is evidence from recent papers and assessment on formaldehyde that the effects of it are due to DNA–protein adducts, which are shown to have a threshold (Schmid and Speit, 2006; Speit et al., 2007; UKCOM, 2007). The conclusions from UK Committee on Mutagenicity of Chemicals in Food, Consumer Products and the Environment are as follows: "There was no convincing evidence from *in vivo* mutagenicity studies in experimental animals and from biomonitoring studies of genotoxicity in workers exposed to formaldehyde for a direct *in vivo* systemic mutagenic effect of inhaled formaldehyde. A secondary mechanism might be involved in the genotoxic effects documented in peripheral blood lymphocytes in the biomonitoring studies reviewed. For occupational and environmental exposure to formaldehyde, the pattern of metabolism and distribution of formaldehyde indicate that a threshold for *in vivo* systemic mutagenicity is likely (UKCOM, 2007)."

We agree with the conclusions by UKCOM. Systemic exposure after inhalational exposure of formaldehyde is negligible and thus would not present a germ cell hazard. However, local genotoxic effects in somatic cells should be taken consider in hazard communication.

5.10. o-Anisidine [90-04-0] (J-GHS ID 0083), Cat. 2

Negative results were obtained from a rodent micronucleus test and several *in vivo* genotoxicity tests with somatic cells. On the other hand, a transgenic mouse mutation test showed a positive result. The positive finding was supported by the positive results from *in vitro* gene mutation tests (DFG, 1998; IARC, 1999b; ECB, 2002a). Based on the positive result from the transgenic gene mutation model assay, GHS Category 2 was assigned. *o*-Anisidine

is categorized as a Category 3 mutagen in the EU Annex I, but not classified as GCM in the MAK List.

5.11. Glycidol [556-52-5] (J-GHS ID 0098), Cat. 2

Positive results in a rodent cytogenetic evaluation were supported by the positive findings *in vitro*. There were no data on mutagenicity or genotoxicity in germ cells (ACGIH, 2001; Bingham et al., 2001b; CERI, 2002a; DFG, 2003b). GHS Category 2 was assigned. Glycidol is categorized as a Category 3 mutagen in the EU Annex I, but not classified as GCM in the MAK List.

5.12. Vinyl chloride [75-01-4] (J-GHS ID 0113), Cat. 2

A negative result was obtained from a rodent dominant lethal test. However, positive results were obtained from *in vivo* mutagenicity and genotoxicity tests with somatic cells including a human epidemiological study, and from *in vitro* mutagenicity tests (ECETOC, 1998; ATSDR, 2004; CERI-NITE, 2004d). Based on the positive results from *in vivo* tests with somatic cells, GHS Category 2 was assigned. Vinyl chloride is not categorized as a mutagen or GCM in the EU Annex I or the MAK List, respectively.

5.13. 1,4-Dioxane [123-91-1] (J-GHS ID 0125), NC

Almost all tests conducted were negative. One positive result was obtained in a mouse bone marrow micronucleus test, but it was not confirmed by other reports. Conflicting results were obtained in DNA damage tests in rodents, and where positive findings were observed these were only at high doses. All *in vitro* mutagenicity tests were negative (CERI-NITE, 2004a). Based on the negative results in *in vivo* and *in vitro* tests, NC was assigned. 1,4-Dioxane is not categorized as a mutagen or GCM in the EU Annex I or the MAK List, respectively. Several evaluation documents have been issued on 1,4-dioxane (NICNAS, 1998; ECB, 2002b; DFG, 2003a; ATSDR, 2006). These documents were not used in the J-GHS, because only one review document written in Japanese was used by the Classifier. Negative results in a rodent liver UDS assay and a positive result in rodent liver micronucleus test were also described in the EU (ECB, 2002b) and US documents (ATSDR, 2006). However, the positive result at high doses in the liver micronucleus test is non-relevant to humans.

A practical issue identified is a shortage of information collection, though it gave no influence of the classification result.

5.14. 4,4′-Methylenebis(2-chloroaniline) [101-14-4] (J-GHS ID 0130), Cat. 2

Positive results were obtained from rodent micronucleus tests and *in vivo* genotoxicity tests with somatic cells. There were no data on mutagenicity or genotoxicity in germ cells (IARC, 1993; CERI-NITE, 2005b; NTP, 2005b). Based on the positive findings from *in vivo* tests with somatic cells, GHS Category 2 was assigned. 4,4′-Methylenebis(2-chloroaniline) is not categorized as a mutagen or GCM in the EU Annex I or the MAK List, respectively.

5.15. 3,3′-Dichlorobenzidine [91-94-1] (J-GHS ID 0138), Cat. 2

Positive results were obtained from rodent cytogenetic evaluations and *in vivo* genotoxicity tests with somatic cells. There was no data on mutagenicity or genotoxicity in germ cells (IARC, 1982; DFG, 1992b; ATSDR, 1998; IPCS, 1998; CERI, 2002b). Based on the positive findings from *in vivo* tests with somatic cells, GHS Category 2 was assigned. 3,3′-Dichlorobenzidine is not categorized as a mutagen or GCM in the EU Annex I or the MAK List, respectively.

5.16. Nitrilotriacetic acid [139-13-9] (J-GHS ID 0170), Changed to NC from Cat. 1B

A dominant lethal test, chromosomal aberration test, micronucleus test and SCE analysis with rodent cells all gave negative results. However, aneuploidy was detected in mouse spermatocytes with nitrilotriacetic acid trisodium salt (CAS No. 5064-31-1). Both negative and positive results were obtained in *in vitro* mutagenicity tests in which the trisodium salt was mainly used (IARC, 1999b; CERI, 2002c). Based on the positive finding for germ cell aneuploidy, GHS Category 1B was assigned. Nitrilotriacetic acid and its trisodium salt are not listed in the EU Annex I and the MAK List.

Due to the positive finding in germ cells, despite the negatives in somatic cells *in vivo*, re-evaluation was performed on these chemical using additional references. Mouse dominant lethal tests gave negative results (column 1.3), but nitrilotriacetic acid trisodium salt induced meiotic aneuploidy (hyperhaploidy) in mouse spermatocytes (column 2.1) (Costa et al., 1988). This result was supported by positive finding in a rat kidney micronucleus test (column 3.3) (Robbiano et al., 1999). On the other hand, a negative result was reported in a mouse chromosomal aberration test (aneuploidy, column 3.1), mouse bone marrow micronucleus test, and mouse SCE test (column 5.2) (IARC, 1990, 1999b). *In vitro* chromosomal aberration tests showed negative or positive results in CHO cells and human peripheral lymphocytes or in rat kangaroo kidney cells, respectively (column 6.1) (IARC, 1999b). *In vitro* micronucleus tests gave a positive result in hamster CL-1 cells and primary kidney cells from rats and humans (column 6.2) (IARC, 1999b; Robbiano et al., 1999).

The following conclusion is made in the re-evaluation. Standard *in vivo* mutagenicity tests including dominant lethal test, chromosomal aberration test, and micronucleus tests showed negative results. A positive result was obtained in a test which measured mouse germ cell aneuploidy, and this is supported by a positive finding in an *in vivo* rat kidney micronucleus test. On the other hand, it is not supported by the mouse chromosomal aberration test (negative in aneuploidy) and mouse bone marrow micronucleus test. Some *in vivo* tests used the trisodium salt, which might have different toxicokinetics, and the *in vivo* rat kidney micronucleus test is not a standard test. Therefore, a positive result for mouse germ cell aneuploidy is not considered to be sufficient evidence, and also there is no clear evidence of *in vivo* somatic cell mutagenicity. Overall, we propose that nitrilotriacetic acid or its trisodium salt should be assigned NC in GHS GCM.

Practical issues complicate the classification identified are (1) insufficient review; no critical review of the positive finding for mouse germ cell aneuploidy, and (2) shortage of information collection; non-citation of IARC (1990) and Robbiano et al. (1999).

5.17. 1,3-Dibromopropane [109-64-8] (J-GHS ID 0539), CNP

Positive results were obtained from both an *in vitro* chromosomal aberration test and an Ames test (JECDB, 2006a,b). No information was obtained in *in vivo* mutagenicity or genotoxicity tests. Classifier requested experts to judge classification of this chemical based on the practical decision tree (Fig. 2). Experts reviewed these data and information on other dihaloalkanes or dihaloalkenes including 1,2-dibromoethane, 1,2-dichloropropane, 1,2-dichloroethane, 1,3-dichloropropane and 1,3-dichloropropene (IARC, 1999a; HSDB, 2002). These halogenated compounds are usually

positive in *in vitro* mutagenicity tests, but are negative in *in vivo* micronucleus tests, mouse dominant lethal tests, and the spermatocyte UDS assay. There is no clear evidence or suggestion that 1,3-dibromopropane should be classified as Category 2. Therefore, the experts concluded that 1,3-dibromopropane should be assigned as CNP. 1,3-Dibromopropane is not listed in the EU Annex I, and not categorized as a GCM in the MAK List.

5.18. Ethanol [64-17-5] (J-GHS ID 0662), Changed to NC from Cat. 1B

Conflicting results were obtained in *in vivo* heritable and germ cell mutagenicity tests with rodents. Some rodent dominant lethal tests showed positive results as well as positive results in chromosomal aberration (aneuploidy) tests in mouse spermatocytes, and negative results were also obtained in both tests in different studies. Chromosomal aberration tests in mammalian bone marrow cells showed negative results. Conflicting results were obtained in *in vivo* micronucleus tests with rodent somatic cells, and a positive result was obtained in an SCE test in mouse fetal hepatocytes. All *in vitro* mutagenicity tests were negative (IARC, 1988; DFG, 1999; Bingham et al., 2001a). Based on positive results in germ cells including dominant lethal tests, GHS Category 1B was assigned. Ethanol is not categorized as mutagen in the EU Annex I, but as GCM Category 5 in the MAK List.

Due to the mixture of positive and negative findings reported in mutagenicity or genotoxicity tests from *in vivo* germ or somatic mutagenicity tests and from all of the *in vitro* mutagenicity tests evaluated, re-evaluation was performed.

Conflicting results were obtained in rodent dominant lethal tests (column 1.3). Waters et al. also summarized positive results of dominant lethal tests both in rats and mice (Waters et al., 1994). However, the following conclusions for dominant lethal effect of ethanol were drawn in the review documents: (1) dominant lethal mutations were observed in mice given bolus doses of highly concentrated ethanol solutions. For rats, such findings have only been reported after very high doses which produced marked symptoms of systemic toxicity (DFG, 1999); (2) using a weight of evidence approach, it was concluded that ethanol does not induce dominant lethality in assays using standard regulatory approved methodologies (Phillips and Jenkinson, 2001; OECD, 2004a), and; (3) the majority of studies showing positive results can be criticized on the grounds of inadequate numbers of animals or on the methods used to score, evaluate or distinguish between early and late fetal deaths (OECD, 2004a).

In the chromosomal aberration test with germ cells including spermatocytes or spermatogonia, conflicting results were obtained (column 2.1). For aneugenic effects of ethanol in germ cells, the following conclusions were drawn in the review documents: (1) mutagenic potential seems to be weak, is limited to the induction of aneuploidy and could be demonstrated to date only with very high doses of at least 5 g/kg, which produced systemic toxicity, and only in mice (DFG, 1999); (2) findings could be due to an aneugenic effect during meiosis but convincing evidence is lacking and alternative non-genotoxic mechanisms are feasible (Phillips and Jenkinson, 2001), and; (3) many variables may affect the outcomes (Pacchierotti and Ranaldi, 2006).

The cytogenetic analysis with somatic cells showed negative results, however, positive findings were also reported (column 3.1 and 3.3). Significant increases of micronuclei in bone marrow cells from rats given a diet containing ethanol might be due to the increase of cell division as is induced by erythropoietin (Phillips and Jenkinson, 2001). For micronucleus induction by ethanol in bone marrow cells, the review documents concluded that there is no convincing evidence that ethanol induces micronuclei in the bone marrow of rodents (Phillips and Jenkinson, 2001; OECD, 2004a).

The majority of studies on sister chromatid exchange (SCE) induction *in vivo* were positive, although the effects have generally been small (column 4.1 and 5.2) (IARC, 1988; DFG, 1999; Phillips and Jenkinson, 2001). Negative results have also reported for SCE induction (column 5.2). For SCE induction by ethanol, Phillips and Jenkinson pointed out the possibility of disturbance of the metabolism, nutritional or hormonal status of the animal treated at high dose levels of ethanol (Phillips and Jenkinson, 2001).

A human monitoring study found an association between alcohol drinking and aneuploidy in sperm from young men (column 2.4) (Robbins et al., 1997), which has been reviewed by Phillips and Jenkinson (2001) and Pacchierotti and Ranaldi (2006). Several studies for SCE induction in humans (column 5.5) have suggested that alcoholics have higher SCE frequencies in their lymphocytes than non-alcoholics. However, these studies frequently failed to control for smoking and other confounding factors which may have influenced the results (IARC, 1988).

All standard *in vitro* mutagenicity tests showed negative results (column 6) (IARC, 1988; DFG, 1999; Phillips and Jenkinson, 2001; OECD, 2004a).

A recent IARC monograph mentioned that numerous reports have shown that human alcoholics have a higher frequency of chromosomal aberrations, SCE and micronuclei in their peripheral lymphocytes. The data from studies in animals suggest that ethanol causes DNA damage in target tissues (IARC, 2007). However, the following overall conclusions are suitable for the purpose of classification of industrial chemicals: (1) it is concluded that there is no significant evidence that ethanol is a genotoxic hazard according to the criteria normally applied for the purpose of classification and labeling of industrial chemicals (Phillips and Jenkinson, 2001), and; (2) the balance of evidence is that ethanol is not genotoxic. There is very little evidence to suggest that ethanol is genotoxic in somatic cells and it may have a very limited capacity to induce genetic changes *in vivo* but under very specific circumstances and at very high doses achievable in humans only by deliberate oral ingestion (OECD, 2004a). Therefore, we propose that ethanol should be assigned NC in GHS GCM. Deliberate high oral intake of ethanol in alcoholic beverages over a long period might be genotoxic; however, GHS does not require foods or beverages to be labeled to indicate the presence of hazardous materials (UN, 2007).

Practical issues identified are (1) shortage of information collection; Non-citation of Phillips and Jenkinson (2001) and Screening Information Data Set (SIDS) (OECD, 2004a), (2) discrepancy of international or national review evaluation depend on year; for example, change from MAK GCM Category 2 (DFG, 1999) to Category 5 (Adler et al., 2000; DFG, 2007), and (3) necessity of careful evaluation of single positive findings in some tests, especially with non-standard methods.

5.19. Sodium chlorite [7758-19-2] (J-GHS ID 1109), Changed to NC from Cat. 2

Conflicting results were obtained in rodent cytogenetic evaluations. A mouse micronucleus test by intraperitoneal injection gave a positive result, but a negative result was obtained in a test using gavage dosing in a chromosomal aberration test. Positive results were obtained in *in vitro* mutagenicity tests. There were no data on mutagenicity or genotoxicity in germ cells (IARC, 1991; ECB, 2000; EPA, 2000; RTECS, 2003). Based on the positive result in the micronucleus test by i.p. dosing, GHS Category 2 was assigned. Sodium chlorite is not listed in the EU Annex I or the MAK List.

Since the route of administration yields different results, a re-evaluation was performed. Mouse bone marrow chromosomal aberration tests and mouse bone marrow micronucleus test were negative by single or multiple oral administrations (column 3.1 and 3.3) (Meier et al., 1985; Hayashi et al., 1988; IARC, 1991;

EPA, 2000). On the other hand, single i.p. dosing induced micronuclei in mice (Hayashi et al., 1988; IARC, 1991; EPA, 2000; RTECS, 2003). An IARC review noted the importance of the chemical properties of sodium chlorite: in aqueous acid solutions, chlorite forms chlorous acid, which rapidly decomposes to chlorine dioxide, chlorate and chloride (IARC, 1991). Sodium chlorite is not considered to be a somatic cell mutagen at relevant exposures in humans, based on the negative result in micronucleus test by p.o. dosing, in which sodium chlorite decomposes in the stomach. Therefore, we propose that sodium chlorite should be assigned NC in GHS GCM.

Practical issues identified are (1) insufficient review; no consideration of chemical properties, and (2) discrepancy of weighting of evidence; positive when dosed i.p., but negative *in vivo* when dosed by the p.o. route.

5.20. 1-Chloro-2-nitrobenzene [88-73-3] (J-GHS ID 1184), Changed to CNP from Cat. 2

Data from one *in vivo* genotoxicity test with rodents is available. DNA single strand breaks in liver and kidneys were observed in mice (DFG, 1992a; IARC, 1996; CERI, 1999; OECD, 2001). Conflicting results were obtained in chromosomal aberration tests with cultured mammalian cells and in bacterial reverse mutation tests. In this case (i.e., positive in *in vivo* germ or somatic cell genotoxicity test which is supported by positive in *in vitro* mutagenicity test), an expert review should have been requested originally (see Fig. 2). However, GHS Category 2 was assigned without expert judgment. 1-Chloro-2-nitrobenzene is not categorized as mutagen or GCM in the EU Annex I or the MAK List, respectively.

Conflicting results were shown in *in vitro* mutagenicity tests. In addition, a single positive finding in *in vivo* genotoxicity test should be viewed with care. Also, no expert review was conducted in this case. Therefore, a follow-up review by experts was conducted. The positive finding in DNA damage as DNA single strand breaks was identified by the alkaline elution technique after i.p. dosing to male mice (column 5.4). A dose–effect relationship was not obtained, and the route of administration should be taken into consideration (OECD, 2001). An *in vitro* mammalian cell gene mutation test was negative using the V79 *hprt* assay (column 6.3) (DFG, 1992a; OECD, 2001). Positive effects in a cytogenetic test *in vitro* and a bacterial reverse mutation test were weak (column 6.1 and 6.4) (DFG, 1992a; OECD, 2001). There is no definitive evidence of *in vivo* somatic cell mutagenicity by 1-chloro-2-nitrobenzene. Therefore, we propose that 1-chloro-2-nitrobenzene should be assigned as CNP.

6. Issues identified in classification

Several issues in classification are revealed in the review process of acrylonitrile, phenol, nitriloacetic acid, ethanol, sodium chlorite and 1-chloro-2-nitrobenzene.

6.1. Sources of information on mutagenicity or genotoxicity of chemicals

The GHS is based on currently available information on toxicity of chemicals and it does not require any additional testing to classify a chemical substance. Categorization is based on the criteria for assessing classification and on the existing/available test data/information. Therefore, it is important to know where to find the information necessary for classification and, more importantly, how to correctly interpret these data. Several types of information sources are available. These include review documents, peer-reviewed papers, industry based reports, abstracts, or databanks, etc. The most reliable source is international or national review documents in terms of the quality, availability and suitability of information that has to be used in decision making. Peer-reviewed papers and industry based reports have high quality and suitability, but low availability. Databanks have high availability, but low quality. Abstracts should not be used for classification without any supportive information. One of the major factors of the different classifications was the different sources used. These resulting classifications may differ to a significant degree, leading to varying hazard communication. The age of the data differ among sources. Newer information will be available from more recent documents, and this information could result in changed assessment of chemicals. Therefore, the timeliness of data is an important consideration as previous classifications may be revised when new data becomes available. The use of data in one major information source (Table 2) led to unsuitable classification in the J-GHS. It is important that all available information (e.g., review documents) should be collected and used in a complementary fashion. It is noted that the other information sources, e.g., USEPA GENE-TOX database (http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?GENETOX) and UKCOM statements (http://www.iacom.org.uk/statements/index.htm) were not used in the J-GHS (Table 2). These sources are also useful for GCM classification. Classification based on old or limited information will possess lower reliability. The evaluation of test results in each information source should be checked with multiple sources of information, if available. Original peer-reviewed papers are the best source for assessing difficult and comprehensive test results: these should be included in information collection, if possible. Different classification results from different information source sets have also been noted by The United Nations Institute for Training and Research (UNITAR, 2008b). It might be necessary to develop an internationally-constructed and maintained information database for general Classifiers.

If mutagenicity/genotoxicity data are not available in the list of information sources, Classifiers should search original peer-reviewed papers using PubMed (http://www.ncbi.nlm.nih.gov/pubmed/), for example. In the case of no data in any available information sources, the substance will be assigned as "No data to make classification [as Classification not possible]".

6.2. Expertise of Classifiers

The GHS is originally designed as a self-classification system. The hazard classification process under the GHS is highly technical in nature, and requires a certain background and level of expertise of Classifiers to perform it accurately. If a Classifier lacks understanding of the GHS classification criteria, the effort should be repeated or reviewed carefully before communicating hazard or risk. Many mutagenicity or genotoxicity tests exist today; and many test results have been reported. Research oriented and non-validated studies are included. Classifiers should understand well the classification criteria and suitable materials for classification. A summary or abstract may omit important information or discussion. Therefore, Classifiers should fully study review documents, text, tables, and/or figures, and not only the summary or abstract. Evaluation and interpretation of the test results sometimes differ between the authors of original papers and of review documents. In addition, Classifiers should note that classification will be conducted based on hazard identification and assessment, not on risk characterization for humans. The people who have the scientific knowledge, experimental skill and expertise in toxicology (preferably genotoxicity) should become Classifiers. A deep knowledge of standard mutagenicity testing protocols is also required.

6.3. Expert judgment on data quality and weight of evidence

The GHS criteria for determining health hazards do not depend entirely on test methods. However, the methods used should sci-

entifically sound, validated and accepted according to international scientific standards (e.g., peer-review, authorization by international bodies). Though not all endpoints of the existing substances have been investigated, there is a huge data base of published literature and of files in research institutes and in industry. Not all these data have been developed under standardized test methods or according to the requirements of Good Laboratory Practice (GLP), i.e. they are not equally adequate, valid and relevant. However, it may be difficult for those who are not genetic toxicologists to evaluate the quality of test data. There are a variety of types of test methods and multiple test results in mutagenicity or genotoxicity tests. The determination of the quality of test data is a critical point for the classification of GCM. Therefore the evaluation of data quality frequently has to be done by an expert. Expert judgment plays an important role in making weight of evidence determinations in interpreting data for hazard classification of substances. When multiple data for one endpoint exist, the so-called "total weight of evidence approach" must be applied. GHS defines weight of evidence as follows: all available information bearing on the determination of toxicity is considered together, including the results of valid *in vitro* tests, relevant animal data, and human experience such as epidemiological and clinical studies and well-documented case reports and observations. Both positive and negative test results are assembled together in the weight of evidence determination. However, a single positive study performed according to good scientific principles and with statistically and biologically significant positive results may justify classification (UN, 2007). Genetic toxicology experts must consider all available data (both positive and negative), weigh it with respect to validity, and finally reach a conclusion. Useful data/information can come from different sources, e.g. from human experience, from experimental investigations in animals, from *in vitro* tests, or from similar substances (UN, 2007; UNITAR, 2008a).

Recent analysis demonstrates that *in vitro* mammalian cell tests have high sensitivity to carcinogens (i.e., above 65%), while showing very low specificity (i.e., below 45%) to non-carcinogens that results in false positive findings (Kirkland et al., 2005). Species-specific differences in metabolism are known in animals used in *in vivo* tests. Different mutagenic or genotoxic responses might be obtained. Therefore, it is important to understand the mechanism and/or mode of action of mutagenicity, and to use weight of evidence approaches for assessing the results.

Any discrepancy in classification will be based on the different weighting evidence used from expert to expert. Also, scientific progress will affect expert judgments. Styrene provides an example of the impact of these factors. A draft EU Risk Assessment Report on styrene has been published in November 2007 (ECB, 2007a). This report contains in depth discussions on whether styrene can be classified as a Category 3 mutagen in the EU classification scheme (corresponding to Category 2 GCM in GHS) (ECB, 2006a,c, 2007b). Exhaustive data collection and review were performed. The conclusion is "based on standard regulatory tests, there is no convincing evidence that styrene possesses significant mutagenic/clastogenic potential *in vivo* from the available data in experimental animals". Therefore, the EU classification of styrene as a mutagen Category 3 is not justified. In the J-GHS, styrene was classified as a Category 2 GCM based on the positive findings both *in vivo* micronucleus tests and a human biomonitoring study on micronucleated peripheral lymphocytes. At the time of the J-GHS effort, the draft EU Risk Assessment Report had not been published. We agree with the conclusion in the EU draft report for the purpose of classification and labeling at present time. Epidemiological studies will be needed for further discussion on the effect of styrene to industrial workers. The case of styrene reveals that harmonization of expert judgment is not easy, and is not static.

Recently, many discussion papers on data evaluation by weight of evidence and mode of action approaches have been published (Butterworth, 2006; Hoffmann and Hartung, 2006; Kirkland et al., 2007; Thybaud et al., 2007a,b). Determination of a genotoxic mode of action depends on mutagenicity and/or genotoxicity tests with bacteria, cell cultures, and whole animals. In addition, differences in data quality for published data are dramatic. A weight of evidence approach is essential to judge whether the chemical should be classified as GCM (Butterworth, 2006). Harmonization or consensus of weighting of mutagenicity or genotoxicity tests is needed for non-experts in GCM classification.

6.4. Decision logic and practical decision tree

The practical decision tree (Fig. 2) has been prepared by combining the decision logic of Fig. 1 with GHS classification criteria of Table 1 for germ cell mutagenicity. The tree flows from upstream (i.e., evidence of heritable germ cell mutagenicity) to downstream (i.e., evidence of germ cell mutagenicity *in vivo*, somatic cell mutagenicity *in vivo*, and then mutagenicity *in vitro*). The decision tree is simple and clear when definitive test result(s) exist. However, the results of heritable germ cell mutagenicity tests including the dominant lethal test are emphasized in the decision tree. The positive findings of a dominant lethal test had an impact in the classification of ethanol despite a lack of clear supportive evidence of mutagenicity in *in vivo* and *in vitro* tests (see Section 5.18). In addition, positive findings are given more weight than negative ones for decision making in the tree. Some of the results of classification in J-GHS were overestimated. A complete data set of mutagenicity or genotoxicity tests including *in vitro* tests is not usually available, and the results are sometimes inconsistent. *In vitro* tests employ a metabolic activation system (e.g., rat liver S9) to mimic *in vivo* situation. However, the S9 might be able to produce a metabolite that is not produced in humans. In such case, a positive response in *in vitro* tests in the presence of S9 would be irrelevant for humans. In addition rat liver S9 may not be able to produce a metabolite(s) that is formed in humans. If this is known, genotoxicity data should be sought for such metabolites, in their own right. Ideally tests should be supported by positive controls similar in structure to the test compounds in question. For evaluation of *in vivo* tests, the dose levels, route of administration, and target tissue exposure should be considered. As positive responses as a result of changes in core body temperature and increases in erythropoiesis following prior toxicity to erythroblasts are known in rodent micronucleus tests (Tweats et al., 2007), the consideration to such secondary effects is also needed, as these effects do not necessarily indicate genotoxicity by the test chemical under normal exposure conditions. Therefore, careful evaluation of the test results (negative or positive) is important (see Section 5.20). Though the decision tree is useful for the GCM classification a "total weight of evidence approach" using all available data is required for classification. Expert judgment is required in some cases in the practical decision tree (Fig. 2).

7. Conclusions

The usefulness of various information sources (Table 2), the examples of different mutagenicity or genotoxicity tests (Table 3), the practical decision tree (Fig. 2), and the definition of GCM (Table 4) for GHS classification have been demonstrated. GHS criteria for germ cell mutagenicity have been adopted in worldwide, and are becoming standard for hazard classification. In Japan, the GHS system has been employed in the Amended Industrial Safety and Health Law for labeling of hazardous chemicals (enforcement date, December 1, 2006). In the EU, the European Commission adopted the "Proposal for Regulation of the European Parliament

and of the Council on classification, labeling and packaging of substances and mixtures, and amending Directive 67/548/EEC and Regulation (EC) No 1907/2006". The proposed Regulation would align the EU system of classification, labeling and packaging substances and mixtures with the GHS. The preparation of detailed guidance for the application of the GHS criteria is under development for the REACH framework (the Registration, Evaluation and Authorisation of Chemicals, a new regulatory approach for chemicals in the EU) (ECHA, 2008a,b,c). After implementation of GHS in each country, the classification of chemicals will be conducted by (hopefully) experts in classification within chemical suppliers, i.e., manufacturers or importers. Illustrations, practical guide and supporting explanations for classification of GCM are helpful for them in order to classify chemicals using scientifically principles. Classification should be performed by Classifiers with high expertise using high quality information sources. It is clear that suitable classification depends on the weight of evidence and reliability of the data. Genetic toxicologists as experts should consider data quality and reliability, and critically review several authoritative documents including original articles to support the classification of chemicals. Finally, it is noted that the results of GHS classification are not inflexible; they will be revised by the consideration of new information including new test data and/or by the elucidation of the mechanism or mode of action of the chemical.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

Acknowledgment

This work was supported in part by the Health and Labor Sciences Research Grants (H18-Chemistry-General-009 and H21-Chemistry-General-004).

References

ACGIH (American Conference of Governmental Industrial Hygienists), 2001. Documentation of the threshold limit values for chemical substances, Glycidol.
Adler, I.D., Anrae, U., Kreis, P., Neumann, H.-G., Thier, R., Wild, D., 2000. Recommendation for the categorization of germ cell mutagens. Int. Arch. Occup. Environ. Health 73, 428–432.
ATSDR (US Agency for Toxic Substances and Disease Registry), 1998. Toxicological profile, 3,3′-Dichlorobenzidine. Available from: <(http://www.atsdr.cdc.gov/toxprofiles/tp108.pdf> (accessed 20.4.2009).
ATSDR, 2004. Toxicological profile, Vinyl chloride, draft. Available from: <http://www.atsdr.cdc.gov/toxprofiles/tp20.pdf> (2007, final version, accessed 20.4.2009).
ATSDR, 2006. Toxicological profile, 1,4-Dioxane. Available from: <http://www.atsdr.cdc.gov/toxprofiles/tp187.pdf> (2007, draft for public comment, accessed 20.4.2009).
Bingham, E., Cohrssen, B., Powell, C.H. (Eds.), 2001a. Patty's Toxicology, fifth ed., vol. 6, Ethanol. John Wiley and Sons, New York, pp. 382–394.
Bingham, E., Cohrssen, B., Powell, C.H. (Eds.), 2001b. Patty's Toxicology, fifth edition, vol. 6, Glycidol. John Wiley and Sons, New York, pp. 1132–1134.
Bulsiewicz, H., 1977. The influence of phenol on chromosomes of mice (Mus Musculus) in the process of spermatogenesis. Fol. Morph. 36, 13–22.
Butterworth, B.E., 2006. A classification framework and practical guidance for establishing a mode of action for chemical carcinogens. Regul. Toxicol. Pharmacol. 45, 9–23.
CERI (Chemicals Evaluation and Research Institute), 1999. Chemical hazard data sheet, 98-21, *o*-Chloronitrobenzene (in Japanese). Available from: <http://qsar.cerij.or.jp/SHEET/F98_21.pdf> (accessed 20.4.2009).
CERI, 2002a. Chemical hazard data sheet, 2000-17, 2,3-Epoxy-1-propanol (Glycidol), revised (in Japanese). Available from: <http://qsar.cerij.or.jp/SHEET/F2000_17.pdf> (accessed 20.4.2009).
CERI, 2002b. Chemical hazard data sheet, 2000-19, 3,3′-Dichlorobenzidine, revised (in Japanese). Available from: <http://qsar.cerij.or.jp/SHEET/F2000_19.pdf> (accessed 20.4.2009).
CERI, 2002c. Chemical hazard data sheet, 2000-23, Nitrilotriacetic acid, revised (in Japanese). Available from: <http://qsar.cerij.or.jp/SHEET/F2000_23.pdf> (accessed 20.4.2009).
CERI-NITE (Chemicals Evaluation and Research Institute-National Institute of Technology and Evaluation), 2003. Hazard assessment report, 64, Acrylonitrile, ver. 1.0 (in Japanese). Available from: <http://www.safe.nite.go.jp/pdf/No-64_1.1.pdf> (2005, ver. 1.1, accessed 20.4.2009).
CERI-NITE, 2004a. Hazard assessment report, 13, 1,4-Dioxane, ver. 1.1 (in Japanese). Available from: <http://www.safe.nite.go.jp/pdf/No-13_1.1.pdf> (accessed 20.4.2009).
CERI-NITE, 2004b. Hazard assessment report, 35, Acrylamide, ver. 1.0 (in Japanese). Available from: <http://www.safe.nite.go.jp/pdf/No-35_1.1.pdf> (2006, ver. 1.1, accessed 20.4.2009).
CERI-NITE, 2004c. Hazard assessment report, 63, Aniline, ver. 1.0 (in Japanese). Available from: <http://www.safe.nite.go.jp/pdf/No-63_1.1.pdf> (2006, ver. 1.1, accessed 20.4.2009).
CERI-NITE, 2004d. Hazard assessment report, 75, Chloroethylene (Vinyl chloride), ver. 1.1 (in Japanese). Available from: <http://www.safe.nite.go.jp/pdf/No-75_1.1.pdf> (accessed 20.4.2009).
CERI-NITE, 2005a. Hazard assessment report, 32, Phenol, ver. 1.0 (in Japanese). Available from: <http://www.safe.nite.go.jp/pdf/No-32.pdf> (accessed 20.4.2009).
CERI-NITE, 2005b. Hazard assessment report, 33, 3,3′-Dichloro-4,4′-diaminodiphenylmethane (4,4′-Methylenebis(2-chloroaniline)), ver. 1.0 (in Japanese). Available from: <http://www.safe.nite.go.jp/pdf/No-33.pdf> (accessed 20.4.2009).
CERI-NITE, 2005c. Hazard assessment report, 71, Formaldehyde, ver. 1.1 (in Japanese). Available from: <http://www.safe.nite.go.jp/pdf/No-71_1.1.pdf> (accessed 20.4.2009).
Costa, R., Russo, A., Zordan, M., Pacchierotti, F., Tavella, A., Levis, A.G., 1988. Nitrilotriacetic acid (NTA) induces aneuploidy in *Drosophila* and mouse germline cells. Environ. Mol. Mutagen. 12, 397–407.
Dearfield, K.L., Cimino, M.C., McCarroll, N.E., Mauer, I., Valcovic, L.R., 2002. Genotoxicity risk assessment: a proposed classification strategy. Mutat. Res. 521, 121–135.
DFG (Deutsche Forschungsgemeinschaft), 1992a. Occupational Toxicants, 4, *o*-Chloronitrobenzene. WILEY-VCH, Weinheim, pp. 107–114.
DFG, 1992b. Occupational Toxicants, 5,3,3′-Dichlorobenzidine. WILEY-VCH, Weinheim, pp. 99–107.
DFG, 1998. Occupational Toxicants, 10, o-Anisidine. WILEY-VCH, Weinheim, pp. 1–13.
DFG, 1999. Occupational Toxicants, 12, Ethanol. WILEY-VCH, Weinheim, pp. 129–165.
DFG, 2003a. Occupational Toxicants, 20, 1,4-Dioxane. WILEY-VCH, Weinheim, pp. 105–133.
DFG, 2003b. Occupational Toxicants, 20, Glycidol. WILEY-VCH, Weinheim, pp. 179–190.
DFG, 2007. List of MAK and BAT values, Commission for the investigation of health hazards of chemical compounds in the work area, Report No. 43. Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, Germany.
ECB (European Chemicals Bureau), 2000. International Uniform Chemical Information Database (IUCLID), Sodium chlorite. Available from: <http://ecb.jrc.it/esis/esis.php?PGM=hpv&DEPUIS=autre> (accessed 30.6.2005).
ECB, 2002a. EU Risk Assessment Report, 15, *o*-Anisidine. Available from: <http://ecb.jrc.ec.europa.eu/DOCUMENTS/Existing-Chemicals/RISK_ASSESSMENT/REPORT/o-anisidinereport025.pdf> (accessed 20.4.2009).
ECB, 2002b. EU Risk Assessment Report, 21, 1,4-Dioxane. Available from: <http://ecb.jrc.ec.europa.eu/DOCUMENTS/Existing-Chemicals/RISK_ASSESSMENT/REPORT/dioxanereport038.pdf> (accessed 20.4.2009).
ECB, 2002c. EU Risk Assessment Report, 28, Acrylic acid. Available from: <http://ecb.jrc.ec.europa.eu/DOCUMENTS/Existing-Chemicals/RISK_ASSESSMENT/REPORT/acrylicacidreport028.pdf> (accessed 20.4.2009).
ECB, 2003. EU Risk Assessment Report. Cadmium Oxide, Draft: Final version, 75, 2007. Available from: <http://ecb.jrc.ec.europa.eu/DOCUMENTS/Existing-Chemicals/RISK_ASSESSMENT/REPORT/cdoxidereport302.pdf> (final, vol. 75, cadmium oxide, Part II Human Health, accessed 20.4.2009).
ECB, 2004a. EU Risk Assessment Report, 32, Acrylonitrile. Available from: <http://ecb.jrc.ec.europa.eu/DOCUMENTS/Existing-Chemicals/RISK_ASSESSMENT/REPORT/acrylonitrilereport029.pdf> (accessed 20.4.2009).
ECB, 2004b. EU Risk Assessment Report, 50, Aniline. Available from: <http://ecb.jrc.ec.europa.eu/DOCUMENTS/Existing-Chemicals/RISK_ASSESSMENT/REPORT/anilinereport049.pdf> (accessed 20.4.2009).
ECB, 2006a. Classification proposal for styrene, Danish Environmental Protection Agency, November 2006, ECBI/19/06 Add. 1, part I. Available from: <http://ecb.jrc.it/classification-labelling/MEETINGS/public.htm> (accessed 30.7.2008).
ECB, 2006b. EU Risk Assessment Report, 64, Phenol. Available from: <http://ecb.jrc.ec.europa.eu/DOCUMENTS/Existing-Chemicals/RISK_ASSESSMENT/REPORT/phenolreport060.pdf> (accessed 20.4.2009).
ECB, 2006c. Proposal for harmonized classification and labeling of styrene, U065 (UK), Styrene, ECBI/19/06 Add. 11. Available from: <http://ecb.jrc.it/classification-labelling/MEETINGS/public.htm> (accessed 30.7.2008).
ECB, 2007a. EU Draft Risk Assessment Report, Styrene, R034_0711_hh November 2007. Available from: <http://ecb.jrc.it/DOCUMENTS/Existing-Chemicals/RISK_ASSESSMENT/DRAFT/R034_0001_0711_env_hh.pdf> (accessed 30.7. 2008).
ECB, 2007b. Industry response to the classification proposal for styrene, Submitted by the Danish Environmental Protection Agency, CEFIC Styrenics Steering Committee, January 2007, ECBI/19/06 Add. 6. Available from: <http://ecb.jrc.it/classification-labelling/MEETINGS/public.htm> (accessed 30.7.2008).
ECB, 2008. EU Annex I, Annex I to Directive 67/548/EEC on Classification and Labelling of Dangerous Substances. Available from: <http://ecb.jrc.it/classification-labelling/search-classlab/> (accessed 30.6.2008).

ECETOC (European Center of Ecotoxicology and Toxicology of Chemicals), 1998. Technical report 31, The mutagenicity and carcinogenicity of vinyl chloride: a historical review and assessment.

ECHA (European Chemicals Agency), 2008a. Guidance on information requirements and chemical safety assessment, Chapter R.3: information gathering, May 2008. Available from: <http://guidance.echa.europa.eu/docs/guidance_document/information_requirements_r3_en.pdf?vers=20_08_08> (accessed 20.4.2009).

ECHA (European Chemicals Agency), 2008b. Guidance on information requirements and chemical safety assessment, Chapter R.4: evaluation of available information, May 2008. Available from: <http://guidance.echa.europa.eu/docs/guidance_document/information_requirements_r4_en.pdf?vers=20_08_08> (accessed 20.4.2009).

ECHA (European Chemicals Agency), 2008c. Guidance on information requirements and chemical safety assessment, Chapter R.7a: endpoint specific guidance, May 2008. Available from: <http://guidance.echa.europa.eu/docs/guidance_document/information_requirements_r7a_en.pdf?vers=20_08_08> (accessed 20.4.2009).

EPA (US Environmental Protection Agency), 2000. Integrated Risk Information System (IRIS), Chlorite (sodium salt), Toxicological review of chlorine dioxide and chlorite, EPA/635/R-00/007, September 2000. Available from: <http://cfpub.epa.gov/ncea/iris/index.cfm> (accessed 12.10.2007).

EPA, 2002a. IRIS, Phenol. Available from: <http://cfpub.epa.gov/ncea/iris/index.cfm> (accessed 15.10.2007).

EPA, 2002b. Toxicological review of phenol, September 2002, EPA/635/R-02/006. Available from: <http://cfpub.epa.gov/ncea/iris/index.cfm> (accessed 15.10. 2007).

European Communities, 2001. Commission directive 2001/59/EC of 6 August 2001, Offi. J. Eur. Commun. L225. (accessed 21.8.2001).

Hayashi, M., Kishi, M., Sofuni, T., Ishidate Jr., M., 1988. Micronucleus tests in mice on 39 food additives and eight miscellaneous chemicals. Food Chem. Toxicol. 26, 487–500.

Hoffmann, S., Hartung, T., 2006. Toward an evidence-based toxicology. Hum. Exp. Toxicol. 25, 497–513.

HSDB (Hazardous Substance Data Bank), 2002. 1,3-Dichloropropane, HSDB Number 5482, Last revision data, Oct 2002. Available from: <http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB> (accessed 12.6.2008).

IARC (International Agency for Research on Cancer), 1982. IARC Monographs on the evaluation of the carcinogenic risks to humans, 29, Some Industrial Chemicals and Dyestuffs. IARC, Lyon, France.

IARC, 1988. IARC Monographs on the evaluation of the carcinogenic risks to humans, 44, Alcohol Drinking. IARC, Lyon, France.

IARC, 1990. IARC Monographs on the evaluation of the carcinogenic risks to humans, 48, Some Flame Retardants and Textile Chemicals, and Exposures in the Textile Manufacturing Industry. IARC, Lyon, France.

IARC, 1991. IARC Monographs on the evaluation of the carcinogenic risks to humans, 52, Chlorinated Drinking-water; Chlorination By-products; Some Other Halogenated Compounds; Cobalt and Cobalt Compounds. IARC, Lyon, France.

IARC, 1993. IARC Monographs on the evaluation of the carcinogenic risks to humans, 57, Occupational Exposures of Hairdressers and Barbers and Personal Use of Hair Colourants; Some Hair Dyes, Cosmetic Colourants, Industrial Dyestuffs and Aromatic Amines. IARC, Lyon, France.

IARC, 1994. IARC Monographs on the evaluation of the carcinogenic risks to humans, 58, Beryllium, Cadmium, Mercury, and Exposures in the Glass Manufacturing Industry. IARC, Lyon, France.

IARC, 1996. IARC monographs on the evaluation of the carcinogenic risks to humans, 65, Printing processes and printing inks, carbonblack and some nitro compounds. IARC, Lyon, France.

IARC, 1999a. IARC Monographs on the evaluation of the carcinogenic risks to humans, 71, Re-evaluation of Some Organic Chemicals, Hydrazine and Hydrogen Peroxide. IARC, Lyon, France.

IARC, 1999b. IARC monographs on the evaluation of the carcinogenic risks to humans, 73, Some Chemicals that Cause Tumours of the Kidney or Urinary Bladder in Rodents, and Some Other Substances. IARC, Lyon, France.

IARC, 2007. IARC Monographs on the evaluation of the carcinogenic risks to humans, 96, Consumption of Alcoholic Beverages and Ethyl Carbamate (Urethane). Available from: <http://monographs.iarc.fr/ENG/Meetings/96-alcohol.pdf> (accessed 20.4.2009).

IPCS (International Program on Chemical Safety), 1989. Environmental Health Criteria (EHC), 89, Formaldehyde. Available from: <http://www.inchem.org/documents/ehc/ehc/ehc89.htm> (accessed 20.4.2009).

IPCS, 1998. Concise International Chemical Assessment Document (CICAD), 2, 3,3′-Dichlorobenzidine. Available from: <http://www.who.int/ipcs/publications/cicad/en/cicad02.pdf> (accessed 20.4.2009).

IPCS, 2001. Concise International Chemical Assessment Document (CICAD), 29, Vanadium pentoxide and other inorganic vanadium compounds. Available from: <http://www.who.int/ipcs/publications/cicad/en/cicad29.pdf> (accessed 20.4.2009).

IPCS, 2002. Concise International Chemical Assessment Document (CICAD), 39, Acrylonitrile. Available from: <http://www.who.int/ipcs/publications/cicad/cicad39_rev.pdf> (accessed 20.4.2009).

JECDB (Japan Existing Chemical Data Base), 2006a. In vitro chromosomal aberration test of 1,3-dibromopropane in cultured Chinese hamster cells (in Japanese). Available from: <http://dra4.nihs.go.jp/mhlw_data/home/paper/paper109-64-8f.html> (accessed 2.9.2006).

JECDB, 2006b. Reverse mutation test of 1,3-dibromopropane in bacteria (in Japanese). Available from: <http://dra4.nihs.go.jp/mhlw_data/home/paper/paper109-64-8e.html> (accessed 2.9.2006).

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

Kirkland, D.J., Aardema, M., Banduhn, N., Carmichael, P., Fautz, R., Meunier, J.R., Pfuhler, S., 2007. *In vitro* approaches to develop weight of evidence (WoE) and mode of action (MoA) discussions with positive in vitro genotoxicity results. Mutagenesis 22, 161–175.

Meier, J.R., Bull, R.J., Stober, J.A., Cimino, M.C., 1985. Evaluation of chemicals used for drinking water disinfection for production of chromosomal damage and sperm-head abnormalities in mice. Environ. Mutagen. 7, 201–211.

Morita, T., Asano, N., Awogi, T., Sasaki, Y.F., Sato, S., Shimada, H., Sutou, S., Suzuki, T., Wakata, A., Sofuni, T., Hayashi, M., 1997. Evaluation of the rodent micronucleus assay in the screening of IARC carcinogens (groups 1, 2A, and 2B). The summary report of the 6th collaboration study by CSGMT/JEMS MMS. Mutat. Res. 389, 3–122. (Erratum, Mutat. Res. 391, 259–267, 1997).

Morita, T., Hayashi, M., Morikawa, K., 2006. Globally harmonized system on hazard classification and labeling of chemicals and other existing classification systems for germ cell mutagens. Genes Environ. 28, 141–152.

NICNAS (Australia National Industrial Chemical Notification and Assessment Scheme), 1998. PECAR, PEC/7, 1,4-Dioxane. Available from: <http://www.nicnas.gov.au/publications/car/PEC/PEC7/PEC7_Full_Report_PDF.pdf> (accessed 20.4.2009).

NITE (National Institute of Technology and Evaluation), 2005a. GHS Classification Manual, Ed. Interministerial Committee on GHS, ver. 20 October 2005. Available from: <http://www.safe.nite.go.jp/english/pdf/ghs_manual_e.pdf> (accessed 20.4.2009).

NITE, 2005b. Technical Guidance on the GHS classification, Ed. Interministerial Committee on GHS. Available from: <http://www.safe.nite.go.jp/english/pdf/ghs_guidance_e.pdf> (accessed 20.4.2009).

NTP (US National Toxicology Program), 2005a. Testing Information Data Base, Cadmium Oxide. Available from: <http://ntp.niehs.nih.gov:8080/index.html?col=010stat> (accessed 2.12.2005).

NTP, 2005b. Testing Information Data Base, 4,4′-Methylenebis(2-chloroaniline). Available from: <http://ntp.niehs.nih.gov:8080/index.html?col=010stat> (accessed 28.12.2005).

NTP, 2005c. Testing Information Data Base, Phenol. Available from: <http://ntp.niehs.nih.gov:8080/index.html?col=010stat> (accessed 28.12.2005).

NTP, 2005d. Testing Information Data Base, Vanadium Pentoxide. Available from: <http://ntp.niehs.nih.gov:8080/index.html?col=010stat> (accessed 12.12. 2005).

OECD, 2001. Screening Information Data Set (SIDS) Initial Assessment Report, 1-Chloro-2-nitrobenzene. Available from: <http://www.chem.unep.ch/irptc/sids/OECDSIDS/CHLORONITROB.pdf> (accessed 20.4.2009).

OECD, 2004a. Screening Information Data Set (SIDS) Initial Assessment Report, Ethanol. Available from: <http://www.chem.unep.ch/irptc/sids/OECDSIDS/64175.pdf> (accessed 20.4.2009).

OECD, 2004b. Screening Information Data Set (SIDS) Initial Assessment Report, Formaldehyde. Available from: <http://www.chem.unep.ch/irptc/sids/OECDSIDS/FORMALDEHYDE.pdf> (accessed 20.4.2009).

Pacchierotti, F., Ranaldi, R., 2006. Mechanisms and risk of chemically induced aneuploidy in mammalian germ cells. Curr. Pharm. Des. 12, 1489–1504.

Phillips, B.J., Jenkinson, P., 2001. Is ethanol genotoxic? a review of the published data. Mutagenesis 16, 91–101.

Pratt, I.S., Barron, T., 2003. Regulatory recognition of indirect genotoxicity mechanisms in the European Union. Toxicol. Lett. 140–141, 53–62.

Robbiano, L., Carrozzino, R., Puglia, C.P., Corbu, C., Brambilla, G., 1999. Correlation between induction of DNA fragmentation and micronuclei formation in kidney cells form rats and humans and tissue-specific carcinogenic activity. Toxicol. Appl. Pharamcol. 161, 153–159.

Robbins, W.A., Vine, M.F., Truong, K.Y., Everson, R.E., 1997. Use of fluorescence in situ hybridization (FISH) to assess effects of smoking, caffeine, and alcohol on aneuploidy load in sperm of healthy men. Environ. Mol. Mutagen. 30, 175–183.

RTECS (Registry of Toxic Effects of Chemical Substances), 2003. US National Institute for Occupational Safety and Health (NIOSH), Sodium chlorite, RTECS No. VZ4800000, Last revision data, August 2003. Available from: http://csi.micromedex.com (accessed 30.6.2005).

Schmid, O., Speit, G., 2006. Genotoxic effects induced by formaldehyde in human blood and implications for the interpretation of biomonitoring studies. Mutagenesis 22, 69–74.

Speit, G., Schmid, O., Fröhler-Keller, M., Lang, I., Triebig, G., 2007. Assessment of local genotoxic effects of formaldehyde in humans measured by the micronucleus test with exfoliated buccal mucosal cells. Mutat. Res. 627, 129–135.

Spencer, P.J., Gollapudi, B.B., Waechter Jr., J.M., 2007. Induction of micronuclei by phenol in the mouse bone marrow: I. Association with chemically induced hypothermia. Toxicol. Sci. 97, 120–127.

Thybaud, V., Aardema, M., Casciano, D., Dellarco, V., Embry, M.R., Gollapudi, B.B., Hayashi, M., Holsapple, M.P., Jacobson-Kram, D., Kasper, P., MacGregor, J.T., Rees, R., 2007a. Relevance and follow-up of positive results in *in vitro* genetic toxicity assays: an ILSI-HESI initiative. Mutat. Res. 633, 67–79.

Thybaud, V., Aardema, M., Clements, J., Dearfield, K., Galloway, S., Hayashi, M., Jacobson-Kram, D., Kirkland, D., MacGregor, J.T., Marzin, D., Ohyama, W.,

Schuler, Suzuki, H., Zeiger, E., 2008b. Expert working group on hazard identification and risk assessment in relation to *in vitro* testing. Strategy for genotoxicity testing: hazard identification and risk assessment in relation to *in vitro* testing. Mutat. Res. 627, 41–58.

Tweats, D.J., Blakey, D., Heflich, R.H., Jacobs, A., Jacobsen, S.D., Morita, T., Nohmi, T., O'Donovan, M.R., Sasaki, Y.F., Sofuni, T., Tice, R., 2007. Report of the IWGT working group on strategies and interpretation of regulatory *in vivo* tests. I. Increases in micronucleated bone marrow cells in rodents that do not indicate genotoxic hazards. Mutat. Res. 627, 78–91.

UKCOM (UK Committee on Mutagenicity), 2007. Formaldehyde: Evidence for Systemic Mutagenicity, COM/07/S5, November 2007. Available from: <www.advisorybodies.doh.gov.uk/com/formalde.htm> (accessed 10.12.2008).

UN (United Nations), 2003. Globally Harmonized System of Classification and Labelling of Chemicals (GHS), New York and Geneva. Available from: <http://www.unece.org/trans/danger/publi/ghs/ghs_rev00/00files_e.html> (accessed 20.4.2009).

UN, 2005. Globally Harmonized System of Classification and Labelling of Chemicals (GHS), First revised edition, New York and Geneva. Available from: <http://www.unece.org/trans/danger/publi/ghs/ghs_rev01/01files_e.html> (accessed 20.4.2009).

UN, 2007. Globally Harmonized System of Classification and Labelling of Chemicals (GHS), Second revised edition, New York and Geneva. Available from: <http://www.unece.org/trans/danger/publi/ghs/ghs_rev02/02files_e.html> (accessed 19.8.2008).

UNITAR (United Nations Institute for Training and Research), 2007. WSSD Global GHS Partnership Annual Report 2007. Available from: <http://www.unitar.org/cwm/publications/pag_ghs/pag13/PAG_13-3_Guide.pdf> (accessed 19.8.2008).

UNITAR, 2008a. Course 2: classifying chemicals according to the GHS, part 1: understanding classification, part 2: classification (Draft version 27 June 2008). Available from: <http://www.unitar.org/cwm/ghs/ghs14.html> (accessed 19.8.2008).

UNITAR, 2008b. Implementation of GHS, Development of lists of classification, UN/SCEGHS/15/INF.32, Fifteenth session, Geneva, 9–11 July 2008. Available from: <http://www.unece.org/trans/doc/2008/ac10c4/UN-SCEGHS-15-inf32e.pdf> (accessed 19.8.2008).

Waters, M.D., Stack, H.F., Jackson, M.A., Bridges, B.A., Adler, I.D., 1994. The performance of short-term tests in identifying potential germ cell mutagens: a qualitative and quantitative analysis. Mutat. Res. 341, 109–131.