SCCS/1532/14
Revision of 22 October 2014

Scientific Committee on Consumer Safety

SCCS

ADDENDUM

to the SCCS's Notes of Guidance (NoG) for the Testing of Cosmetic Ingredients and their Safety Evaluation, 8th Revision (SCCS/1501/12)

This Addendum replaces the section 3-4.7 Mutagenicity/Genotoxicity and 3-4.8 Carcinogenicity of the NoG, from pages 26 to 29.

The SCCS adopted this Addendum on 9 April 2014 by written procedure

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Three independent non-food Scientific Committees provide the Commission with the scientific advice it needs when preparing policy and proposals relating to consumer safety, public health and the environment. The Committees also draw the Commission's attention to the new or emerging problems which may pose an actual or potential threat.
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In addition, the Commission relies upon the work of the European Food Safety Authority (EFSA), the European Medicines Agency (EMA), the European Centre for Disease prevention and Control (ECDC) and the European Chemicals Agency (ECHA).

SCCS

The Committee shall provide opinions on questions concerning all types of health and safety risks (notably chemical, biological, mechanical and other physical risks) of non-food consumer products (for example: cosmetic products and their ingredients, toys, textiles, clothing, personal care and household products such as detergents, etc.) and services (for example: tattooing, artificial sun tanning, etc.).

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This opinion has been subject to a commenting period of six weeks after its initial publication. Comments received during this time have been considered by the SCCS and discussed in the subsequent plenary meeting. Where appropriate, the text of the relevant sections of the opinion has been modified or explanations have been added. In the cases where the SCCS after consideration and discussion of the comments, has decided to maintain its initial views, the opinion (or the section concerned) has remained unchanged. Revised opinions carry the date of revision.

Keywords: SCCS, scientific opinion, Regulation 1223/2009, SCCS's Notes of Guidance (NoG), Mutagenicity/Genotoxicity, Carcinogenicity

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3-4.7 Mutagenicity

Mutagenicity refers to the induction of permanent transmissible changes in the amount or structure of the genetic material of cells or organisms. These changes may involve a single gene or gene segment, a block of genes or chromosomes. The term clastogenicity is used for agents giving rise to structural chromosome aberrations. A clastogen causes breaks in chromosomes that result in the loss or rearrangement of chromosome segments. Aneugenicity (aneuploidy induction) refers to the effects of agents that give rise to a change (gain or loss) in chromosome number in cells, resulting in cells that do not have an exact multiple of the haploid number [2006/1907/EC].

Genotoxicity is a broader term and refers to processes which alter the structure, information content or segregation of DNA and are not necessarily associated with mutagenicity. Thus, tests for genotoxicity include tests which provide an indication of induced damage to DNA (but not direct evidence of mutation) via, for example sister chromatid exchange (SCE), DNA strand breaks, DNA adduct formation or mitotic recombination, as well as tests for mutagenicity [see also 2006/1907/EC, ECHA 2008a].

As a general recommendation, the SCCS is of the opinion that the evaluation of the potential for mutagenicity of a cosmetic substance to be annexed in the Regulation (EC) No 1223/2009 should include tests to provide information on the three genotoxic endpoints, namely 1) mutagenicity at the gene level, 2) chromosome breakage and/or rearrangements (clastogenicity), and 3) numerical chromosome aberrations (aneuploidy). This recommendation represents the actual consensus of international groups of scientific experts [Muller et al., 2003, Dearfield et al., 2011, 2006/1907/EC, EFSA 2011], and of an expert advisory committee [COM 2011]. Several well-established *in vitro* mutagenicity / genotoxicity tests are available, described in OECD Guidelines[1] and/or in Regulation (EC) No 440/2008 [2008/440/EC]. The SCCS is of the opinion that for this task only genotoxicity tests, which measure a real mutation endpoint (gene or chromosome mutations), should be preferred. So-called indicator tests which measure primary DNA damage, instead of irreversible DNA damage, without taking into account the consequences of the damage, are not preferred. Moreover, the SCCS recommends that, before undertaking any testing, a thorough review should be carried out of all available data on the substance (both published and data on file) under assessment, including its (physical) chemistry, toxicokinetic and toxicological profile, as well as data on analogous substances.

A concern with regard to existing *in vitro* tests is the occurrence of results that are negative with suspect genotoxic carcinogens or positive with substances assumed to be non-carcinogens. The current view is that cell systems of human origin, p53 and DNA repair proficient, that are genetically (karyotypically) stable and have defined phase 1 and phase 2 metabolism should be preferred (Kirkland et al., 2007; Pfuhler et al., 2011, 2014). A retrospective analysis on cosmetic Annex ingredients conducted between 2000 and 2013 revealed that the cell type (*e.g.* human origin) and the top concentration used did not have a major impact on the outcome (Ates et al., 2014). This may be due to the fact that the majority of evaluated substances by the SCCS were hair dyes with specific structural alerts for genotoxicity. An evaluation by Kirkland et al. [2005] for combinations of two or three assays demonstrated that with an increase in the number of tests, the number of

[1] http://www.oecd-ilibrary.org

'unexpected positives' increases whereas the number of 'unexpected negatives' decreases. Furthermore, Kirkland et al. [2011] showed that the sensitivities of the 2- and 3-test batteries seem quite comparable when an existing database of rodent carcinogens and a new database of *in vivo* genotoxins, together over 950 substances, are considered. Using data from the gene mutation test in bacteria and the *in vitro* micronucleus test appears to allow the detection of all relevant genotoxic carcinogens and *in vivo* genotoxins for which data exist in these databases. The combination of these two assays would cover the three genotoxicity endpoints described above, as the bacterial test detects gene mutations and the *in vitro* micronucleus assay detects both structural and numerical chromosome aberrations. EFSA has already published an opinion in which the use of these 2 tests is recommended as a first step in genotoxicity testing for food and feed safety assessment [EFSA, 2011]. The guidance of the UK Committee on Mutagenicity also recommends the above two tests for stage 1 *in vitro* testing [COM, 2011].

Except for special cases for which the Ames test is not suitable, the SCCS recommends two assays for the base level testing of cosmetic substances, represented by the following test systems:

- Bacterial Reverse Mutation Test [OECD 471] as a test covering gene mutations
- *In vitro* Micronucleus Test [OECD 487] as a test for both structural (clastogenicity) and numerical (aneugenicity) chromosome aberrations

It is known that there are classes of substances for which the bacterial reverse mutation test is not suited. In these cases the test procedure should be modified and a scientific justification should be given. This relates to e.g. biocidal substances and antibiotics due to their high toxicity for bacteria as well as to substances with low bioavailability that do not pass easily through the bacterial wall such as nanomaterials and larger particles (*Salmonella* and *Escherichia* bacteria lack the mechanisms (e.g. endocytosis) to incorporate particles). In such cases, the results from a bacterial reverse mutation test are not reliable and a gene mutation test in mammalian cells (*hprt* test, mouse lymphoma assay) should be performed (see also Guidance on safety assessment of nanomaterials in cosmetics SCCS/1484/12). It should also be noted that cytochalasin B can interfere with the uptake of particles by mammalian cells; therefore, in an *in vitro* micronucleus test focused on binuclear cells, the addition of cytochalasin B needs to occur after treatment with the nanoparticles (Pfuhler et al., 2013). Except in specific cases mentioned above for which Ames test is not suitable, the two tests base level strategy is recommended.

Although most tests will give clearly positive or clearly negative results, in some cases the outcome may be considered inconclusive or equivocal. Equivocal refers to a situation where some but not all the requirements for a clear positive or clear negative result have been met. A substance giving an equivocal test result should be reinvestigated using the same test method, but varying the conditions (including sampling more cells) to obtain conclusive results. A justification for the test modifications should be given. Inconclusive means a situation where no clear result was achieved due to a limitation of the test or the test-procedure. In this case, repeating the test under modified conditions should produce a clear result or another test should be performed. A justification for the test modifications or for choosing another test should be given.

Cells should be exposed to the test substance both in the presence and absence of an appropriate metabolic activation system. The most commonly used system is a cofactor-

supplemented S9-fraction prepared from the livers of rodents (usually rat) treated with enzyme-inducing agents such as Aroclor 1254 or a combination of phenobarbital and β-naphthoflavone. The choice and concentration of a metabolic activation system may depend on the class of chemical being tested. In some cases, it may be appropriate to utilise more than one concentration of S9-mix. For azo dyes and diazo compounds, using a reductive metabolic activation system is recommended [Matsushima 1980; Prival et al., 1984].

In order to demonstrate that the result obtained is due to treatment with the substance, it is essential to demonstrate exposure of the bacteria or cells. A way to demonstrate exposure is through cytotoxicity. In the Ames test, a reduction in the number of spontaneous revertant colonies and/or clearing of the bacterial background lawn is sufficient to indicate cytotoxicity and thus exposure to the substance. The other tests, measuring the induction of micronuclei or gene mutations (as for instance in the case of nanoparticles) in mammalian cells require longer exposure to ensure that the cells divide and undergo through at least (and sometimes at most) one round of replication in order to maximise the probability of detecting a mutagen, aneugen or clastogen acting at a specific stage in the cell cycle. A sufficient number of cell divisions is required (depending on test) to convert DNA damage into the genetic endpoint scored by the test. Therefore, cytotoxicity measures based on cell proliferation are preferred and, consequently, have been incorporated into the revised OECD Test Guidelines.

In the *in vitro* micronucleus test, Fowler et al. (2012a, 2012b) have shown that the use of relative population doubling (RPD) or relative increase in cell counts (RICC) helps to improve the specificity of the *in vitro* micronucleus test. If cytochalasin B is used to obtain binuclear cells, determination of the reduction in the number of binuclear cells is a justified alternative way to measure cytotoxicity. In mammalian cell gene mutation tests, relative total growth or relative survival (relative cloning efficiency) are the preferred measures of cytotoxicity.

Based on the 2-test base level recommendation, 4 scenarios are possible.

1. Ames test negative, in vitro micronucleus test negative

If the results from both tests are clearly negative in adequately performed tests, it is very likely that the substance has no mutagenic potential. Further testing is not necessary.

2. Ames test negative, in vitro micronucleus test positive

If the *in vitro* micronucleus test is positive, the substance may be considered an *in vitro* mutagen. Further testing may be essential to clarify the clastogenic potential of the substance, and either the Comet assay in mammalian cells or in the 3D-reconstructed human skin model or the micronucleus test in the 3D-reconstructed human skin model should be considered. The 3D-reconstructed human skin model seems a good alternative to bridge the gap between *in vitro* and *in vivo* testing in terms of final hazard assessment.
Mechanistic investigations (toxicodynamics and toxicogenomics) or internal exposure (toxicokinetics) may be helpful in a weight of evidence evaluation.
Expert judgment is mandatory to come to a conclusion.

3. Ames test positive, in vitro micronucleus test negative

If the Ames test is positive, the substance may be considered an *in vitro* mutagen. Further testing can be used to better assess the mutagenic potential of the substance. The performance of an *in vitro* mammalian gene mutation test seems obvious. However, it is not self-evident that negative results from such a test in mammalian cells can on their own overrule the positive results from a bacterial gene mutation test. For the same reasons as above, additional data from the Comet test in mammalian cells and/or in the 3D-reconstructed human skin model as well as mechanistic investigations (toxicodynamics and toxicogenomics) or internal exposure (toxicokinetics) may add to the weight of evidence. Expert judgment is mandatory to come to a conclusion.

4. Ames test positive, in vitro micronucleus test positive

If the results from both tests are clearly positive in adequately performed tests, it is very likely that the substance has mutagenic potential. Further testing is not necessary.

In the near future, there will be more clarity in the meaning of the positive and negative results in the different mutagenicity/genotoxicity tests, which may result in a better and more reliable decision on the mutagenic potential of substances. Recently, in workshops organized respectively by EURL-ECVAM as well as by the SCCS, the importance of mammalian cell test results was analysed for Ames test-positive substances. It was shown that, following a positive result in an Ames test, the result in an *in vitro* micronucleus test alone is not sufficient to discriminate between chemicals that are positive or negative for carcinogenicity and/or genotoxicity *in vivo*. The performance of a mammalian cell gene mutation test is required to provide further evidence. If both mammalian cell tests, covering the genotoxic endpoints, gene mutations, structural chromosome aberrations and aneuploidy, are negative, it is highly unlikely that the Ames-positive chemical is an *in vivo* genotoxin and/or a genotoxic carcinogen. Likewise, if both mammalian cell tests are positive, it is likely that the substance possesses *in vivo* genotoxic or carcinogenic potential. The SCCS considers this approach promising.

Under the EU Cosmetic Regulation (2009/1223/EC), further *in vivo* follow-up testing to confirm or to overrule the positive *in vitro* findings is no longer possible. In cases where a clear positive result cannot be overruled in a weight of evidence approach with additional tests, the substance has to be considered as a mutagen.
A positive *in vitro* result in genotoxicity testing is also seen as indicative for the carcinogenic potential of substances.

3-4.8 Carcinogenicity

Substances are defined as carcinogenic if they induce tumours (benign or malignant) or increase their incidence, malignancy or shorten the time of tumour occurrence when they are inhaled, ingested, dermally applied or injected [ECB 2003]. It is often differentiated between "genotoxic carcinogens" for which the most plausible mode of carcinogenic action includes the consequences of genotoxic effects [ECB 2003] and "non-genotoxic carcinogens" which are carcinogenic due to mechanisms other than direct interactions with DNA.

Under the testing/marketing ban taken up in the EU Cosmetic Regulation, *in vivo* testing is prohibited for the purpose of this Regulation. The decision on the carcinogenic potential of mutagenic or genotoxic substances may be made on the outcome of *in vitro* mutagenicity tests. A positive *in vitro* result in mutagenicity testing is seen as indicative for the carcinogenic potential of substances.

At present generally accepted alternative *in vitro* methods with OECD guidelines to determine the carcinogenic potential of substances are not available. However, there are promising new *in vitro* approaches which may be helpful to recognise genotoxic as well as non-genotoxic carcinogenic substances. By determining the cellular transformation potency, a step in the multihit/multistep process of carcinogenesis, the *in vitro* Cell Transformation Assay (CTA), have the potential to detect both genotoxic and non-genotoxic carcinogens. The CTA may be considered as providing additional information to more routinely employed *in vitro* tests and may be used as a follow-up assay for confirmation of *in vitro* positive results from genotoxicity assays, typically as part of a weight of evidence assessment (Doktorova *et al*., 2012). OECD has prepared Draft Test Guidelines for the "*In Vitro* Carcinogenicity: Syrian Hamster Embryo (SHE) Cell Transformation Assay" and the "*In Vitro* Carcinogenicity: Bhas 42 Cell Transformation Assay" (the Bhas 42 cell line was established by the transfection of the v-Ha-ras oncogene into the BALB/c 3T3 A31-1-1 cell line). In addition to *in vitro* mutagenicity/genotoxicity tests (see above), data from *in vitro* CTA tests may be considered in a weight of evidence approach.

Without the 2-year bioassay, it is very difficult if not impossible to conclude on the carcinogenicity of substances. As far as genotoxic substances are concerned, in vitro mutagenicity tests are quite well developed. Due to the relation between mutations and cancer, these genotoxicity tests can be seen as a pre-screen for cancer. A positive result in one of the genotoxicity tests may be indicative to consider a substance as putatively carcinogenic. In combination with the CTA, measuring cell transformation, one step in the multistep cancer process, this indication may be stronger.
The situation is different for the non-genotoxic carcinogens. Before the animal testing and marketing ban, non-genotoxic carcinogens were detected by the (sub-)chronic repeated dose studies, including the carcinogenicity test. Alternatives for these in vivo tests to detect non-genotoxic carcinogens, however, are not available with the exception of the CTA but discussions are still ongoing with respect to its use as a test for non-genotoxic carcinogens.

Also worldwide research is ongoing with regard to *in vitro* toxicogenomics for the detection of mutagenesis or carcinogenesis. The idea is that by global gene expression profiling via microarray technology, gene patterns covering diverse mechanisms of substance-induced genotoxicity can be extracted. These gene patterns/biomarkers can be further used as a follow-up of positive findings of the standard *in vitro* mutagenicity/genotoxicity testing battery [Goodsaid et al., 2010, Doktorova et al., 2012a, Magkoufopoulou et al., 2012]. In addition to *in vitro* mutagenicity/genotoxicity tests (see above), data from *in vitro* tests combined with toxicogenomics may also be considered in a weight of evidence approach.

The animal testing ban under the EU Cosmetics Regulation will, however, also have a strong impact on the ability to conduct a quantitative risk assessment for carcinogenic potential of cosmetics ingredients and contaminants. This impact is not only due to the ban on the cancer bioassay itself, but also on *in vivo* mutagenicity/genotoxicity testing which may provide data for semi-quantitative risk assessment (Hernandez et al., 2011, Dybing and Sanner, 2005).

Recently, JRC published one report reviewing the current state of the art of genotoxicity test methods and testing strategy (JRC 2013).

REFERENCES

2008/440/EC - Commission Regulation (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH). *Official Journal L 142, 31/05/2008 p.1.*

2009/1907/EC - Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), establishing a European Chemicals Agency, amending Directive 1999/45/EC and repealing Council Regulation (EEC) No 793/93 and Commission Regulation (EC) No 1488/94 as well as Council Directive 76/769/EEC and Commission Directives 91/155/EEC, 93/67/EEC, 93/105/EC and 2000/21/EC. *Official Journal L 396, 30/12/2006, p.1. Corrigendum in Official Journal L 136, 29/05/2007, p.3.*

2009/1223/EC - Regulation (EC) No 1223/2009 of the European Parliament and of the Council of 30 November 2009 on cosmetic products (recast). *Official Journal L 342, 22/12/2009 p.59.*

Adler, S.; Basketter, D.; Creton, S.; Pelkonen, O.; van Benthem, J.; Zuang, V.; Andersen, KE.; Angers-Loustau, A.; Aptula, A.; Bal-Price, A.; Benfenati, E.; Bernauer, U.; Bessems, J.; Bois, FY.; Boobis, A.; Brandon, E.; Bremer, S.; Broschard, T.; Casati, S.; Coecke, S.; Corvi, R.; Cronin, M.; Daston, G.; Dekant, W.; Felter, S.; Grignard, E.; Gundert-Remy, U.; Heinonen, T.; Kimber, I.; Kleinjans, J.; Komulainen, H.; Kreiling, R.; Kreysa, J.; Leite, SB.; Loizou, G.; Maxwell, G.; Mazzatorta, P.; Munn, S.; Pfuhler, S.; Phrakonkham, P.; Piersma, A.; Poth, A.; Prieto, P.; Repetto, G.; Rogiers, V.; Schoeters, G.; Schwarz, M.; Serafimova, R.; Tähti, H.; Testai, E.; van Delft, J.; van Loveren, H.; Vinken, M.; Worth, A.; Zaldivar, J.M. Alternative (non-animal) methods for cosmetics testing: current status and future prospects- 2010.
Archives of Toxicology 85(5), 367-485 (2011).

Ates G, Doktorova TY, Pauwels M, Rogiers V (2014). Retrospective analysis of the mutagenicity/genotoxicity data of the cosmetic ingredients present on the Annexes of the Cosmetic EU legislation (2000-12). Mutagenesis. 29: 115-121.

Benigni R, Bossa C, Tcheremenskaia O. In vitro cell transformation assays for an integrated, alternative assessment of carcinogenicity: a data-based analysis.Mutagenesis. 2013 Jan;28(1):107-16.

Benigni R, Bossa C, Battistelli CL, Tcheremenskaia O.IARC classes 1 and 2 carcinogens are successfully identified by an alternative strategy that detects DNA-reactivity and cell transformation ability of chemicals. Mutat Res. 2013 Dec 12;758(1-2):56-61. doi: 10.1016/j.mrgentox.2013.09.006. Epub 2013 Sep 27.

COM, 2011. Guidance on a Strategy for genotoxicity Testing and mutagenic hazard assessment of Chemical substances. Committee on Mutagenicity of Chemicals in Food, Consumer Products and the Environment (COM).
http://www.iacom.org.uk/publications/documents/StrategyGuidanceCOMconsultation3.pdf

Dearfield KL, Thybaud V, Cimino MC, Custer L, Czich A, Harvey JS, Hester S, Kim JH, Kirkland D, Levy DD, Lorge E, Moore MM, Ouédraogo-Arras G, Schuler M, Suter W, Sweder K, Tarlo K, van Benthem J, van Goethem F, Witt KL. (2011) Follow-up actions from positive results of *in vitro* genetic toxicity testing. Environ Mol Mutagen. 52:177-204.

ECB (European Chemicals Bureau) (2003). Technical Guidance Document on Risk Assessment in support of Commission Directive 93/67/EEC on Risk Assessment for new notified substances, Commission Regulation (EC) No 1488/94 on Risk Assessment for existing substances and Directive 98/8/EC of the European Parliament and of the Council concerning the placing of biocidal products on the market. Doc. EUR 20418 EN/1, European Communities (2003).

ECHA, 2008. Guidance on information requirements and chemical safety assessment Chapter R.4: Evaluation of available information. Available at: http://guidance.echa.europa.eu/docs/guidance_document/information_requirements_r4_en.pdf?vers=20_08_08.

EFSA, 2011. Scientific opinion on genotoxicity testing strategies applicable to food and feed safety assessment, EFSA Scientific Committee, European Food safety Authority, Parma, Italy. EFSA Journal 9, 2379-2447.

Doktorova TY, Pauwels M, Vinken M, Vanhaecke T, Rogiers V (2012). Opportunities for an alternative integrating testing strategy for carcinogen hazard assessment? Crit RevToxicol. 42: 91–106.

Fowler P, Smith R, Smith K, Young J, Jeffrey L, Kirkland D, Pfuhler S, Carmichael P (2012). Reduction of misleading ("false") positive results in mammalian cell genotoxicity assays. II. Importance of accurate toxicity measurement. Mutat Res.747, 104-117.

Fowler P, Smith K, Young J, Jeffrey L, Kirkland D, Pfuhler S, Carmichael P. Reduction of misleading ("false") positive results in mammalian cell genotoxicity assays. I. Choice of cell type (2012). Mutat Res 742: 11-25.

Goodsaid FM, Amur S, Aubrecht J, Burczynski ME, Carl K, Catalano J, Charlab R, Close S,

Cornu-Artis C, Essioux L*, et al.* (2010). Voluntary exploratory data submissions to the US

FDA and the EMA: experience and impact. Nature reviews. Drug discovery 9: 435-445.

Hernandez L, Slob W, van Steeg Harry, van Benthem J (2011). Can carcinogenic potency be predicted from *in vivo* genotoxicity data? A meta-analysis of historical data. Environ Mol Mutagen 52: 518-528.

JRC 2013. EURL ECVAM Strategy to Avoid and Reduce Animal Use in Genotoxicity Testing. Joint Research Centre, Ispra (VA), Italy. http://publications.jrc.ec.europa.eu/repository/bitstream/111111111/30088/1/jrc_report_en_34844_online.pdf

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

Kirkland D, Reeve L, Gatehouse D, Vanparys P (2011). A core *in vitro* genotoxicity battery comprising the Ames test plus the *in vitro* micronucleus test is sufficient to detect rodent carcinogens and *in vivo* genotoxins. Mutat Res 721:27-34.

Magkoufopoulou C, Claessen SM, Tsamou M, Jennen DG, Kleinjans JC, van Delft JH (2012). A transcriptomics-based *in vitro* assay for predicting chemical genotoxicity *in vivo*. Carcinogenesis 33: 1421-1429.

Matsushima T., Sugimura T., Nagao M., Yahagi T., Shirai A., Sawamura M. (1980). Factors modulating mutagenicity microbial tests. In: Short-term test systems for detecting carcinogens (Eds. Norpoth K.H. and Garner R.C.) Springer, Berlin-Heidelberg-New York, 273-285.

Muller L., Blakey D., Dearfield K.L., Galloway S., Guzzie P., Hayashi M., Kasper P., Kirkland D. MacGregor J.T., Parry J.M., Schechtman L., Smith A., Tanaka N., Tweats D., Yamasaki H (2003). Strategy for genotoxicity testing and stratification of genotoxicity test results- report on initial activities of the IWGT Expert Group. Mutat Res 540, 177-181.

OECD 471 OECD Guideline for testing of chemicals - Guideline 471: Bacterial Reverse Mutation Test. Organization for Economic Cooperation and Development, Paris, adopted 26 May 1983, last updated 21 July 1997.

OECD 487 OECD Guideline for testing of chemicals - Guideline 487: *In vitro* Mammalian Cell Micronucleus Test (MNvit). Organization for Economic Cooperation and Development, Paris, adopted 22 July 2010.

Pfuhler S, Fautz R, Ouedraogo G, Latil A, Kenny J, Moore C, Diembeck W, Hewitt NJ, Reisinger K, Barroso J (2014). The Cosmetics Europe strategy for animal-free genotoxicity testing: project status up-date. Toxicol *In Vitro* 28: 18-23.

Pfuhler S, Elespuru R, Aardema MJ, Doak SH, Maria Donner E, Honma M, Kirsch-Volders M, Landsiedel R, Manjanatha M, Singer T, Kim JH (2013). Genotoxicity of nanomaterials: refining strategies and tests for hazard identification. Environ Mol Mutagen 54: 229-239.

Pfuhler S, Fellows M, van Benthem J, Corvi R, Curren R, Dearfield K, Fowler P, Frötschl R, Elhajouji A, Le Hégarat L, Kasamatsu T, Kojima H, Ouédraogo G, Scott A, Speit G (2011). *In vitro* genotoxicity test approaches with better predictivity: summary of an IWGT workshop. Mutat Res 723: 101-107.

Prival, M.J., Bell S.J., Mitchell V.D., Peiperl M.D., Vaughan V.L. (1984) Mutagenicity of Benzidine and Benzidine-congener dyes and selected monoazo dyes in a modified Salmonella assay. Mutat Res 136, 33-47.

Sanner T, Dybing E (2005). Comparison of carcinogenic and *in vivo* genotoxic potency estimates. Basic Clinical Pharmacol Toxicol 96: 131–139.