Committee on CARCINOGENICITY

Committee on Carcinogenicity of Chemicals in Food, Consumer Products and the Environment (COC)

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Hazard Identification and Characterisation: Conduct and Interpretation of Animal Carcinogenicity Studies

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

Hazard Identification and Characterisation: Conduct and Interpretation of Animal Carcinogenicity Studies

Preface

1. This guidance statement provides advice on hazard identification and characterisation of chemical carcinogens. It is part of a series of guidance statements by the Committee on Carcinogenicity of Chemicals in Food, Consumer Products and the Environment. It should be read in conjunction with the other guidance statements, in particular, G01 on the overall strategy of risk assessment of chemical carcinogenicity, G05 on defining a point of departure and potency estimates in carcinogenic dose response, and G06 on cancer risk characterisation methods.

2. In our overarching document, 'A Strategy for the Risk Assessment of Chemical Carcinogens' (G01), the Committee reaffirms its view, that the most appropriate information to use for positive identification of carcinogenic hazard is clear evidence from well conducted epidemiology studies. However, these may not have sufficient power to identify the *absence* of carcinogenic hazard. Where this is the case, identification of a carcinogenic hazard is based upon a review of the animal carcinogenicity data on a chemical and any knowledge of effects on human health. This will include determining whether a chemical is genotoxic or likely to be carcinogenic through a non-genotoxic mechanism, which is discussed further within this statement.

3. The objective of an animal carcinogenicity study is to treat groups of animals (a control group and at least 3 groups receiving increasing amounts of the compound under test) by an appropriate route of exposure for a major portion of their life span and to observe the animals for the development of neoplastic lesions during or after exposure.

4. Animal carcinogenicity studies were initially intended only for the identification of carcinogenic hazard of a chemical but the purpose has now expanded beyond hazard identification to providing also quantitative data for risk characterisation. This can lead to compromise in the design of the studies, for example, the use of more

groups containing fewer animals may enhance the data available for risk characterisation but reduce the likelihood of identifying a carcinogenic hazard. OECD Test Guidelines 451 and 453 state that a sufficient number of animals should be used so that a biological and statistical evaluation is possible and recommend that this should be at least 50 animals of each sex in each dose group (OECD, 2009).

5. All carcinogenicity studies should abide by the principles of humane euthanasia and test animals should be observed carefully and frequently, and any animals exhibiting clear signs of severe pain or distress should be humanely killed. Carcinogenicity studies are usually carried out in rats and mice and, for similar animal welfare reasons, ideally these should be caged in small groups of the same sex, and not individually. Also, the testing of substances at potentially irritating or corrosive concentrations/doses should be avoided.

Conduct of Carcinogenicity Studies

6. As stated by the International Programme on Chemical Safety, the design, conduct and completeness of reporting of experimental findings in toxicological studies on mammalian species are of critical importance in determining the validity and relevance of results. Toxicological results from adequate animal systems signal anticipated effects in humans. Thus, negative results cannot be assessed from an inadequate study, and full evaluation of a positive effect is confounded by incomplete reporting from poorly designed or poorly conducted studies. However, positive findings cannot be ignored. Studies should be of good scientific quality and follow standard guidelines and recognized good laboratory practices (GLPs) wherever possible (IPCS, 1999).

7. A number of test guidelines are available for carcinogenicity studies, for example, OECD guidelines: Test 451 ‘Carcinogenicity Studies’ and Test 453 ‘Combined Chronic Toxicity and Carcinogenicity Studies’ (OECD, 2009). Also, the OECD has published a Guidance Document on the Conduct and Design of Chronic Toxicity and Carcinogenicity studies to support these test guidelines which discusses the following topics: mode of toxicological action, study design, and statistical and dose response analysis (OECD, 2012). This document is recommended as a source of detailed information on the conduct of carcinogenicity studies and no further advice will be given here.

8. Guidance has also been issued by the International Conference on Harmonisation of Testing for Pharmaceuticals (ICH) on the carcinogenicity testing of human pharmaceuticals (ICH, 1995, 1997, 2008 and 2012), by the Committee for Proprietary Medicinal Products (CPMP, 2002) and by the US Environmental Protection Agency (EPA) on carcinogen risk assessment (US, 2005). For guidance on dose selection, two publications by the International Life Sciences Institute (ILSI) are recommended. These are ‘Principles of the Selection of Doses in Chronic Rodent Bioassays’ (ILSI, 1997) and ‘Issues in the Design and Interpretation of Chronic Toxicity and Carcinogenicity Studies in Rodents: Approaches to Dose

Selection' (Rhomberg et al., 2007). These reports provide theoretical and practical guidance on factors that influence dose selection in carcinogenicity studies.

Statistical Analysis of Results

9. The critical endpoint of a carcinogenicity study is the type and number of neoplasms occurring at each dose level, but information such as time to tumour detection is also important. The main aim of the statistical analysis is to determine whether exposure to the test compound is associated with an increase in development of neoplasms. The statistical methods most appropriate for the analysis of the data collected should be established at the time of designing the study and staff with appropriate statistical expertise should be involved in both the design of the study and analysis of results. Advice on available methods can be found in OECD Test Guidelines 451, 452 and 453 (2009), in OECD Guidance Document (2012) and in the EPA guidance (2005). The standard for determining statistical significance of neoplastic incidence is a comparison of neoplasms in dosed animals with those in concurrent control animals. However, further interpretation is needed to determine whether any increase in dosed animals is biologically significant. Additional insights about both statistical and biological significance can come from an examination of historical control data. These can be useful if there are indications that the concurrent control data are appreciably 'out of line' with those from recent previous studies. Further information is available in OECD (2012).

Hazard Characterisation

10. Hazard characterisation involves a qualitative description of the nature of the hazard and a quantitative description of the change in effect caused by differing doses of a chemical substance after a certain exposure time i.e. the dose-response relationship. The purpose of analysing the dose-response relationship is to investigate the magnitude of response (in terms of severity or incidence) within the dose range used in the study. This helps to estimate, ultimately, the risk from exposure to the concentrations of the chemical in the environment, food etc. These concentrations are usually much lower than those used in animal studies. The relationship between dose and response may also be used to aid hazard characterisation by allowing a comparison of carcinogenic potency. However, other important factors that can affect this relationship and should be considered further are: the absorption, distribution, metabolism and excretion (ADME) of the chemical, its mode of action (MOA), and the variability in susceptibility between species and among humans. In particular, how the dose-response relationship is used in the final assessment of risk will depend on whether or not the carcinogenic response occurs as the result of genotoxic activity. Although dose-response relationships may be evident in animal studies, the relevance and applicability to the human dose-response should be assessed on a case-by-case basis, because of the uncertainties introduced when extrapolating between species. A further uncertainty is the extrapolation of results seen at the high doses used in animal studies to produce an estimate of risk at levels of human exposure.

Genotoxic vs. non-genotoxic carcinogens

11. Animal carcinogenicity studies have been used for more than 50 years to determine whether chemicals might cause cancer in humans. Inherent in this testing is the assumption that the observation of neoplasms in animals is directly relevant to the risk of cancer in humans. When assessing the risks from a chemical carcinogen, it is important to consider the mechanism(s) by which the chemical causes cancer, in particular, whether a genotoxic MOA is involved i.e. whether DNA-reactivity is a key step in the carcinogenic process.

12. Genotoxic potential should be assessed according to the guidance issued by the Committee on Mutagenicity of Chemicals in Food, Consumer Products and the Environment (COM, 2011). In this guidance, the COM proposes a strategy for evaluating the available data on the genotoxicity of a substance, and recommends appropriate tests to conduct in the absence of sufficient data, as well as suitable *in vitro* and *in vivo* follow-up tests where it is necessary to further characterise the genotoxic hazard.

13. Non-genotoxic carcinogens are those chemicals for which there is sufficient evidence of carcinogenicity from epidemiological or animal studies, and good evidence of an absence of genotoxic activity (on the basis of the COM Guidance on the assessment of genotoxic hazard). Some information about MOA is necessary for an adequate consideration of such carcinogens (see below).

14. Moreover, the data from a carcinogenicity study may help to indicate whether or not a chemical is a genotoxic or non-genotoxic carcinogen. Genotoxic carcinogens tend to produce cancer at a number of sites, whereas non-genotoxic agents are more specific in their action, according to their more specific MOA.

15. In the case of genotoxic carcinogens, it is generally assumed that they have the potential to be carcinogenic in humans. Thus, it is assumed that any exposure to an *in vivo* genotoxicant is associated with some damage to DNA and, consequently, an increased risk of mutation leading to an increased risk of adverse health effects, including cancer. In a number of instances, a biologically meaningful threshold for genotoxicity has been established. However, such chemicals are still considered as potentially carcinogenic in humans, albeit the subsequent risk characterisation may demonstrate that the levels to which humans are exposed are below this threshold and therefore unlikely to pose a risk to health (COM, 2010). However, for non-genotoxic carcinogens, it is not always the case that they have the potential to be carcinogenic in humans: examples of tumours seen in rodents but not relevant to man include those specific to the male rat kidney following α2μ-globulin accumulation in tubular cells, and thyroid follicular cell carcinomas in rodents after exposure to substances capable of causing thyroid gland enlargement (goitrogens) (ECETOC, 1996).

16. In 2001, the International Programme on Chemical Safety (IPCS) proposed a structured approach for the assessment of the overall weight of evidence for a

postulated MOA (Sonich-Mullin et al., 2001) and, subsequently, the Risk Sciences Institute of the International Life Sciences Institute (ILSI/RSI) proposed a human relevance framework (HRF) which extends the IPCS MOA approach by incorporating a systematic evaluation and comparison of animal and relevant human data (Cohen et al., 2003, 2004; Meek et al., 2003). More recently, IPCS has developed a HRF based on the IPCS MOA framework and the ILSI/RSI HRF (Boobis et al., 2006) and this was recently updated in a review (Meek et al., 2014). The utility of this framework was demonstrated when it was used to show that there is clear evidence of a MOA involving cytotoxicity and cell proliferation for formaldehyde-induced nasal tumours in rats and mice and that this MOA is considered relevant to humans, despite limitations in the human data (McGregor et al., 2006). These frameworks are of value in assessing carcinogenic risk. The HRF provides a systematic approach for the evaluation of whether the key events in the MOA of carcinogenic responses in experimental animals would be plausible in humans. Recently, the OECD has developed guidance on adverse outcome pathways (AOPs), which share many characteristics of and build on the concepts of the MOA framework and these areas are being followed by the COC (see CC/2016/08).

Hazard characterisation of genotoxic carcinogens

17. For carcinogens with genotoxic activity, in the absence of mechanistic data to suggest a threshold for genotoxicity, or carcinogens where no threshold for effect has been or can be identified, it is prudent to assume that no threshold for carcinogenicity exists. Therefore, ideally, exposure should be zero or as low as reasonably practicable (ALARP). If this is not possible (for example, in the case of a food or environmental contaminant), the Committee advocates using the study data to derive a benchmark dose, the dose associated with a pre-specified change in response. The derivation of this parameter is described in Guidance Statement G05. The benchmark dose can then be used by risk assessors to derive a Margin of Exposure (see G06) which indicates the level of concern associated with likely exposures and can be used to advise on the appropriate risk management action.

Hazard characterisation of non-genotoxic carcinogens

18. For most non-genotoxic carcinogens, it is accepted that there is a threshold dose below which no effect occurs. Non-genotoxic carcinogens produce cancer by perturbing normal physiology or biochemistry. For example, they may have a hormonal effect or cause damage to tissues which stimulates proliferative changes and this may result in a spontaneous mutation which leads to hyperplastic and neoplastic changes. Therefore, neoplasms occur as a secondary effect arising from the initial toxic effect, for which a 'threshold' dose may be identified (Ashby *et al.*, 1996). It follows that these substances are unlikely to pose a carcinogenic risk at dose levels at and below the given threshold that does not produce the primary toxic effect (Williams, 2001). Human relevance frameworks (see paragraph 16) may enhance the clarity and transparency of the risk assessment.

19. Where there is adequate evidence for a plausible, non-genotoxic MOA which supports a threshold for carcinogenicity, an exposure level can be derived at or below which there is estimated to be no risk of carcinogenicity in humans. Where the carcinogenicity data are obtained from animal studies, the MOA should be relevant to humans. The derived exposure level should be based on a point of departure for carcinogenicity or, more likely, on a precursor event linked to tumour induction. The point of departure is divided by an appropriate uncertainty factor to take account of potential interspecies and intraspecies (interindividual) differences in susceptibility (see Guidance Statement G06).

Summary

20. The design, conduct and completeness of reporting of experimental findings in toxicological studies on mammalian species are of critical importance in determining the validity and relevance of results. Toxicological results from adequate animal systems signal anticipated effects in human. Readers are referred to the OECD Test Guidelines 451, 452 and 453 (OECD, 2009) and the accompanying Guidance Document (OECD, 2012) as a source of information on the conduct and statistical analysis of carcinogenicity studies for non-pharmaceuticals and to the ICH guidance for the carcinogenicity testing of human pharmaceuticals (ICH, 1995, 1997, 2008 and 2012).

21. Hazard characterisation involves a qualitative description of the nature of the hazard and a quantitative description of the change in effect caused by differing doses of a chemical substance after a certain exposure time i.e. the dose-response relationship. A critical factor to consider is whether the chemical tested is genotoxic or non-genotoxic. If genotoxic, the Committee advocates using the study data to derive a benchmark dose, the dose associated with a pre-specified change in response. If non-genotoxic, it should be established whether the mode of action is relevant to humans. If so, the Committee recommends deriving an exposure level at or below which there is estimated to be no risk of carcinogenicity in humans, which should be based on a point of departure for carcinogenicity or, more likely, for a precursor event linked to tumour induction. The point of departure is divided by an appropriate uncertainty factor to take account of potential interspecies and intraspecies (interindividual) differences in susceptibility to give an acceptable or tolerable exposure level for humans, see G06 for more information.

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