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

**COC Annual Report for 2008**

Members are invited to comment on the attached draft text for the COC 2008 Annual Report.

Secretariat  
February 2009

## **Preface**

The Committee on Carcinogenicity of Chemicals in Food, Consumer Products and the Environment (COC) evaluates chemicals for their carcinogenic potential in humans at the request of UK Government Departments and Agencies. The membership of the Committee, agendas and minutes of meetings, and statements are all published on the internet (<http://www.iacoc.org.uk/>).

During 2008 the Committee has considered a number of interesting items. We began our consideration of the complex problem of the assessment of mixtures of chemicals for carcinogenicity. This proved a difficult task due to the inevitable lack of data on carcinogenicity testing of mixtures and the need to attempt to draw conclusions from short-term studies and epidemiological findings. The Committee intends to produce a statement on this topic in 2009.

The committee was also asked to advise the UK National Coordinator for the Organisation for Economic Cooperation and Development (OECD) Test Guidelines on the planned revision of the guidelines for chronic toxicity and carcinogenicity studies and the associated Guidance Document. The Committee has agreed to draft a chapter on the investigations undertaken in studies of this type, which will include advice on histopathology.

I would like to thank the members and secretariat of the Committee for the work they have undertaken during the past year. We look forward to new challenges in 2009.

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## **Carcinogen-DNA adducts as a biomarker for cancer risk**

Researchers seeking to understand the mechanism by which a chemical causes cancer in a particular (target) tissue may measure the levels of adducts derived from the chemical which are bound to DNA. At the April meeting, the Committee considered a methodological paper by Rundle (2008; *Mutation Research*, Volume 600: pp 23-36) on the use of adducts as a biomarker for cancer risk. The paper suggests that epidemiological studies which seek to establish an association between carcinogen-DNA adduct levels and the risk of cancer often fail to incorporate fundamental epidemiological principles into their methods. The author described a number of studies which have investigated associations between DNA-carcinogen adduct levels and cancer, and he addressed a number of methodological issues common to these studies, such as the use of target tissue versus surrogate tissue and how this choice impacts on the selection of controls, the use of inappropriate statistical analyses, and small sample sizes. A number of suggestions were made to improve study designs to overcome these issues in the future.

The Committee considered that researchers are aware of the limitations of using surrogate tissues to measure DNA adducts. It also pointed out that, even if adduct levels were measured in samples of target tissue rather than in surrogate tissue, they may also lack relevance to the underlying pathological condition. This is because only certain cell types in the tissue will be targets, and only a limited number of adducts are causal, with the majority occurring at non-critical sites or in non-critical genes. The Committee considered that it was an over-simplification to argue that target tissue samples will overcome a major limitation of adduct determination and to dismiss the value of adducts in surrogate tissues. Researchers were aware of the limitations of using surrogate tissues.

The Committee noted that adducts measured at the time of diagnosis may not reflect exposure at the critical period and may be affected by the pathology of the condition suffered by the patient. It recommended that lymphocyte fractions of blood samples could be stored in biobanks and used for biomarker analysis.

## **Age as an independent risk factor for chemically-induced acute myelogenous leukaemia in children**

In 2006 the COC discussed the question of whether there are age-related differences in susceptibility to carcinogenesis. At the November 2008 meeting, the Committee considered a recent review by Pyatt et al (2007; *J Toxicol Env Health B*, Volume 10(5): pp 379-400) which had tested the assumption that children are inherently up to 10-fold more sensitive than adults to genotoxic carcinogens. It had done this using data on the development of secondary or therapy-related acute myelogenous leukemia (t-AML) in children who had received treatment with high dose chemotherapy and/or radiation. This disease is well established as a potential long-term consequence of exposure to such treatment. The review had investigated the effect of age at treatment on a child's susceptibility to developing t-AML.

Members noted that there was little information, which had led the authors of the review to draw cautious conclusions. The Committee concluded the data presented did not give cause to think that children are more susceptible than adults, although the evidence was not strong enough to rule out such an effect.

Members also noted that the dose of chemotherapy administered to children is often scaled by body surface area, using an algorithm incorporating height and weight, whereas, in a risk assessment of chemicals which cause leukaemia, exposure would be scaled

relative to metabolic rate (oxygen demand) on the basis of an exponent of body weight. It was acknowledged that, where susceptibility of the subpopulation is the result of increased exposure, this would normally be incorporated into the risk assessment by separate assessment of the exposure of the subpopulation, with emphasis on children's specific exposure assessment.

### **Mode of Action/Human Relevance Framework**

The International Programme on Chemical Safety (IPCS) Mode of Action (MOA) Framework is a conceptual framework for considering data on the mode of action of chemical carcinogens. The COC considered aspects of the MOA Framework in 1999 and, in 2004, considered a related topic, the Human Relevance Framework (HRF), which had been developed by a working group sponsored by the US Environmental Protection Agency and the International Life Science Institute (ILSI) Risk Science Institute (RSI). The HRF systematically considers the weight of evidence of hypothesized modes of action in animals and their potential human relevance for cancer.

In 2008, the Committee discussed recent developments made by the IPCS in the continuing evolution of HRFs. The IPCS HRF entails answering a series of three questions followed by a statement of confidence, analysis and implications. The COC considered 3 case studies which had used the IPCS HRF as an approach to determine the sufficiency of evidence and the relevance of an animal MOA for humans. These case studies entailed 3 different MOAs: 1) sustained cytotoxicity and regenerative proliferation leading to nasal tumours following exposure to formaldehyde, 2) direct alkylation of DNA leading to tumours in multiple sites following exposure to 4-aminobiphenyl, 3) increased hepatic clearance of thyroxin leading to thyroid tumours following exposure to thiazopyr.

The COC considered that the IPCS HRF was a valuable evolution of the work on this concept and proposed that the IPCS HRF approach should be used on a case-by-case basis in its future evaluations of chemicals.

The Committee also reviewed a paper by Sielken et al (2005; Scand J Work Environ Health, Volume 31, Suppl 1: pp151-5). This paper described a dose-response modelling approach to provide statistical insight into the relative likelihood of different mechanisms of action in cancer dose-response studies. The paper provided two examples based on time-to-tumour data for mammary fibroadenoma and adenocarcinoma in female Sprague-Dawley rats exposed to a pesticide in the diet. The examples considered how 34 different dose metrics (i.e. a measure of exposure to the pesticide or a measure of the biological activity potentially generated by the exposure if a specific mechanism of action applies) related to the incidence of fibroadenoma and adenocarcinoma and demonstrated how maximum likelihood statistical methodology could be used to provide an indication of the mechanism of action of the pesticide.

The Committee considered that it was unclear how the dose metrics and the different variables were identified and chosen for inclusion as no references were cited in the paper. It questioned the statistical robustness of the approach and considered that, although the most likely mechanism of action for the unidentified pesticide in the above examples was found to be hormonal, no other data were provided to show that it acted through a hormonal mechanism and therefore the assumption made in the paper was unwarranted. Moreover, no data were provided on the other dose metrics used. The Committee concluded that there may well be potential value in the approach suggested, but that more work was required. Before applying this approach to a specific example, it would be necessary to have alternative endpoints linked to a MOA.

The Committee also heard a short presentation by a PhD student at Imperial College London on the weight of evidence in framework approaches to cancer hazard identification.

**Preliminary report by the EU Scientific Committees on Consumer Products, on Health and Environmental Risks, and on Emerging and Newly-Identified Health Risks on “Risk assessment methodologies and approaches for mutagenic and carcinogenic substances”**

The Committee was invited to comment on a preliminary report by the EU Scientific Committee on Consumer Products (SCCP), the Scientific Committee on Health and Environmental Risks (SCHER) and the Scientific Committee on Emerging and Newly-Identified Health Risks (SCENIHR).

The Committee considered the report to be well considered and up-to-date. However, it expressed concern about the discussion of the T25 method, which has been proposed for use in risk assessment. Several organisations no longer support this methodology due to its reliance on the lowest tested dose and lack of consideration of dose response which makes the methodology inherently more variable than the Benchmark Dose Modelling (BMD) approach. Also, the T25 method does not incorporate uncertainty in the analysis of the data. Committee members were concerned that the report suggests that the T25 and BMDL<sub>10</sub> are equivalent. Most organisations considered that the BMDL<sub>10</sub> was considerably superior to the T25 and that, where it was not possible to determine a BMDL<sub>10</sub>, it would not be possible to derive an informative T25. The Committee also criticised the fact that the *post hoc* justification of the uncertainty factor of 10,000 commonly used in the Margin of Exposure (MoE) approach. This justification had never been adopted by the European Food Safety Authority (EFSA) and it was not clear that there was any reference to this specific derivation.

The Committee considered that the report should refer to the International Programme on Chemical Safety (IPCS) mode of action (MoA) framework since it is critical to understand whether there is likely to be a genotoxic MoA underlying the carcinogenicity of a chemical. It also noted that the text does not reflect the more refined framework for application of the Threshold of Toxicological Concern (TTC) by Kroes et al (2004; Food and Chemical Toxicology, Volume 42: pp 65–83). This methodology should not be used indiscriminately and consideration should be given to whether the chemicals under consideration are adequately represented by the database used to develop the TTC approach.

**Pyrrrolizidine alkaloids in food**

The COC was asked by the COT for advice on the carcinogenicity of pyrrolizidine alkaloids (see paragraphs ....).refer to appropriate COT section. The COC considered data on the mutagenicity and carcinogenicity of seven pyrrolizidine alkaloids (riddelliine, lasiocarpine, clivorine, petastitenine, senkirkine, symphytine and monocrotaline) and on the chemicals dehydroheliotridine and dehydroretronecine, which are the metabolites of many pyrrolizidine alkaloids. The Committee assessed whether the evidence was sufficient to conclude that each of the pyrrolizidine alkaloids had carcinogenic activity. As a general comment, the Committee noted that some of the data was rather old.

The most data were available for riddelline and lasiocarpine. Riddelliine is positive in a range of *in vitro* and *in vivo* assays for genotoxicity. In a carcinogenicity study conducted by the US National Toxicology Programme (NTP), it induced an increased incidence of liver haemangiosarcomas in both rats and mice, and of alveolar and bronchiolar neoplasms in female mice. The COC concluded that there was good evidence that riddelliine was genotoxic and carcinogenic and that it would be prudent to assume that at

least part of its carcinogenic effect was due to a genotoxic mechanism. Lasiocarpine is positive in *in vitro* assays for genotoxicity but has not been tested in *in vivo* studies. In an NTP carcinogenicity study in rats, it induced an increased incidence of liver angiosarcoma in both sexes. In more limited studies in rats by either dietary or parenteral administration, treatment-related angiosarcomas of the liver and liver cell carcinomas were seen. The Committee decided that the database was less extensive than that for riddelliine but, due to the similarities in tumour profiles, concluded that it was also carcinogenic and likely to have a genotoxic mechanism

Clivorine shows conflicting results in *in vitro* assays for genotoxicity and has not been tested in *in vivo* assays. In a limited rat study in which clivorine was administered in drinking water, it induced an increased incidence of haemangioendothelial sarcoma of the liver. The COC considered that there were not enough *in vivo* data to reach a definite conclusion but clivorine was likely to have carcinogenic properties, based on its structure and limited evidence that it induced the same tumour type as riddelliine and lasiocarpine. Petasitenine is positive in *in vitro* assays for genotoxicity but has not been tested in *in vivo* studies. In a limited rat study in which petasitenine was administered in drinking water, there was a treatment-related increased incidence of liver haemangioendothelial sarcomas and liver cell adenomas. The Committee concluded that petasitenine would be likely to have carcinogenic properties, based on the structure and tumour type induced in the rat study.

Senkirkine has shown largely positive results in *in vitro* assays for genotoxicity but has not been tested in standard *in vivo* studies. In a limited study in male rats using parenteral administration, there was a treatment-related increased incidence of liver cell adenomas. The Committee concluded that there was insufficient evidence to conclude that senkirkine had carcinogenic activity. No genotoxicity data have been found on symphytine. In a limited study in male rats using parenteral administration, there was a treatment-related increase in liver haemangioendothelial sarcoma and a small treatment-related increase in liver cell adenomas. The COC concluded that it was probable that symphytine had carcinogenic activity based on the structure and the limited evidence that it induced angiosarcomas.

Monocrotaline has shown conflicting results in *in vitro* assays for genotoxicity and a positive result in one *in vivo* assay. In a limited study in male rats using parenteral administration, there were treatment-related increases in a number of tumours, principally liver cell carcinomas and pulmonary adenocarcinoma. From the available evidence and commonality of structure, the Committee concluded that the data were sufficient to conclude that monocrotaline had carcinogenic activity but with a different tumour profile to the other pyrrolizidine alkaloids. The mode of action was unclear. The metabolite dehydroretronecine has shown positive results in *in vitro* assays for mutagenicity but no genotoxicity data were found for dehydroheliotridine. The Committee found no convincing evidence of carcinogenicity for these metabolites.

The Committee decided that benchmark dose modelling on riddelliine and lasiocarpine, which had been carried out by the Secretariat, could be used as a basis for a Margin of Exposure approach to the risk assessment of pyrrolizidine alkaloids. A BMDL<sub>10</sub><sup>1</sup> of 0.073 mg/kg bw/day for lasiocarpine, based on the angiosarcoma incidence in the NTP study, should be used for any Margin of Exposure approach to the risk assessment. The Committee further agreed that a “Cumulative Assessment Group” approach, as described in the opinion of an EFSA Scientific Panel on methodologies for the assessment of cumulative and synergistic risks from pesticides, would be appropriate for pyrrolizidine

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<sup>1</sup> The lower confidence limit on the benchmark dose associated with a 10% response.

alkaloids in view of the evidence for a common tumour pattern for several of these compounds.

### **Revision of OECD Test Guidelines for carcinogenicity studies**

The Organisation for Economic Cooperation and Development (OECD) is currently revising its Test Guidelines for carcinogenicity studies and chronic toxicity studies. The purpose of these guidelines is to enable mutual acceptance of data by different regulatory authorities around the world and hence to reduce costs and use of animals. The OECD is also preparing a Guidance Document to accompany the revised guidelines. The first chapter, on dose selection, was discussed by the Committee at the April meeting when it was also asked whether the UK should offer to draft any of the other planned chapters. Members considered that, although much guidance already exists on histopathology, it would be important to bring it into an OECD context, and recommended that the UK should propose leading on this chapter.

The COC was also asked for advice on the guidelines in July, when it was informed that one of the principal issues under consideration for the revision of the Test Guideline for carcinogenicity testing (no. 451) is the required duration of the studies, in particular, how to deal with high levels of mortality before scheduled termination of the study. Specifically:

- if there is excess mortality in the high dose group and other treated groups. The Committee commented that this scenario would indicate a seriously flawed study and would recommend abandoning it at that point.
- if there is excess mortality in treated groups other than the high dose group. The Committee advised that there would be concern about study design and technical handling since the deaths would probably not be compound related. They considered that, on balance, it would be better to run the study to completion
- if there is excess mortality in the controls only, or in controls and one or more treated groups. It was noted that what action was taken would depend on how much survival is reduced. The COC recommended the continuation of the study only if the number of surviving animals is similar across the groups. It would be important to establish that the study still had sufficient power to detect effects at the level of concern.

The COC was also asked for comments on a paper by Roth et al (2007; Toxicologic Pathology, Volume 35: pp 1040-1043) which discussed excess mortality in two-year rodent carcinogenicity studies. The committee considered that the paper was suited more to testing of pharmaceuticals, where a risk/benefit analysis was required, than to other chemicals for which a hazard identification is needed. The paper was a reasonable qualitative description of potential strategies but failed to justify the details and included many "rules of thumb" of unknown origin. Many relevant issues had not been discussed in the paper.

The COC also considered whether the wording in the "Duration of Study" section of the 1981 Test Guideline 451 should be revised, and if so, how. The current text states that overall survival should be 50% for a negative study to be acceptable. The COC advised that the wording should be revised but the proposals set out in the Roth paper were not acceptable. The Committee also agreed with the proposal that the normal duration of carcinogenicity studies in mice should be revised to 2 years.

The Committee considered that the method of analysis to be used should be explicit at the outset and both data analysis and study design should be clearly linked to the primary objective of the study. Therefore, it was important that the key requirements for study design and data analysis were included in the Test Guideline, so that they become obligatory under the Mutual Acceptance of Data agreement and thus avoided rejection of completed studies or the need for duplication.

### **Horizon scanning**

The COC undertakes “horizon scanning” exercises at regular intervals to identify new and emerging issues which have the potential to impact on public health. A number of topics were identified by the secretariat for consideration by the Committee at the 2008 exercise. From these and Committee members’ own proposals, the COC considered that the following topics should be taken forward:

- RNA related effects as mechanism of carcinogenicity
- Endogenous DNA adducts
- Carcinogenic risk of carbon nanotubes
- Carcinogenic risk of exposure to environmental tobacco smoke in childhood
- Possible carcinogenic hazard from dietary insulin-like growth factor 1 (IGF-1)

### **Ongoing topics**

#### *Carcinogenicity of mixtures*

The COC is discussing current developments in the assessment of chemical mixtures with regard to carcinogens and their modes of action. A statement is expected in 2009.

#### *Update review of epidemiological studies on cancer incidence near municipal solid waste incinerators*

The COC published a statement on municipal solid waste incinerators and cancer in 2000. In 2008, the Committee reviewed the results of new epidemiological studies published in the scientific literature since that date. A statement is expected in early 2009.

### **Statements of the COC (to be appended)**

#### *Chlorinated drinking water and cancer*

#### *Non-Hodgkin’s lymphoma*