CC/2014/15

COMMITTEE ON CARCINOGENICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT (COC)

Guidance Statements:

G07- Alternatives to the 2-year Bioassay – Introduction, parts a and b - first draft

1. This paper, prepared by the PHE Toxicology Unit, is provided as the first draft of the Guidance statement on Alternatives to the 2-year bioassay. A preliminary discussion paper on this topic was presented at the COC meeting in November 2013 and this first draft statement was presented at the meeting in July 2014 but due to time constraints at both meetings, there was insufficient time to discuss the papers. Since then Members have been asked to provide their opinions and comments on the first draft of the guidance statement. A few comments were received and incorporated.

2. The statement has been divided into 4 parts as follows:

a. *in vivo* assays
b. cell transformation assays (CTA's),
c. developing methodologies and strategies (for example toxicogenomics)
d. alternative testing paradigms (for example evaluation using histopathology and proliferative markers in sub-chronic rodent studies)

Parts a) and b) are provided today (part b. is a link to the COM's recent statement on the assays).

3. Part A of the guidance statement has been drafted with the intention of providing guidance specifically on the alternative in vivo assays to a second species carcinogenicity study in a carcinogenicity testing strategy as listed in ICH Guideline S1B (ICH 1998). If appropriate, this could form a recommendation not to use the assay.

4, Three approaches have been considered: transgenic mouse models, *in utero* models and initiation promotion models. A short introduction and evaluation of each model is provided. This is based on previous guidance and a comprehensive, but not systematic, review of the recent literature of the performance of the assays. It is noted that the *in utero* and initiation promotion models have not been widely used, that there are limited chemicals and the protocols are more experimental. In addition some of the conclusions of these papers may be contentious, and thus Members opinions on these approaches and data interpretation are sought. Key, recent reviews of the transgenic assays have been considered: Eastmond et al 2013 - a review of the three transgenic assays p53 +/-, rasH2 and Tg.AC and Nambiar and Morton 2013 – a recent interpretation of rasH2 model (papers provided in Annex 1).

5. It is recognised that many of the assays described are not widely utilised in current carcinogenicity testing strategies. A Member stated that the goal of any revisions to the Carcinogenicity Testing strategy should be to: (a) reduce the cost, time and number of animals used in assessing carcinogenic potential and (b) to improve the accuracy of the prediction of carcinogenic potential for humans. It is his opinion that any Committee evaluations of approaches aiming to replace the traditional 2-species x 2-year bioassays should focus on Alternative Strategies to Carcinogenicity Testing (intended to form part (d) of this Guidance) and not on the alternative in vivo assays described in here and forming part (a) this Guidance. His email is provided (in Annex 2) and can form a starting point for Members discussions if appropriate.

6. A Strategy to replace the 2-year bioassays was outlined in the Committee's Horizon Scanning 2013 (CC/2013/14 – available here: http://webarchive.nationalarchives.gov.uk/20140506122027/http://www.iacoc.org.uk/papers/documents/CC-2013-14AnnexAandAnnexcoversHorizonScanning2013.pdf) and it is our intention to consider this and include Committee recommendations as part of the Guidance series (d). The ICH is currently reviewing the strategy described in Guideline S1. However, the timetable for review of the strategy is uncertain. It is also noteworthy that this strategy is intended to support the testing of pharmaceuticals.

7. Questions for Members

With regards to the current Guidance Statement on Alternatives to the 2 year bioassay – (a) in vivo assays. :

- Given the ongoing debate surrounding the best strategies for improved identification of human carcinogens, do Members wish to continue with reviewing the alternative in vivo models and produce guidance on the use of these assays?
- If so:
    - What are Members views of the overall structure and content of the guidance?
    - Do Members wish to continue to consider the *in utero* and initiation/promotion models, and if so how?
    - What are Members opinions of the use of the transgenic assays as replacements for a second species 2-year bioassay? Can a conclusion of their usefulness be drawn?
    - Do Members agree with the recommendations put forward by CAMM (para 15)?
    - What are Members overall conclusions?

With regards to the development of new strategies to carcinogenicity testing (d):

- Do Members have any preliminary comments or ideas for how this topic could be covered in the future?

PHE Toxicology Unit/Secretariat
October 2014

COMMITTEE ON CARCINOGENICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT (COC)

G07 - Alternatives to the 2-year Bioassay

General Introduction

1. This guidance statement comprises of four parts which together provide an overview of approaches the which have been proposed as alternatives to the 2-year rodent bioassay,

   a. *in vivo* assays

   b. cell transformation assays (CTA's),

   c. developing methodologies and strategies (for example toxicogenomics)

   d. alternative testing paradigms (for example evaluation using histopathology and proliferative markers in sub-chronic rodent studies)

It is part of the Committee of Carcinogenicity (COC) guidance statement series which provides the Committee's views on all aspects of carcinogen risk assessment. It should be read in conjunction with G03 Hazard Identification and Characterisation: Conduct and Interpretation of Animal Carcinogenicity Studies.

2. The conduct of 2-year bioassays in two species, usually rat and mouse, has underpinned carcinogenicity risk assessment since the standard assay was developed in the 1960's (Cohen, 2010a). The objective of these long-term studies is to observe animals for the development of neoplastic lesions following exposure to a test substance for a major part of their life-span. The studies are usually designed to conform to closely defined test protocols and procedures (OECD GL 451, and detailed in G03).

3. A large body of data is available, particularly from the US National Toxicology Program (NTP), which has evaluated a large number of known carcinogens using the standard 2-year bioassay. Carcinogenicity testing strategies were developed taking into consideration the assumptions that biologically, humans and animals are intrinsically similar and that carcinogenesis is a multistage process (Boobis et al., 2009). However it has become evident that the conditions under which chemicals are tested are not necessarily relevant to human exposure (for example, the use of the maximum tolerated dose [MTD]) or that some modes of carcinogenic action (MOA) are not relevant to human risk assessment. Furthermore, standard carcinogenicity study protocols involve the use of large numbers of animals (approximately 500 of each species) and with increasing concern surrounding unnecessary or poorly designed studies, efforts are being made to reduce animal use and to develop more refined testing strategies.

4. The use of both rat and mouse 2-year bioassays in assessing carcinogenic potential of chemicals has been subjected to close scrutiny. Several detailed evaluations of datasets have been undertaken with a view to assessing the utility of the mouse bioassay and the relevance of non-genotoxic liver only rodent carcinogens. In an assessment by Schach von Wittenau & Estes (1983) (cited by Alden et, 1996) of 273 chemicals tested in both rats and mice, 206 were positive in both species whilst only 9 were positive in the mouse, and negative or inconclusive in the rat. Similarly in an assessment by Huff et al (1991), 18/313 chemicals tested in both rats and mice in NTP studies were positive only in the mouse (ie 5.7%) (cited by Alden et al., 1996). Of 202 pesticides evaluated in the European Union, only 3 produced tumours only in mice (Billington et al., 2010). In a further review of data from 710 chemicals which had been tested in both the mouse and rat bioassays – only 3 compounds were identified that produced tumours other than, or in addition to, the liver in the mouse by a non-genotoxic MOA. Mouse only non-genotoxic liver carcinogens have been considered an unreliable indicator of carcinogenic potential for some years (Osimitz et al., 2013).

5. These investigations and analyses suggest that a single two-year rodent assay is sufficient for cancer hazard identification. This view is endorsed by the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) which indicates that bioassay data from only one species (e.g the rat) is required for evaluation of carcinogenic potential, when supported by appropriate mutagenicity and pharmacokinetic studies and a study from a short-term *in vivo* assay, such as a transgenic mouse model (ICH S1B, 1998). As well as alternative *in vivo* models, *in vitro* cell transformation assays have been developed as alternative methods to detect carcinogenic potential, in particular for use in testing scenarios where *in vivo* testing is not permitted (e.g cosmetics testing). Furthermore, recent developments in 'omics technologies such as genomics, proteomics and metabolomics enable detailed examinations of chemically-induced changes in the regulation of genes, proteins and metabolite profiles respectively. They are considered useful in providing insight into the mode and mechanism of action of the effects of chemicals, including carcinogenicity risk assessment.

6. The following guidance documents present the Committees opinions and views on the use of all assays with the potential to be used as alternatives to the 2-year rodent bioassay in a carcinogenicity testing strategy.

G07 Alternatives to the 2-year bioassay –

a) *In vivo* assays

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1. Three alternative types of model are presented: transgenic mouse assays, *in utero*/neonatal models and initiation promotion models, with a view to assessing their usefulness as a replacement for the 2-year bioassay in a second species in a carcinogenicity testing strategy.

2. A number of alternative animal models for the prediction of carcinogenesis have been developed. In a regulatory setting, ICH Guideline S1B (ICH 1998) supports the use of certain alternative models instead of a second species (usually, but not exclusively the mouse) in the carcinogenicity testing strategy for the evaluation of human pharmaceuticals. It states the following:

Several experimental methods are under investigation to assess their utility in carcinogenicity assessment. Generally, the methods should be based on mechanisms of carcinogenesis that are believed relevant to humans and applicable to human risk assessment. Such studies should supplement the long term carcinogenicity study and provide additional information that is not readily available from the long term assay. There should also be consideration given animal numbers, welfare and the overall economy of the carcinogenic evaluation process. The following is a representative list of some approaches that may meet these criteria and is likely to be revised in the light of further information.

a) The initiation-promotion model in rodent. One initiation-promotion model for the detection of hepatocarcinogens (and modifiers of hepatocarcinogenicity) employs an initiator, followed by several weeks of exposure to the test substance. Another multiorgan carcinogenesis model employs up to five initiators followed by several months of exposure to the test substance.

b) Several transgenic mouse assays including the p53+/- deficient model, the Tg.AC model, the TgHras2 model, the XPA deficient model, etc.

c) The neonatal in utero rodent tumorigenicity model.

i) Transgenic (Tg) animal models

3. A number of genetically modified mouse strains have been developed with the aim of providing models to facilitate the quick and accurate detection of chemical carcinogens. The mice develop tumours much more rapidly than wild-type mice as the transgenic modifications involve genes critical to the carcinogenic process. This underpins their utility in risk assessment strategies. The International Life Sciences Institute (ILSI) and the Health and Environmental Science (HESI) co-ordinated a research and validation programme of work which evaluated the most commonly used models; the $p53^{+/-}$ hemizygous knockout mouse, the rasH2 model and the Tg:AC skin model. The COC evaluated this programme of work and other alternative models for carcinogenicity testing (the $Xpa^{-/-}$ and $Xpa^{-/-}$ p53 $^{+/-}$ transgenic mice model and the neonatal mouse model) in 2002. A statement was generated (COC/02/S3 - hyperlink).

4. The overall conclusion was: *The COC agreed an overall conclusion that none of the models used in the ILSI/HESI Alternative Cancer Test programme were*

suitable as a replacement for the mouse carcinogenicity bioassay (the primary purpose for the development of these models) and that further research should look to identify models with a greater relevance to mechanisms of carcinogenicity in humans. Of the animal models assessed there was evidence that p53$^{+/-}$ transgenic mouse model could identify some genotoxic carcinogens. There was insufficient data to suggest that the animal models under consideration (RasH2, Tg.AC, Xpa, Xpa/P53+/- and p53+/-) provide essentially similar results. Since the initial COC review, a number of studies and overviews evaluating the utility of these models have been published and these have been considered for the current guidance.

p53+/- hemizygous knockout mouse

5. p53$^{+/-}$ knockout mice are heterozygous for the tumour suppressor gene p53 - a point mutation in the remaining allele gives rise to a short latency period to tumour development. However they have a low spontaneous tumour rate at 9 months thus making them sensitive to the detection of chemically-induced tumours, particularly those caused by genotoxic chemicals (French et al 2001; Pritchard et al 2003). The standard protocol involves daily oral dosing for 26 weeks, 3 dose groups, 15 mice/sex/group and extensive macroscopic and histopathological examination of tissues at the end of the study period. The ILSI HESI project on Alternatives to Carcinogenicity Testing (ACT) examined early assay performance, spontaneous tumour incidences and results of commonly used positive controls [e.g p-cresidine] and provides a comprehensive evaluation of the assay (Storer et al 2001). Of the 16 genotoxic human and/or rodent carcinogens evaluated, 12 were positive (75%), whilst only 2/22 (9%) of non-genotoxic rodent carcinogens were positive. The non-carcinogens examined, both genotoxic (4) and not genotoxic (6), were negative. The conclusion that the p53$^{+/-}$ model is sensitive to genotoxic carcinogens but not non-genotoxic carcinogens remains following further evaluations of the assay data (Jacobsen-Kram et al 2004; Storer et al 2010).

6. The p53 knockout model also has the ability to identify hormonal, carcinogenic mechanisms (Diethylstilboestrol [DES], 17β-estradiol) and immunosuppressive carcinogens (cyclosporine A), although it is noted that the results are inconsistent (Storer et al 2001; Alden et al 2002). Some concerns have been raised within the pharmaceutical industry with regards to assay performance during a review of the use of the assay. This includes some negative results in the p53$^{+/-}$ model following a positive *in vitro* clastogenicity response (Storer et al 2010). More recently, an evaluation of 52 NTP-tested chemicals (37 positives, 15 negatives) showed concordance of p53 mouse with NTP mouse carcinogens was 57% (Eastmond et al 2013) It is noted that 11 of the NTP mouse carcinogens were not detected by any of the transgenic models.

rasH2 model

7. The rasH2 model is a hemizygous transgenic mouse which carries the human *c-Ha-ras* gene with a point mutation and its own promoter elements (Morton et al 2002). These mice develop spontaneous and chemically induced tumours more rapidly than their non-transgenic counterparts and this enhanced sensitivity to neoplasia underpins the utility of this model for carcinogenic risk assessment. The standard protocol is essentially the same as for the p53 model although the use of

25/sex/group is also reported (Nambiar and Morton 2013). A positive control response can be elicited by a single dose of N-methyl-N-nitrosourea (MNU).

8. Data from the ILSI HESI ACT trial indicate the utility of the rasH2 model for detecting both genotoxic and non-genotoxic chemicals (Usui et al 2001). The 2/3 genotoxic human carcinogens tested were positive (cyclophosphamide, phenacetin) whilst melphalan generated equivocal results. DES and 17-β- estradiol were positive and negative respectively, and the immunosuppressive cyclosporine A was equivocal. Of the 11 non-genotoxic rodent carcinogens tested, 10 were negative; clofibrate gave equivocal and positive results in two separate studies. Analyses of 37 IARC classified chemicals indicated an 81% accuracy when assessing assay performance with regards to human carcinogenicity (Pritchard et al 2003). More recent test results provide some evidence that the rasH2 assay also has the capacity to identify some non-genotoxic rodent carcinogens (namely clofibrate, DEHP and Wy-14643, ethylenethiourea, ethylacrylate, 1,4-dioxane, troglitazone), though the majority of the assays of this class of chemicals were negative (Storer et al 2010).

9. A recent report reviews data from studies evaluating 10 chemicals in the rasH2 model in pharmaceutical laboratories and compared outcomes with the conclusions from 2-year rat bioassays (Nambiar and Morton 2013). All chemicals tested were negative in genotoxicity tests. Two of the 10 chemicals were positive in the rasH2 model. Both of these chemicals were also positive in rat 2-year bioassays at the same histological sites and were also associated with proliferative findings in the target organs. Non-genotoxic MOA's were assumed for these chemicals. A review of the spontaneous tumours and histology in rasH2 mice from 11 studies indicated little variation in the background incidence and consistent qualitative and quantitative responses with MNU as the positive control (Nambiar et al 2012). These studies provide control data, which aids the interpretation of studies and supports the use and interpretation of this model as an alternative to the mouse 2-year assay. Another review of NTP chemicals tested in mice indicated an overall 82% concordance of the rasH2 assay with the mouse 2-year bioassay (16/20 positives, 7/8 negatives) (Eastmond et al 2013).

Tg. AC skin model

10. Tg.AC transgenic mice are hemizygous for mutant *v-Ha-ras* and can be considered as genetically 'initiated' due to the presence of this transgene. This model differs from the other two models as the most commonly used protocol involves topical application of the test chemical and the induction of squamous cell papillomas or carcinomas as the endpoint (Tennant et al 2001). The protocol comprises topical application of the test chemical (*to shaved skin?*) 3 times/week for 26 weeks (*it is unclear if this is a standardized protocol*). Evaluation of the assay in the ILSI HESI ACT indicated that the Tg.AC model detects both genotoxic and non-genotoxic human carcinogens but only 9/14 chemicals positive in a standard 2-year bioassay with a variety of carcinogenic modes of action were demonstrated to be active in the model. The number of false positives was low (1/14) therefore the model is not considered over-sensitive (Tennant et al 2001).

11. In a separate evaluation 27/35 (77%) chemicals were accurately predicted for carcinogenesis (23 carcinogens, 12 non carcinogens) (Pritchard et al 2003). A recent review indicates a 82% concordance between the Tg.AC assay and NTP

mouse carcinogens (12/23 positives, 8/10 negatives) (Eastmond et al 2013). It is considered that the Tg:AC model is able to predict both genotoxic and non-genotoxic carcinogenesis when they are applied dermally. However, there are some concerns with regards to tumourigenesis caused by inflammatory or irritant properties of chemicals (Lynch et al 2007). Therefore the Tg.AC mice model may be unreliable for general use if interpretation of a positive result is complicated by confounding inflammation.

XPA-/- and XPA-/-p53+/- models

12. *There are no new substantive data available since the last COC review. Should this model continue to be considered?*

Evaluation of the transgenic animal models

13. A comprehensive overview and evaluation of all three assays ($p53^{+/-}$, Tg.AC and rasH2) used various combinations of the models, with and without consideration of rat 2-year bioassay data, to predict the carcinogenicity of 99 chemicals. It was concluded that correct identification of human carcinogens and non-carcinogens was 74-81% (whilst the similar evaluation of 2-year bioassay data was 69%). However some IARC 1 and 2A carcinogens were not identified and there were also a few false positives (Pritchard et al 2003). A more recent evaluation of the three principle models suggests that used alone, these assays would miss some probable human carcinogens (phenacetin, 17β-estradiol) (Storer et al 2010). Furthermore, several issues of concern have been highlighted: methodological uncertainties, such as the effect of sample size on assay sensitivity and variability in spontaneous tumour frequencies and reproducibility issues have been raised, together with questions on how the dose-response data can be used for human risk assessment (Eastmond et al 2013).

14. A survey devised by the Carcinogenicity Alternative Mouse Models (CAMM) working group (Long et al. 2010) elicited 21 responses (90% of responses were from pharmaceutical organisations and 75% had used CAMM to support product development). The most commonly used model was the $p53^{+/-}$ mouse model with fewer laboratories using the rasH2 mouse model. There was only one example where the regulatory agency had rejected the submitted data. Feedback from agencies on study design was most often concerned with dose selection. The most common positive control used was p-cresidine for the $p53^{+/-}$ model and urethane and MNU for the rasH2 model. However, it was considered by some respondents (5/15) that a positive control was not required if the model was well characterized within their laboratory. The tissues/organs which require pathological examination in the positive control animals is still under debate (i.e. all or only target organs). The importance of dose level selection was also discussed.

15. From the survey the following recommendations were proposed by CAMM. [*what does the committee think of these proposals?]*

- Positive control groups need not be included in every study but a study using them should be conducted periodically (every 2-3 years) to ascertain model consistency

- Positive control compounds should consistently show positive responses at a dose which shows the sensitivity of the model but not at a dose which increases mortality
- The numbers of animals used should be large enough to demonstrate the positivity of the positive control or 10-15 animals
- Positive control groups are included to monitor for possibility of genetic drift in the transgenic strains of mice.
- Recommendations for the extent of histopathological examination – all tissues from all animals with peer review – however there may be some caveats such that tissues only from control and high dose are studied in the first instance (as for current carcinogenicity study)
- Recommendations for diagnostic criteria – standardized nomenclature for mouse neoplasms WHO/IARC. Whilst the majority of neoplasms in CAMM are similar to those in normal mice, it is noted that there is an on-going evaluation and classification of tumours specific to CAMM.
- Recommendations for historical control data – these data will be used in qualitative manner to add to the weight of evidence and identification of chemical induced effects. The occurrence of incidence of proliferative lesions. Laboratories encouraged to share data to more rapidly evaluate the animal model and aid in interpretation of chemical induced effects.

Committee's evaluation of transgenic models

16. The Committee conclude that the p53 model……. The rasH2 model…… the Tg.AC model……

17. The Committee's overall conclusion is that the use of hemizygous p53$^{+/-}$, Tg.AC and rasH2 mouse models to replace the conventional mouse long-term bioassay is supported, that the assays have been shown to perform adequately and are not overly sensitive. However, currently they are only supported when undertaken in addition to a rat 2-year bioassay (ICH S1B). It is noted that in a typical carcinogenic risk assessment strategy, chemicals with genotoxic properties will have been identified using the standard genotoxicity testing battery. Therefore the p53$^{+/-}$ assay is considered less useful than the rasH2 model as it is not able to predict chemicals with the potential to be carcinogenic in the absence of DNA reactivity. Accordingly there is increasing use of the rasH2 mouse model in strategies supporting the development and risk assessment of human pharmaceuticals in accordance with ICH S1B.

18. The Committee note that transgenic assays can also provide insight into carcinogenic mode of action. For example they may be useful for investigating chemicals where a high dose causes organ specific cytotoxic responses leading to cell proliferation or where the carcinogenic MOA is attributable to pharmacodynamics action. Attention is drawn to the need for rigorous optimization of protocols and validation of study designs, and it is recommended that attempts are made to improve the understanding of false positives and negatives.

ii) *In utero*/neonatal exposure models of carcinogenesis

19. The Committee evaluated the rat neonatal model of carcinogenesis in 1998 as part of the ICH initiative and the conclusions are provided in a statement (COC/99/C1–hyperlink). It was noted that there was very limited validation data and concluded that the available information showed tumour yields with genotoxic carcinogens were highly dependent on the strain of animal, age at start of treatment, and treatment protocol. There were no validation data regarding the use of short-term neonatal rodent bioassays for the identification of non-genotoxic carcinogens. Overall, the Committee concluded that there was no current evidence to support the use of the neonatal mouse or rat bioassays as part of the regulatory testing strategy for human medicines.

20. More recently Huff et al. (2008) discussed the advantages of intrauterine exposure, including the changes which may occur pre-natally, sensitizing cells to growth promotion in later life. It has been proposed that exposures during foetal development may represent an important factor in increasing childhood and adult cancer incidence (Perera 2011).

21. There are a number of published studies which examine the mechanism of arsenic-induced carcinogenesis using prenatal exposure dosing regimens. Mice exposed to arsenic in drinking water trans-placentally and then throughout neonatal and adult life developed tumours in lung, liver, gallbladder, ovaries and uterus, a different pattern of tumours from that seen when not exposed in utero (Tokar et al 2011). Waalkes et al. (2006a; 2006b) conducted studies where arsenic was given transplacentally with or without diethylstilbestrol and demonstrated the hypersensitizing effects of arsenic given pre-natally, including the exacerbation of tumours in the urogenital tract and liver induced by diethystiboestrol. They examined the MOA's which may impact on arsenic-induced murine carcinogenesis following exposure at different life-stages (Waalkes et al., 2007). Ahlborn et al. (2009) exposed mice to arsenic during gestation only or up to a year after birth. There was an increased incidence and severity of urogenital proliferative lesions but decreases in liver and adrenal tumours compared to exposure to adults only.

Committee's evaluation of the in utero/neonatal model

22. The Committee considers that whilst ICH SB1 supports the use of the neonatal mouse model, there are limited data available, the majority of which are investigations of endocrine disruption or arsenic MOA's*. The studies were designed on a case-by–case basis and as such there is no single protocol. Therefore the Committee concludes that the model is not suitable as a general replacement for a 2-year bioassay.*

iii) Initiation Promotion models

23. In the Solt Farber model, rats are treated with a single dose of diethyl nitrosamine (DEN) as an initiator, followed by partial hepatectomy and repeated treatment with the test compound for several weeks to stimulate the formation of glutathione-S-transferase positive (GST+) foci which are considered to be pre-neoplastic lesions. The method was originally published in 1976 (Solt, 1976) and was developed and refined to become what is known as the Ito Liver model (Ito et

al., 1996; Ito et al., 2003). This is a medium term treatment strategy and is based on the recognition that a large number of known carcinogens (genotoxic and non-genotoxic; >50% is quoted) are hepatocarcinogens in rodent bioassays and it is believed that the mode of action of many is mitogenic by stimulating hepatocyte proliferation. A multi-organ model based on the same principles was subsequently developed

24. A recent evaluation of this model examined an 8 week protocol in which 6 week old rats are given a single intraperitoneal (ip) dose of DEN (200mg/kg) or saline, followed 2 weeks later by administration of the test compound for 6 weeks and partial hepatectomy to stimulate liver growth during week 3. At week 8, livers are assessed for GST-P+ foci. An increase is considered to be indicative of hepatocarcinogenic potential of the test chemicals (Tsuda et al. 2010). Of the 159 compounds, 61 of 66 rodent liver carcinogens were identified as positive, 10 of 43 which were carcinogens but not in the liver (non-hepatocarcinogens) and 1 of 50 non-carcinogens were positive in this assay.

25. The multi organ model was developed with the goal of identifying the carcinogens not detected by the Ito liver model. The principle of the assays are the same ; rats are given an ip injection of DEN (100mg/kg) and methyl nitrosouerea (MNU; 20mg/kg), a s.c. injection of 1,2-dimethylhydrazine (40mg/kg) and 0.05% N-butyl-N-(4-hydroxy-di-n-propylnitrosamine) and 0.1% 2,2'-dihydroxy-di-n-propylnitrosamine in the drinking water for 4 weeks, as initiators at various sites, followed by the test chemical for a further 24 weeks. At the end of the dosing period organs (liver, lung, kidney, bladder, upper digestive tract and intestines) are examined for the presence of pre-neoplastic and neoplastic foci (as described in (Fukushima et al., 1991; Ito et al., 1996). Forty four chemicals were examined in the published study - 12 of the 12 rodent liver carcinogens, 10 of the 11 non-hepatocarcinogens and 0 of the 1 non-carcinogens were positive in this assay.

26. Although these models of carcinogenesis were developed some time ago, the Committee note that there are few other studies using this methodology published in the literature other than those published by the originators of the protocol and a methodical, systematic review of its accuracy in predicting carcinogenic potential has not been undertaken.

Committees evaluation of the initiation promotion models

27.

G07 = Alternatives to the 2-year bioassay

b) Cell transformation assays (CTA's)

The COC's sister committee, the COM, recently undertook a detailed review of the available cell transformation assays. The assays considered were: SHE ph6.7 or pH7.0; BALB/c 3T3; C3H10T1/2; Bhas 43. A statement was produced in which it was concluded that to date, the CTA's are not suitable for use in a regulatory testing strategy for carcinogenicity. However, they may have value in predicting rodent carcinogenicity if used in the scenario where *in vitro* positive results were obtained for a cosmetic ingredient and no *in vivo* testing is allowed. It is noted that the OECD is pursuing the improvement and validation of the cell transformation assays and the Committees (COM and COC) are actively involved in monitoring and contributing to their development.

The COM statement is available here:

http://iacom.org.uk/statements/documents/COM12S4-CellTransformationAssayStatementfinalforinternet.pdf

G07 = Alternatives to the 2-year bioassay

c) Developing methodologies and strategies (for example toxicogenomics)

To be added at a later date

G07 = Alternatives to the 2-year bioassay

d) Alternative testing paradigms (for example evaluation using histopathology and proliferative markers in sub-chronic rodent studies)

To be added at a later date

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