

**MUT/09/04**

**COMMITTEE ON MUTAGENICITY OF CHEMICALS IN FOOD CONSUMER  
PRODUCTS AND THE ENVIRONMENT (COM)**

**Draft Annual report for 2008**

## **Preface 2008**

The Committee on Mutagenicity (COM) provides advice on potential mutagenic activity of specific chemicals at the request of UK Government Departments and Agencies. Such requests generally relate to chemicals for which there are incomplete, non-standard or controversial data sets for which independent authoritative advice on potential mutagenic hazards and risks is required. Frequently recommendations for further studies are made.

During 2007, the Committee provided advice on a wide range of topics including genotoxicity of acrylamide, chemical mixtures, phenol and the assessment of mutagenic impurities in pesticides. A large proportion of COM business was devoted to the evaluation of acrylamide and its genotoxic metabolite glycidamide.

The COM initiated a revision of its guidance document (Guidance on a Strategy for Testing of Chemicals for Mutagenicity) which had been published in 2000, and initiated a review of the use of Toxicogenomics in genotoxicity evaluation.

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## **DRAFT**

### **COMMITTEE ON MUTAGENICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT (COM)**

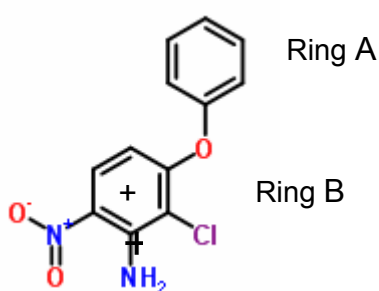
#### Preface

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## Aclonifen

2.1 The COM was asked for advice by the Pesticides Safety Directorate (PSD) on a pesticide active ingredient new to the U.K. which is undergoing evaluation through the independent Advisory Committee on Pesticides (ACP). The referral statement was as follows: 'ACP requested advice on the mutagenicity of Aclonifen and the genotoxicity risk assessment of the postulated metabolites hydroquinone and phenol. The referral does not include carcinogenicity data or the evaluation of mode of action for tumours in rodents observed in long-term carcinogenicity bioassays with Aclonifen'.

2. Aclonifen (2-chloro-6-nitro-3-phenoxyaniline) (figure 1.) is a selective systemic herbicide used for pre-emergence control of grass and broad leaved weeds in a range of crops.



+ = position of uniformly radiolabelled phenoxyaniline ring  
Figure 1. Aclonifen

2.2 The COM considered a large amount of data on Aclonifen, which included an extract from the detailed record of ACP consideration of Aclonifen, extracts from draft EU assessment report on metabolism and genotoxicity of Aclonifen, which presented information on structure, use as a pesticide, ADME studies, toxicology, mutagenicity, carcinogenicity and reproduction, data from mutagenicity test reports on Aclonifen, copy of the report on the investigation of the potential for DNA-binding of Aclonifen and the revised position paper from the data holder on the cleavage of the diphenyl ether bond of Aclonifen. The data holder Bayer Crop Science submitted a presentation which was circulated to Members and in addition a revision to a report on cleavage of the diphenyl ether bond in the Aclonifen molecule. The COM considered Aclonifen at its meeting on 23 October 2008.

2.3 The COM reached a number of conclusions as shown below;

- i) The COM agreed that further data on Aclonifen metabolism was required. This could involve more *in vivo* tests with specific analysis for the formation of hydroquinone and phenol. Alternatively, it might be possible to undertake comparative *in-vitro* studies using rodent and human tissues (with specific measurement of hydroquinone and phenol formation). It was considered this could provide evidence that exposure to Aclonifen was unlikely to be associated with significantly increased genotoxic risk, although this would not preclude the possible

need for additional mutagenicity tests dependent on the outcome of the metabolism studies.

ii) The COM noted the approach to risk assessment had not been considered during the presentation, but that the data holder had included a proposed Margin of Exposure approach in the submission dated 13 August 2008. This would need to be considered further when appropriate metabolism data were available.

2.4 The COM agreed a statement which is reproduced at the end of this report.

### **Impurities**

2.5 The COM had been informed of a published literature survey to evaluate the lowest detectable level of response in the Ames test for mutagens during the Horizon scanning exercise for 2007. The approach adopted by the authors might have potential wider generic use which could be valuable for the review of the COM strategy and also for generic advice to Government Departments. (*Kenyon MO et al Regulatory Toxicol, Pharmacol, 48, 75-86, 2007.*) The COM reviewed this publication and also considered other recent publications which had considered a rationale for determining, testing, controlling specific impurities in pharmaceuticals that possess potential for genotoxicity.

*Kenyon MO et al Regulatory Toxicol, Pharmacol, 48, 75-86, 2007*

2.6 A literature survey of 454 mutagens tested in the Ames test was undertaken to estimate the lowest effective concentrations for a variety of classes of mutagens and to develop an understanding of the sensitivity of the test system. Overall for most representative classes, all compounds were detected at 2500 µg/plate. In a further analysis by class, the authors reported that only a small number of compounds had LECs that were greater than 250 µg/plate. Overall, the authors estimated that 85% of mutagenic impurities in an API should be detected in Ames tests if present at ≥5% assuming the Active Pharmaceutical Ingredient (API) is tested up to 5000 µg/plate. The literature review had been supported by a number of Ames tests of pharmaceutical agents undertaken in the presence of excess mannitol (to represent excess API) and verapamil and diltazem (two highly metabolised medicines). Members agreed that many impurities in APIs were present at less than 5% and it was likely such impurities would need to be isolated and tested separately in order to evaluate their potential mutagenic hazard. A negative result in Ames tests for a test material containing impurities below 5% would not provide reassurance that the impurity had been tested adequately.

Muller L et al *Regulatory Toxicology and Pharmacology*, 44, 198-211, 2006. and EMEA guidance(CHMP/SWP/5199/02, 28 June 2006)

2.7 Members acknowledged that the approach suggested was specific to pharmaceuticals and provided guidance on assessing genotoxic impurities in APIs particularly in relation to decisions on safety in respect of clinical trials. The TTC approach was based on assessment of likely intakes of impurities (i.e. a *de minimus* risk value (Threshold of Toxicological Concern (TTC) (1.5 µg/person/day)) could be identified for any chemical, including those of unknown toxicity, taking chemical structure into consideration). The TTC was originally applied to foodstuffs (e.g. impurities present in flavour materials and food contact materials) was introduced as a way of prioritising action on those most likely to cause the greatest risk. This had translated in some areas into the proposal that TTC level could be used to derive conclusions on acceptability.

2.8 The COM agreed the proposed approach had an advantage in aiding assessment of risk/benefits from clinical trials. Members agreed that it was not possible to conclude that scaling intakes resulted in the same mutagenic risk. Members noted that the EMEA guideline limit for genotoxic impurities in APIs could exceed the TTC for life-threatening illnesses.

### **Chemical Mixtures**

2.9 The COM expressed an interest in the evaluation of the mutagenicity of chemical mixtures during the 2005 and 2006 horizon scanning exercises. One recommendation from COM was to consider the possible occurrence of synergistic interactions regarding mutagenic effects of chemical mixtures, the possible mechanisms for any synergistic effects and the implications of such a finding for risk assessment. It is possible that if synergistic effects between two or more *in vivo* mutagens occurred then co-exposure to mixtures containing these chemicals might result in a significant increase in the risk of mutagenicity and cancer compared to the risks associated with exposure to the individual chemicals alone. The COM evaluation outlined was intended to build on the work of the COT work on Risk Assessment of Mixtures of Pesticides and similar substances (WiGRAMP)<sup>1</sup> <http://www.food.gov.uk/science/ouradvisors/toxicity/cotwg/wigramp/> which was subsequently extended to encompass other types of chemicals in food ( see 2004 COT Annual report <http://www.food.gov.uk/multimedia/pdfs/cotsection.pdf>) and the ongoing work of the Interdepartmental Group on Health Risks from Chemicals (IGHRC) on the risk assessment of chemical mixtures [http://www.silsoe.cranfield.ac.uk/ieh/ighrc/mixtures\\_document.pdf](http://www.silsoe.cranfield.ac.uk/ieh/ighrc/mixtures_document.pdf). Thus the definitions and nomenclature used to describe interactions regarding mutagenicity induced by chemicals in this statement were taken from these reviews.

2.10 A number of strategies have been considered for the evaluation of chemical mixtures. These include testing whole mixtures (integrative),

fractionation of mixtures to determine mutagenic components (dissective, topdown approach), and investigations of interactions by testing simple combinations, recombined fractions, and spiking of mixtures/fractions (synthetic, bottom up approach). All of these approaches were identified from literature searches with regard to mutagenicity testing, although relatively few studies of whole mixtures were identified. Approximately 110 research papers with potentially relevant information were identified for consideration during the COM review

### COM Discussion and Conclusions

#### Whole mixtures

2.11 The COM considered mutagenicity testing of whole mixtures, and approaches to dissection (fractionation/concentration) of mixtures. The primary purpose of such studies is to monitor mutagenic response in tests of a wide variety of mixtures (for example foods, samples of pollution (air and water) condensates or particles from pyrotechnic mixtures (e.g. cigarette smoke or mixtures of known compounds), hazardous wastes including industrial process effluents and municipal sludges. The COM noted that there were comparatively few data on mutagenicity testing of whole mixtures.. The COM agreed that testing whole mixtures first using an *in vitro* screen (such as the Ames test or SOS chromotest) would have the advantage of picking up evidence of potential interactions, such as synergy, that could be missed by testing individual fractions. However, the failure to detect mutagenicity when complex mixtures (e.g. fried foods) or fractions (e.g. catalytically cracked clarified oil) are tested either *in vitro* or *in vivo* did not prove the absence of potentially mutagenic compounds.

#### Approach to dissection of mixtures

2.12 The COM agreed an outline proposal for a strategy for monitoring mutagenicity of chemical mixtures (in particular occupational and environmental mixtures), based on proposals for evaluating the mutagenicity of mixtures in the published literature but noted that this was only general guidance and a case-by case approach was needed.

#### *Preliminary considerations*

- A. Collect information on chemical composition, and mutagenicity of chemicals in the mixture. Define the purpose of the monitoring approach (is this to monitor overall mutagenic hazard of the mixture, or to monitor the mutagenicity of selected levels of chemicals or groups of chemicals within the mixture?).
- B. Review the literature for appropriate data on sampling, extraction and testing of similar mixtures. Review the mutagenicity test data on the mixture or similar mixtures or the chemicals within the mixture selected for monitoring

#### *Mutagenicity testing*

C. Define *in vitro* testing strategy, focusing on optimising and standardising the approach.

D. Undertake *in vitro* monitoring to validate approach and identify sources of variation and their impact.

E. Consider, if necessary on a case-by-case basis, developing an *in vivo* segment to strategy. (This might include studies to test whether chemical(s) selected for monitoring had *in vivo* mutagenic potential if this was not known. It is unlikely that chemical(s) within a mixture which were known to be *in vivo* mutagens would need to be routinely tested.

#### *Review of strategy*

F. Implement the strategy and use data to inform on risk reduction strategies. It is important to periodically review the results of a monitoring strategy, particularly if there is any evidence for a change in the results being reported. There are many potential sources of variation which could affect the results and it would be important to differentiate between a change in results due to composition of the mixture from a change due to variation in fractionation and/or testing procedures. The inclusion of spiked samples in a strategy for mutagenicity testing of mixtures may be valuable.

#### Approach to evaluation of studies to investigate interactions

2.13. The COM agreed the concept of the 'envelope of additivity' was a helpful approach in the presentation of the results of studies and in the identification of non-interaction (e.g. dose-response and effect additive responses) and interaction responses (e.g. synergy and antagonism). The COM noted the proposed unifying approach of Gennings and colleagues (see Gennings C et al (2005). A unifying concept for assessing toxicological interactions: changes in slope. *Tox Sci*, 88 (2), 287-297.) for application of statistical methods in chemical mixture research which is based on the shape of the dose response curve and changes in the slope of the dose-response curve in studies using two or more chemicals, and agreed that this could be of potential use in evaluating genotoxicity.

#### Review of published studies on interaction between chemicals with regard to mutagenicity.

2.14. The COM noted that the available published literature presented a number of examples where interaction between chemicals with regard to mutagenicity had been reported. However, there was essentially no appropriate independent confirmation of the results in separate tests, or within an appropriate mutagenicity testing strategy for the identification of interactions and therefore no definite conclusions could be reached.

2.15. The COM agreed that the available studies had raised a number of

potential hypotheses for interaction (see paragraph). There was a need for further research regarding such mechanisms, which if confirmed in an appropriate mutagenicity testing strategy might be of potential significance for public health.

The COM agreed a statement which is reproduced at the end of this report.

## Phenol

2.16 HSE asked for advice from COM on phenol (along with hydroquinone) in 1994/95 and in 1999. A copy of the conclusions and the statement agreed in 1999 (published January 2000, COM/00S1). [Hydroquinone is a metabolite of phenol, see figure 1 below]

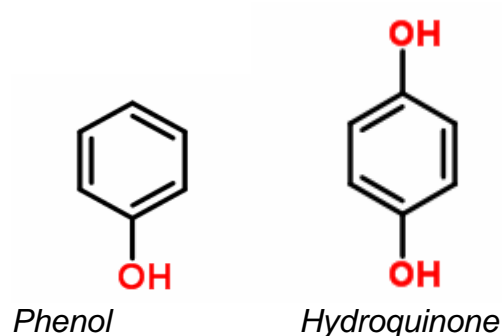


Figure 1.

2.17 In 1994, the COM concluded the *in vitro* mutagenicity data on phenol were of poor quality and results difficult to interpret, but *in vivo* data showed phenol to be a somatic cell mutagen at very high dose levels. (COM noted negative results in long term carcinogenicity bioassays in rats and mice). The COM noted the potential for rapid conjugation and detoxication via the glutathione pathway and that the mutagenicity of phenol appeared to be predominantly related to peroxidase activity and catalase could have a protective role. The COM agreed there was a potential for a threshold mechanism by the oral route of exposure but could not reach a similar conclusion with regard to dermal or inhalation exposure.

2.18. In 1995, the COM considered a submission from industry which provided some metabolism data. Overall the COM concluded that appropriate studies to determine the extent of pre-systemic metabolism following either inhalation or dermal exposure had not been undertaken. The COM provided guidance on the approaches which could be used (including administration of hydroquinone or phenol via a bronchoscope with very early sampling for free and conjugated test substance in the blood).

2.19 In 1999, the COM considered a study on bioavailability and metabolism of hydroquinone after intratracheal instillation in male rats. The results showed free systemic hydroquinone in arterial blood 5-10 seconds after dosing. The COM considered the data suggested the potential for site of contact and

systemic mutagenic effects after inhalation exposure. The COM considered a inhalation exposure transgenic Muta™ mouse study but were unable to draw any conclusions in view of unacceptable levels of DNA packaging in many of the trials in the experiment. The COM noted a small but consistent positive result in bone marrow micronucleus studies in mice given intraperitoneal doses of around 100-160 mg/kg bw.

2.20 The COM agreed a statement (00/S1) in January 2000. The conclusions reached with regard to phenol were similar to those reached in 1994

2.21 In 2003, the COM considered a pre publication report from the Dow Chemical Company which provided results to suggest that the *in vivo* mutagenicity of phenol in the mouse bone marrow micronucleus assay originated from a transient hypothermia induced by high doses of phenol. The COM agreed the data supported a case for a threshold mechanism for the induction of MN in bone marrow of mice but considered publication of the study in a peer-review journal would be necessary before drawing any definite conclusions. A further COM statement was not published in 2003. The relevant study has now been published and was identified during the 2007 COM horizon scanning exercise. Members asked for a review of the paper during the COM horizon scanning exercise. In addition the HPA asked for advice on the genotoxicity of phenol and specifically whether a threshold approach can be used with regard to the risk assessment of genotoxicity of phenol.

#### Introduction to current COM review

2.22. The COM current consideration of phenol covered the period from 1994-2003. The objectives of the current review were i) produce an updated COM statement on phenol, ii) to evaluate the Spencer study on hypothermia and also iii) to consider if any *in vivo* mutagenic effect of phenol can be considered as related to a threshold effect

2.22 The COM agreed with the conclusions reached on phenol in its previous statement (COM/00/S1). (The COM agreed the overall conclusions reached in the draft EU Risk Assessment report which had been provided for members information.)

2.23 The following overall conclusions were agreed.

- a. Phenol is mutagenic *in vitro* in mammalian cells giving rise to gene mutation and chromosomal damage in the presence and absence of exogenous metabolic activation. The mode(s) of action had not been fully elucidated although there was evidence that effects were in part due to oxidative DNA damage

b. Phenol should be regarded as an *in vivo* somatic cell mutagen. The COM confirmed that there was consistent evidence for a small effect at doses below the i.p. LD50.

c. The COM agreed that the published study by Spencer et al 2007 had been well conducted but considered a dose level of 200mg/kg bw i.p would have been valuable. The dose level used in the study of 300 mg/kg bw clearly exceeded the maximum tolerated dose level. The committee considered that the degree and duration of hypothermia reported with phenol was severe and prolonged. Members concurred with the conclusion reached by the study authors and reported in the publication ‘..overall, these studies suggest a role, but not necessarily a causality, for phenol-induced hypothermia in the formation of MN.’

d. The COM concluded that the additional ‘in confidence’ data on thermoregulatory support in phenol treated animals provided inconclusive evidence regarding the role of hypothermia in phenol-induced micronuclei in mice. Thus for phenol-treated animals there was evidence of impaired capacity to modulate temperature compared to controls and a transient hypothermia. It was possible that the application of thermoregulatory control could influence the formation of MN in control and phenol treated mice.

e. The COM concluded that all the available data on phenol suggested phenol should be regarded as a non-threshold *in vivo* systemic mutagen. There is insufficient evidence to support a threshold approach to risk assessment of systemic phenol.

2.24 The COM agreed a statement which is reproduced at the end of this report.

### **Horizon Scanning**

2.26 The annual horizon scanning exercise was intended to provide an opportunity for members and advisers from Government Department/Agencies to discuss and suggest topics for further work. Considerable progress on the items identified in the 2006 horizon scanning exercise had been made, although it was noted that review on mutational spectra had not been initiated and this would be carried over to next years work programme. The primary objective of the 2008/9 horizon scanning exercise was to provide information to aid members’ consideration of the scope and format of the revision of the COM guidance. The committee agreed that the following topics should be considered and could be included in the COM guidance; aneuploidy, mutational fingerprints/spectra, GADD 45 assay, and risk assessment.

2.27. Other suggestions for potential consideration included tissue concentrations in relation to lowest effect dose in carcinogenicity studies (the International Life Sciences Institute (ILSI) was doing some work on this), the

*Pig A* assay (Bryce SM et al Environ Mol Mut, 49, 256-264, 2008), pesticide impurities and nanomaterials.

## **Test Strategies and Evaluation**

### **Review of COM Guidance 2000**

2.28 The current COM mutagenicity testing strategy (2000) was developed to update the strategy document published in 1989 (Report on Health and Social Subject No 35) which had been based on a strategy agreed in 1981. The COM guidance document published in 1989 contained a number of chapters on the basic science of mutations and their significance for human health as well as a testing strategy. The current COM strategy was a scientifically based approach to mutagenicity testing which updated the 1989 guidance, for example, with incorporation of the *in-vitro* micronucleus assay as a test for clastogenicity/aneuploidy and the inclusion of newer approaches to *in-vivo* testing such as use of transgenic animal models. The need to periodically reflect on developments was recognised by COM in 1981 and in 1989. The current COM guidance was not developed in response to a specific regulatory request but reflected the desire of COM members to update their guidance.

2.29 The guidance should produce a scientifically based strategy which can be used for screening compounds (not limited to one sector such as pharmaceuticals), evaluating genotoxicity of existing chemicals (such as contaminants) and providing case-by case guidance in specific circumstances where specific questions regarding a compound arise (e.g. evaluating genotoxicity mode of action in rodent carcinogen target and non target tissues).

2.30 The Committee held two wide ranging scoping discussions during 2008 and during consideration of horizon scanning (see paragraph 2.24 for examples of areas to be considered during revision of the COM guidance). In addition several options for disseminating the COM review were explored including publication of a further booklet on a strategy for genotoxicity testing, a peer review publication and publication of a series of guidance documents on the COM Internet site. The advantage of a series of general guidance statements would be that these could be more readily updated when significant advances in genotoxicity testing and evaluation became available (e.g on identifying thresholds for genotoxicity or the assessment of *in-vivo* mutagenic potency). It was agreed that all three options should be explored.

2.31 The COM agreed to consider the subject of potential thresholds for genotoxins at its February 2009 meeting.

## Ongoing Reviews

### *Acrylamide;*

2.32 In 2007, the HSE requested a further evaluation from the COM regarding the information cited by the Polyelectrolyte Producers Group (PPG) in a letter to the chair of COM (dated 8 May 2007, COM statement 07/02). In view of the widespread dietary exposure to acrylamide, the Food Standards Agency requested that such a review should consider all available genotoxicity data on acrylamide. In 2007, the COM agreed that the EU risk assessment review completed by HSE (EU Risk Assessment report 2002) could be used as a basis for the review, and agreed a strategy for this to be extended with a systematic review of the scientific literature available subsequent to the EU report.

2.33 In 2008 Members reviewed the findings of the EU Risk Assessment Report and were presented with the systematic review of data relating to the genotoxicity of acrylamide and glycidamide published after 1995, and other pre 1995 references that had not been included in the EU risk assessment report. This systematic review, together with several presentations and submissions from the PPG, formed the basis of extensive discussions at each meeting in 2008. This has enabled a detailed statement to be drafted. The Committee expect to receive final comments on the fourth draft of the statement from the PPG in January 2009; with publication of the statement expected soon after, subject to any revisions in light of the submitted comments.

### *Toxicogenomics;*

2.34 The COT/COC/COM held a joint symposium on the issue of genomics and proteomics in October 2001 and published a joint statement in December 2004 on the use of Toxicogenomics in toxicology. This was based on literature review of 50 studies and included information from the International Life Sciences Institute/Health and Environmental Sciences Institute (ILSI/HESI) collaborative programme of research. This topic was identified during the 2006 horizon scanning exercise for an updated review. The DH Toxicology unit drafted a short overview of a number of new relevant *in-vitro* studies, which included data on gene expression changes in studies on DNA adducts and mutagenicity for the October 2007 meeting. A large number of papers had been retrieved, but those selected for review were specifically chosen with the aim of identifying any advancement in the field, which may affect the conclusions drawn in the last statement. The COM considered a draft discussion paper at its October 2008 meeting. A further discussion paper is to be considered in 2009 reporting on the results of the ongoing ILSI/HESI trials

## Statements

### (To be included)

- [COM/08/S1](http://www.iacom.org.uk/statements/documents/MixturesCOMforinternet.pdf)  
<http://www.iacom.org.uk/statements/documents/MixturesCOMforinternet.pdf> **Mixtures**
- [COM/08/S2](#) **Phenol**
- [COM/08/S3](#) **Aclonifen**