

COMMITTEE ON MUTAGENICITY OF CHEMICALS IN FOOD CONSUMER PRODUCTS AND THE ENVIRONMENT (COM)**UPDATE STATEMENT (2008): MUTAGENICITY OF PHENOL****Introduction.***Background to COM review*

1. 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/00S1.¹ [Hydroquinone is a metabolite of phenol, see section on metabolism and figure 1 below]

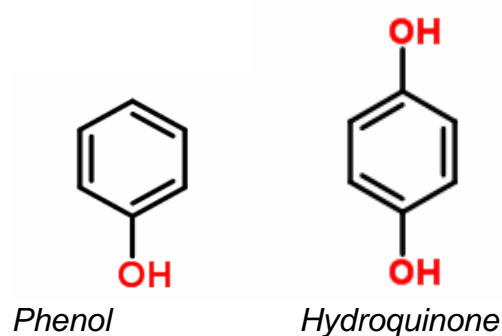


Figure 1.

2. In brief, 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.

3. 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.

4. 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. (Relevant BMMN studies are reviewed in para 14 below).

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

6. 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.² 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

7. The COM consideration of phenol covers a period from 1994-2003. The objectives of the current review is to 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.

8. The COM have considered many of the key studies on phenol in full in the past but over quite a period of time. Thus in order to provide a comprehensive overview of the mutagenicity of phenol, the secretariat have submitted a draft EU risk assessment review which has been provided by HSE (Germany acting as rapporteur) dated 1/09/2005.³ In addition relevant information from important studies on phenol were provided to the COM.

Overview of phenol mutagenicity

In-vitro mutagenicity studies

Bacterial tests

9. The COM agreed that phenol was not mutagenic in standard bacterial mutagenicity tests.³

Mammalian cell gene mutation tests

10. The Committee considered the available mammalian gene cell mutation studies. Phenol induced a dose-related increase in the frequency of *Hprt* mutants in V79 cells in the absence of exogenous metabolic activation (4-fold increase at the top dose). Cell survival at the top dose was 50%.⁴ A positive result had also been documented in SHE cells using the Na⁺/K⁺ and *Hprt* loci in the absence of exogenous metabolic activation at the highest dose tested.⁵ There was no evidence of cytotoxicity reported in this study. Evidence for a positive result had been documented in mouse lymphoma L5178Y cells in the presence and absence of exogenous metabolic activation at dose levels which induced cytotoxicity.⁶ A similar results had also been documented in LY5178Y cells in the presence and absence of exogenous metabolic activation.⁷ Overall it was prudent to conclude a positive response in gene mutation assays in mammalian cells in the presence and absence of exogenous metabolic activation, although the mechanism for the induced effects had not been resolved.

Mammalian cell chromosomal aberration tests

11. Phenol gave a positive result for chromosomal aberrations in CHO cells in the presence and absence of exogenous metabolic activation.⁸ Members noted the increase in the absence of exogenous metabolic activation was approximately 3 fold and there was no evidence for a dose response in the presence of exogenous metabolic activation. Positive results were also reported in a number of micronucleus tests in CHO cells both in presence and absence of exogenous metabolic activation⁹, in V79 cells and human PBLs (both in the absence of exogenous metabolic activation).^{10,11} No evidence for an aneugenic effect of phenol was reported in a test where chromosome number in metaphase spreads were scored and reported (positive results were reported for benzene in the same experiment but a known aneugenic positive control was not used).⁵ Evidence for a moderate increase in both kinetochore positive and negative micronuclei was reported in PBLs indicating some evidence for both clastogenic and aneugenic activity with phenol.¹¹ Overall members considered no definite conclusion regarding the potential for aneugenicity could be drawn from these data.

Studies investigating DNA damage

12. A number of *in-vitro* studies investigating the potential for DNA damage were available. Members noted the evidence for UDS in the absence of exogenous metabolic activation in SHE cells.⁵ Members noted the evidence for ssDNA breaks in mouse lymphoma cells in the presence of exogenous metabolic activation.¹² Members considered the evidence for formation of 8-hydroxy-deoxyguanosine (8-OHdG)³ indicated some potential for oxidative DNA damage but commented that undifferentiated HL60 cells were likely to be more predisposed towards formation of free radicals and oxidative DNA damage than differentiating HL60 cells¹² or normal human peripheral blood lymphocytes¹³ Members noted the evidence for formation of DNA adducts in calf thymus DNA in the presence of horseradish peroxidase and hydrogen

peroxide.¹⁵ The data reported provided some evidence for oxidative DNA damage with phenol but the test system was likely to be predisposed to formation of free radicals and oxidative DNA damage.¹⁵

Conclusion: *In vitro* mutagenicity data

13. Thus phenol was 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.

***In-vivo* mutagenicity studies**

14. The results of available studies considered in the draft EU risk assessment report reported evidence for a 2-2.5 fold induction of BMMN using oral and i.p. doses which equate to or exceed the relevant LD50 in mice. An important conclusion reached by COM during its previous consideration of phenol related to the evidence for a small but consistent *in vivo* BM MN positive effect at dose levels below the i.p. LD50 in mice. Members reconsidered the three key studies supporting this conclusion. Chen and Eastmond used 3 doses of 160 mg/kg phenol i.p. followed by BM sampling 24h after the last dose. There was no discernable effect on the PCE/NCE ratio but signs of toxicity, if observed were not reported. FISH analysis indicated that the positive results were due to chromosome breakage.¹⁶ Mazzarini A et al 1994 reported a significant positive effect following a single i.p. dose of 120 mg/kg bw to a group of 3 CD-1 mice followed by bone marrow sampling 18h after treatment.¹⁷ There was no apparent effect on the PCE/NCE ratio but signs of toxicity, if observed were not reported. Shelby M et al *Env Mol Mutagen*, 21, 160-179, 1993 reported a positive trend test for BM MN induction in two separate studies where male B6C3F1 mice were given i.p. doses of 0, 45, 90 or 180 mg/kg bw phenol on three consecutive days with bone marrow sampling 48 h after the last dose. All animals survived and there was no apparent effect on percent PCEs. However signs of toxicity, if observed, were not reported.¹⁸

15. The COM affirmed its previous assessment of these studies. The COM agreed the overall conclusions reached in the draft EU Risk Assessment report.³ Thus 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.

Evidence regarding mode of action for the *in vivo* mutagenicity of phenol.

Induction of micronuclei by phenol in mouse bone marrow. Association with chemically induced hypothermia. (Spencer et al Tox Sci, 97, 120-127, 2007)²

16. Groups of four male and four female CD-1 mice were dosed i.p. with 0, 50, 150, 200, 300, 400, or 500 mg/kg bw phenol (Hypothermia test). The relative Body Temperature was monitored subcutaneously using

programmable transponders (also used for animal identification) prior to dosing, 5, 30, 60, 90 min and 2h, 3,4,5,6,24 and 48h after dosing. Clinical signs of toxicity were recorded. In the MN test groups of 6 animals/sex were dosed at 30, 100 or 300 mg/kg (separate group dosed p.o. with 120 mg/kg cyclophosphamide, 24 h sampling). BT was measured prior to dosing, and 2,5,24 and 48 h. Animals were killed at 24 or 48h post dose and bone marrow collected. For kinetochore evaluation a group of 6 males was dosed with 300 mg/kg bw phenol (CP (p.o 120 mg/kg bw) and vinblastine (4 mg/kg bw i.p) used as positive controls with 24 h sampling). For MN evaluation 2000 PCEs were scored blind to dosing status. Data were transformed by adding one and taking natural log of adjusted number. Pairwise comparison of data used Dunnett t-test. Kinetochore positive MN-PCEs were compared using Fisher exact test.

17. All mice dosed at 400 mg/kg bw or 500 mg/kg bw died within 24h of dosing. A single male and female in the 300 mg/kg bw group died prior to the 48 h observation time point. No deaths occurred at 200 mg/kg bw and below. Signs of toxicity included reduced activity (200 mg/kg bw and above) and twitching and tremors (at 100 mg/kg bw and above) which were noted shortly after dosing. Surviving mice appeared normal 1h post dose. Males appeared to be more sensitive with a more rapid onset of signs of toxicity and shorter period to death. Predose mean body temperatures in males and females were 36.7°C and 37 °C respectively. Thirty minutes post dose at 300 mg/kg bw mean BT reduced to 32 °C and the mean BT as low as 28 °C 5h post dose in both sexes. BT did not return to baseline within the 48h observation period and was depressed 4-5 °C at the end of the experiment. BT reductions of up to 8 °C were recorded at 400 and 500 mg/kg bw (at up to 6h post dose). Smaller transient reductions in BT were reported at 100, 150 and 200 mg/kg bw. From the information presented in figure1 of the published paper, the reduction at 100 mg/kg bw appears to be around 2 °C with a return to baseline around 2-3h post dose. At 200 mg/kg bw the decrease in BT appears to be around 2-3 °C with a return to baseline at around 4-6h. No evidence for an effect on BT was reported at 50 mg/kg bw.

18. In the MN test one animal dosed at 30 mg/kg bw died (not related to treatment). The authors report phenol related signs of toxicity in about one third of males and one half of females dosed at 300 mg/kg bw (table 1 of the published paper). Signs of toxicity appeared within minutes and had subsided about 1h post dose. There was evidence for very transient signs in animals dosed at 100 mg/kg bw (lasting only several minutes). No treatment related signs of toxicity were reported at 30 mg/kg bw. BT was reported at 24 and 48 h post dose. A 4-5 °C reduction was evident at 24h post dose in both males and females. By 48h the decrease was approximately 7 °C in males and 6 °C in females. BT at these time points was unaffected at 100 mg/kg bw and 30 mg/kg bw. BT was unaffected in CP positive control animals.

19. A statistically significant increase in MN-PCE/1000 PCE was recorded at 300 mg/kg bw at 24 h sampling (male 10.8 c.f. 2.1 in control and 11.3 in females cf 2.5 in controls). At 48 h the mean frequency of MN-PCE/1000PCEs was 18.3 in males and 17.8 in females. The mean percent PCE values was reduced at 24h (all doses) and 48h (in males/females at 300

mg/kg bw). The frequency of MN-PCEs/1000 PCEs was not increased at 30 and 100 mg/kg bw. CP gave the expected positive result.

20. The authors conclude that phenol induced MN formation occurred only in the presence of marked hypothermia.

21. In the kinetochore experiment, a statistically significant increase in the proportion of kinetochore positive MN was observed in phenol treated mice at 300 mg/kg bw. Vinblastine (VB) gave the expected positive result. The proportion of kinetochore positive MN was substantially higher in VB treated mice.

22. In their discussion the authors note the finding of phenol induced hypothermia at doses at or above the MTD was a novel finding. The induction of hypothermia was associated with a NOEL for MN formation and thus phenol induced MN by a secondary mechanism associated with regulation of BT in mice. It was noted that in part, it was possible to speculate that BT affected spindle function thus resulting in kinetochore positive MN. However a proportion of phenol induced MN were clastogenic and might have been due to an effect of phenol, hydroquinone (a metabolite of phenol) or a combination of phenol/hydroquinone. It is noted that the available data on phenol suggest that any direct genotoxic activity is likely to be mediated by oxidative DNA damage and hence would be presumed to have a potential threshold for activity. Overall the authors suggested a role for hypothermia but did not prove causality. The authors suggest further studies to investigate the role of physically induced changes in BT on the induction of MN in phenol treated animals would be an appropriate way forward.

COM conclusions on Spencer et al 2007

23. Members agreed that the study had been well conducted but considered a dose level of 200 mg/kg bw i.p would have been valuable. The dose level 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.’

Additional in-confidence data on thermoregulatory support study

24. Members considered the additional in confidence data on the thermoregulatory support study which had been provided by Dow Chemicals.

25. A full report of the studies undertaken by DOW has been submitted as an in-confidence document. Essentially phase 1 and phase 2 of the study were published in Spencer et al 2007.² Additional studies were undertaken to investigate the approach to thermoregulatory control induced (i.e. applying external heat to prevent hypothermia) in mice dosed with phenol (phase 3) and a rescue experiment was undertaken (phase 3). The objective of the rescue experiment was to obliterate phenol induced MN formation in mice by

appropriate thermoregulatory control. This was not achieved (a statistical increase in MN formation was reported at 24h post dose). The investigators also noted that the application of external heat to control mice also resulted in a statistically significant increase in MN formation at 24h post dose. Overall the results of the rescue study were considered to be inconclusive. A further Telemetry experiment (phase 4) was undertaken to monitor body temperature in phenol dosed and control animals under thermoregulatory control conditions at five minute intervals to provide more comprehensive data on the effectiveness of thermoregulatory support. Thermoregulatory control in control mice resulted in an overall elevation of body temperature compared to animals maintained under normal environmental conditions. 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. In phase 5, the results of kinetochore staining experiments were reported (these data have been published in Spencer et al 2007²).

26. The COM accepted that thermoregulatory support was in practice very difficult to achieve. It was noted the effects resulting from dosing of phenol and also thermoregulatory support would have been stressful to the animals. Members observed that thermoregulatory support had not offset the phenol induction of micronuclei in mice. The application of thermoregulatory support had resulted in evidence for a slight increase in micronuclei formation in control females. However overall the observed induction of micronuclei by phenol could not be discounted. Members were aware that the principal study author had written to the secretariat and had concluded that, at this time, it is tenuous to make a conclusion regarding the mutagenicity of phenol under conditions of altered thermoregulation in the mouse micronucleus test.

Additional published studies on hypo-and hyperthermic induction of micronuclei in rodents.

27. Members considered the generic paper on the role of hypo- and hyperthermia in the formation of micronuclei in rodents.¹⁹⁻²³ Of particular interest was the publication by Tweats DJ et al 2007.²³ These data support the observation that chemical induced hypothermia in mice and hyperthermia in rats and mice may be potential modes of induction of MN in bone marrow. Experimental evidence needed to support hypothermia or hyperthermia as a mode of action for an unknown chemical would include a time course showing the association between core body temperature and MN induction and evidence for reversibility of the chemical induced MN formation by adjusting core body temperature. The assessment of hypothermic induction of MN for a specific chemical also requires evaluation for evidence regarding other modes of genotoxicity. A clear negative *in vitro* package of genotoxicity tests would rule out other modes of genotoxicity when deriving conclusions regarding the role of hypothermia in any observed *in vivo* MN formation. Evidence for positive *in vitro* genotoxicity would suggest other potential modes of genotoxic action *in vivo* which need to be taken into account in the overall assessment.

COM conclusions

28. 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.³ 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 200 mg/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.

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