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MUT/ 03/9

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

2-PHENYLPHENOL: Further consideration of mutagenicity data

Introduction

1) 2-Phenylphenol and its sodium salt are broad spectrum fungicides that are approved in the UK for use as wood preservatives. They are also used as surface biocides in a number of areas. The COM have advised on the mutagenicity of these compounds, specifically in the context of the mechanism of the bladder tumours seen in male rats fed high doses of these compounds, on a number of occasions over the past 15 years, the most recent being in 1997. Some new data are now available. An EU review of the use of 2-phenylphenol and its sodium salt in wood preservation is shortly to be initiated under the Biocidal Products Directive (98/8/EC). At recent discussions of these compounds at the Interdepartmental Secretariat to the Advisory Committee on Pesticides it was decided to recommend that the COM update its earlier opinions on these compounds, using the available published data, and also that the COC be asked to advise on the mechanisms of the bladder tumour induction in male rats in the light of the COM conclusions regarding genotoxicity. This information would be helpful in developing the UK position with regard to the EU in the context of the Biocidal Products Directive.

2) The advice of the COM is thus sought on the mutagenicity of 2-phenylphenol and its sodium salt, and specifically whether the induction of bladder tumours at high dose levels in male rats in the chronic bioassay is likely to have arisen from a genotoxic mechanism.

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Background

3) 2-Phenylphenol and its sodium salt are fairly widely used as biocides in a number of areas including wood preservation and as masonry washes. They give rise to some toxicological concern because of the induction of bladder tumours in chronic studies in male rats at high dose levels (1-2% in the diet). The effects are much less marked in female rats. There was no evidence of the induction of bladder tumours, or tumours at other sites, in an adequate dietary carcinogenicity study in mice. A more detailed summary of the carcinogenicity data is given in Annex A. It has been postulated that the mechanism whereby bladder tumours are induced in the male rat relates to persistent cytotoxic action on the urothelium leading to regeneration, chronic hyperplasia and ultimately tumours. It is important to be able to exclude a genotoxic mechanism, so that a threshold-based approach to risk assessment may be adopted.

4) The issue was initially referred to the COM in 1988. At that time a comprehensive review of the mutagenicity of the compound was undertaken using data from both the published literature, and unpublished data provided by industry. The available data indicated that negative results were consistently obtained in the Salmonella assay, and also in a number of in-vivo assays (bone marrow assay for clastogenicity dominant lethal assay in germ cells and in a covalent binding study that measured DNA binding in the bladder). The conclusion was reached that it was most unlikely that the induction of bladder tumours at high dose levels arose from a genotoxic mechanism.

5) Further studies became available in the 1990's and these were considered by the COM in 1992. Additional assays in Salmonella confirmed the lack of any activity in these assays ^(1,2). New data from mammalian cell assays however did suggest that the compounds had mutagenic potential. This consisted of positive results in mouse lymphoma assays and in an in-vitro cytogenetics assay ^(3,4,5). In addition there was a report that 2-phenylphenol could induce DNA damage in vivo in male rat bladder using DNA alkaline

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elution techniques ⁽⁶⁾. This was in contrast to the earlier study on covalent binding to DNA in rat bladder following exposure to very high levels of 2-phenylphenol which had given negative results ⁽⁷⁾.

6) The COM agreed the following conclusions in 1992:-

- i. Data from several assays to investigate the ability of 2-phenylphenol or its sodium salt to produce gene mutation in *Salmonella typhimurium* were consistently negative.
- ii. Positive results were obtained in in vitro metaphase analysis studies in CHO cells. Mouse lymphoma assays in the presence of exogenous metabolic activation gave positive results in the form of small colonies. These results suggested that a metabolite of 2-phenylphenol had clastogenic potential. These findings conflicted with an earlier in vitro metaphase analysis in a single study.
- iii. Negative results were obtained in bone marrow assays for clastogenicity in vivo and also in germ cells (dominant lethal assay), indicating that any possible clastogenic potential was not expressed in the whole mammal.
- iv. Conflicting results appeared to be obtained in in vivo assays for effects on DNA in bladder epithelium, the target tissue of concern. The Committee therefore wished to see data from a more sensitive in vivo method to investigate adduct formation. There was insufficient evidence to recommend a departure from the use of the safety factor approach for regulation of this compound at the present time.

7) The Advisory Committee on Pesticides reviewed 2-phenylphenol and the advice from the COM in 1993 and agreed to use a threshold approach to the risk assessment. They also requested further data relating to DNA adduct formation in the urinary bladder epithelium to investigate specifically whether a threshold existed. The ACP set a post review data requirement for an in-vivo ³²P post-labelling study, with particular emphasis on the dose-response relationship at doses known to produce a range of hyperplastic and neoplastic lesions in rodent cancer bioassays.

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8) In 1997 industry provided a full report on the ^{32}P post-labelling studies on 2-phenylphenol to examine the potential for formation of DNA adducts in the bladder of rats to the registration authority (HSE). This was considered by the COM in that year. The following conclusions were drawn:-

The Committee expressed concerns regarding the limitations of methodology used in the study entitled “ ^{32}P -post-labelling study of technical grade 2-phenylphenol to examine the potential for the formation of DNA adducts in the urinary bladder of the male rat.” The Committee requested additional analyses of the bladder epithelial samples from this study using an appropriate, sensitive adduct enrichment method for the detection ^{32}P -postlabelled adducts (namely both nuclease P_1 and butanol extraction) and appropriate control experiments to evaluate the fate of 2-phenylphenol DNA adducts during the extraction and enrichment procedures.

9) The Advisory Committee on Pesticides considered these studies and the COM view, together with additional data from specialist feeding studies/carcinogenicity bioassays in 1998. The ACP agreed that the package of additional studies submitted by the data holder fulfilled the post-review requirements and confirmed that a threshold approach remained relevant for risk assessment purposes. There was thus no regulatory requirement for any further testing.

New Published Data (since the comprehensive 1992 review)

Metabolism: potential formation of reactive species

10) The metabolic profile of 2-phenylphenol is now well established^(8, 9, 24). This is shown in Figure 1.

11) In rats the main metabolites are the sulphate and glucuronide conjugates of 2-phenylphenol⁽⁸⁾. A minor pathway involves cytochrome P_{450} oxidation to 2,5- dihydroxybiphenyl which can be conjugated or converted to 2 phenyl –p-benzoquinone. Metabolism is dose dependent. At low doses (50mg/kg or below) it is essentially totally conjugated, mainly

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with sulphate. At 200 – 600 mg/kg the proportion converted to 2,5-dihydroxybiphenyl glucuronide increases disproportionately. Following repeated exposure to 1000mg/kg small amounts of non-conjugated compounds may be formed such as 2-phenyl-p-benzoquinone, with greater amount in the male compared to female rats.

12) There is thus the potential for formation of unconjugated reactive compounds at very high dose levels. Since the concern regarding carcinogenicity in the rat relates solely to the bladder, there has been suggestions of a 2 stage process of metabolic activation with some initial oxidation at high doses in the liver but with the critical formation of reactive species such as phenylhydroquinone (PHQ) and phenylbenzoquinone (PBQ) in the bladder⁽⁹⁾. Both these compounds are cytotoxic and electrophilic and can potentially give rise to protein and DNA adducts.

13) Data have recently been reported on the comparative metabolism of 2-phenylphenol in the male mouse, rat and human using radio labelled compound. The aim was to clarify the mechanistic basis for the differences in carcinogenic potential of the compound in the rat and the mouse⁽¹⁰⁾.

14) Sulphation of 2-phenylphenol was confirmed to be the major metabolic pathway at the low dose level used, namely 15mg/kg in rat, 28mg/kg in mouse and 0.0006mg/kg in man, with 57, 82 and 69% respectively of the urinary activity being present as this conjugate. The glucuronate conjugate of 2-phenylphenol was also present in appreciable amounts (29, 7 and 4% respectively). In addition conjugates of 2-phenylhydroquinone were present (12, 5 and 15% respectively) but no free PHQ or PBQ [Limit of Detection 0.1 – 0.6%].

15) In dose response studies in the rat and the mouse a clear dose dependent increase in total PHQ (free and conjugated) was seen in the rat. However a similar increase was seen in the mouse.

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16) The overall conclusions drawn by the authors of this work was that the minor differences seen in the metabolism of 2-phenylphenol between rat and mouse could not account for the difference in urinary bladder toxicity and tumour response. It was speculated that additional factors such as localised deconjugation of PHQ glucuronide (or sulphate) followed by oxidation to PBQ may be responsible for the effects seen at high dose levels in the male rat

17) This is consistent with earlier hypotheses that activation of 2-phenylphenol occurs as a 2 stage process with cytochrome P450 mediated formation of PHQ in the liver followed by a prostaglandin synthase-mediated oxidation of PHQ to PBQ in the urinary tract. It has been shown that the peroxidase component of prostaglandin H synthase can efficiently convert PHQ to PBQ and there are appreciable levels of this enzyme in rat (and human) bladder epithelium ^(11, 12, 13).

18) Data on the mutagenic potential of these metabolites, relating to oxygen radical generation, will be considered after the further data now available on DNA adducts is summarised.

Studies of DNA adducts in bladder published since the comprehensive review by the COM in 1992

19) The ability of 2-phenylphenol to induce DNA adducts in vitro has been investigated using ³²P-post-labelling techniques and human HL-60 cells ⁽¹⁴⁾. These are a human promyelocytic cell line that has significant myeloperoxidase activity and was used as a model for the activation of PHQ by prostaglandin H synthase in the urinary bladder.

20) Treatment of HL60 cells with 25-100µM PHQ for 8 hours resulted in a dose related increase in total DNA adduct levels. One principal and three minor adducts were identified with a relative distribution of 80:10:6:4. Total adduct levels were in the range 0.26-2.31 adducts/10⁷ nucleotides.

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Treatment of HL-60 cells with 25-250 μ M PBQ for 2 hours resulted in a similar response.

21) It was felt that these results showed that both PHQ and PBQ could form DNA adducts and thus peroxidase activation of 2-phenylphenol in the bladder may play a role in the induction of bladder tumours. However none of the DNA adducts were identified. When purified calf thymus DNA was reacted with PBQ one DNA adduct was identified but this did not correspond with any of the adducts formed in the HL 60 studies

22) Post-labelling studies have also reported adduct formation in vivo using bladder DNA ⁽¹⁵⁾. Rats were fed a diet containing 2% 2-phenylphenol for 13 weeks and DNA was then isolated from excised urinary bladder. Analysis by ³²P post-labelling revealed one major adduct in the bladder from treated rats. The identity of this was not studied but it was thought to be the PHQ semiquinone radical intermediate formed during conversion of PHQ to PBQ. However the DNA used in these studies was isolated from the total bladder, including mucosa, connective tissue of submucosa and smooth muscle wall, and the relationship of these findings to the bladder mucosa epithelium (less than 10% of the total bladder), which is the target tissue, is not clear.

23) In addition to the above there is also one report of the identification of DNA adducts in the skin of mice treated dermally with 2-phenylphenol ⁽¹⁶⁾ (sodium salt) or phenylhydroquinone and using the ³²P post-labelling technique. Topical application of 10mg of 2-phenylphenol resulted in evidence of 2 adducts and 20mg produced 4 adducts none of which were present in untreated skin. Levels were estimated to be in the range of 1.2 – 1.5 f moles/ μ g DNA. These dose levels were relatively high (the top dose being equivalent to ca 800 – 1000 mg/kg). They were known to produce cell proliferation and be effective for tumour promotion in one study in a two stage mouse model (2-phenylphenol is not a complete carcinogen and has given conflicting results in assays for skin promotion using 2 stage

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models). Reduced levels of adducts were seen in animals pre-treated with an inhibitor of cytochrome P-450 (α -naphthylisothiocyanate) or an inhibitor of prostaglandin synthetase (indomethacin) indicating that metabolic activation of 2-phenylphenol was involved in adduct formation.

24) Earlier in-vitro ^{32}P post-labelling studies by the same group had shown that 2-phenylphenol or PHQ, when incubated with DNA in the presence of cytochrome P450 or prostaglandin synthase activation, produced 4 major adducts ⁽¹⁷⁾. The adduct pattern appeared to be the same as in the in-vivo dermal studies. It was speculated that one of the DNA binding metabolites may be PBQ, although in neither study was the chemical structure identified.

25) The DNA adduct study considered by the COM in 1997, and criticised because of the use of insensitive techniques, has now been published, together with more details of the cytotoxicity seen in the rat bladder following sub-chronic exposure to 2-phenylphenol in the diet ⁽¹⁸⁾. Urothelial toxicity and hyperplasia occurred only at dose levels of 8000 and 12500ppm in the diet. No 2-phenylphenol-DNA adducts were detected in the urothelium at any dose level.

26) In the published paper a rationale for the omission of the use of nuclease p_1 enhancement (a major criticism by the COM) was given. This was that the potential 2-phenylphenol adducts are all of the aromatic type and can be resolved and detected using complex mixtures of chaotropic reagents and autoradiography /scanning/phosphorimaging respectively. Thus it was concluded that the tactile removal of non-adducted nucleotides during the initial development of the TLC system would pre-empt the need for nuclease p_1 -mediated enhancement, providing little or no advantage compared to the labelling of a total DNA digest. Moreover it was stated that nuclease p_1 treatment of a total digest may also be a limitation in terms of a complete or partial loss of certain adducts should the structure prove to be preferential substrates for 3^1 dephosphorylation by nuclease p_1 . In view of

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these considerations it was felt that the nuclease p₁ enhancement could be omitted, with reliance on chromatographic procedures for separation of ³²P-post-labelling normal nucleotides from adducts.

27) Both protein and DNA binding in the liver, kidney and bladder have been investigated in a recent study in male F344 rats⁽⁹⁾. In view of the difficulty of analysing the urothelium because of the small amounts available, a highly sensitive analytical technique, accelerated mass spectrometry (AMS), was used. Rats were dosed with 0, 15, 50, 125, 250, 500 and 1000mg/kg 2-phenylphenol, and its radiocarbon analogue, by oral gavage. Animals were killed 24 hours post dose and protein and DNA extracted and the radiocarbon content in the DNA and protein measured by AMS as the ¹⁴C:¹³C isotope ratio.

28) A clear, dose-related (linear or modest curvilinear) relationship was seen with protein binding in liver and kidney over the whole dose range. However in the bladder, protein binding showed a pronounced non-linear relationship, with increases over background being seen only at about 500mg/kg and above. There was no increase seen over background levels in DNA binding at any dose level in bladder, liver or kidney.

29) The authors concluded that these data were consistent with the hypothesis that 2-phenylphenol is an indirect acting carcinogen, and that regenerative hyperplasia due to 2-phenylphenol metabolite induced cytotoxicity and/or binding to protein targets, may play an important role in 2-phenylphenol induced bladder carcinogenesis. It is also consistent with the statement that the mechanism does not involve genotoxicity of 2-phenylphenol or its metabolites.

Genotoxicity of phenylhydroquinone (PHQ) and phenylbenzoquinone (PBQ)

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30) A number of studies have recently been published which have investigated the reactivity of the 2-phenylphenol metabolites PHQ and PBQ, and their ability to produce oxidative damage via reactive oxygen species. These are summarised below.

31) The ability of PBQ and PHQ to induce micronuclei and gene mutations was investigated in a prostaglandin H synthase containing V79 cell culture system ⁽¹⁹⁾. A number of different genotoxic end-points were investigated; mutations at the HGPRT gene as determined by 6-thioguanine-resistant cells; chromosome breakage and chromosome loss, as detected by the formation of micronuclei in the cytokinesis-blocked micronucleus assay, using CREST antibody to distinguish micronuclei containing whole chromosomes (CREST positive) from those containing acentric fragments (CREST negative).

32) No evidence of any dose-related micronuclei induction was seen in studies in V79 cells except in the presence of arachidonic acid supplementation. In the latter case an increase in CREST positive micronuclei (about 3 fold) was seen at concentrations of 125µM and above. However increasing concentrations did not produce any increase in micronuclei above this level. In view of the very odd dose response, with negative results at the 4 concentrations between 0-108µM, and no increase over the 3-fold background value for the 4 concentrations in the range 125 –187 µM, the biological significance of this result is unclear. There was no evidence of mutations in the HGPRT assay.

33) The authors suggested that the induction of aneuploidy in the assays supplemented with arachidonic acid may indicate that this plays a role in the 2-phenylphenol induced tumours in the rat bladder but it is doubtful if any definite conclusions can be drawn from these data

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34) In another study the ability of PHQ and PBQ to produce DNA strand breaks has been investigated in cultured human cells [HL60]. Oxidative damage was measured as the 8 oxo-7,8dihydro-2-deoxyguanosine (8-oxodG) content of DNA from treated cells⁽²⁰⁾. Concentrations of 15µM and above of both PHQ and PBQ produced an increase in 8 oxodG content but PBQ was considerably more effective in this regard. Studies using 32p-5 end labelled DNA fragments obtained from human P-53 tumour suppressor gene and c-Haras-1 proto-oncogene revealed that PBQ plus NADP and, to a lesser extent PHQ induced DNA damage frequently at 1-thymine residues in the presence of copper II. Catalase inhibited this, suggesting that hydrogen peroxide reacts with copper 1 to produce active species causing DNA damage.

35) These data suggest that metabolites of 2-phenylphenol cause oxidative DNA damage through the generation of hydrogen peroxide in cells, and that this may lead to mutations. PBQ appears to be more important than PHQ in this regard.

36) More recently the potential of 2-phenylphenol, PHQ and PBQ to produce oxidative DNA damage has been investigated in V79 cells⁽²¹⁾. Cells were incubated with compound and then analysed for formation of 8-hydroxy-2-deoxyguanosine (8 OHdG) and also for the induction of DNA single strand breaks, in nuclear DNA. Both PHQ and PBQ (but not 2-phenylphenol) significantly increased the function of 8 OHdG in nuclear DNA at 20µM, whereas 8 OHdG had no effect. The biological significance of this is unclear. Both PHG (at 35µM and above) and PBQ (at 25µM and above), but not 2-phenylphenol (at up to 400µM) produced an increase in single strand DNA breaks.

37) The chemical nature of the adducts formed with PBQ and calf thymus DNA, has been investigated⁽²⁵⁾. Reaction occurs preferentially with the 2-deoxyguanosine nucleobase, with formation of PBQ-²N-deoxyguanosine. This adduct was also observed with hepatoma cells were treated with PBQ.

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Investigation of micronuclei induction in rat bladder epithelial cells following oral exposure to 2-phenylphenol

38) There is one recent report of a study to investigate micronuclei induction, cell proliferation and hyperdiploidy in bladder epithelial cells of rats treated with 2-phenylphenol⁽²²⁾. Male Fischer 344 rats were given 2% 2-phenylphenol in the diet for 14 days. Twenty-four hours prior to being killed they were injected with BrdU. Bladder cells were isolated from the luminal surface and single cell preparations prepared and transferred to slides for staining for micronuclei. It was noted that due to the presence of binucleated cells, and the difficulty of distinguishing the membrane, individual intact nuclei were scored as cells and these nuclei were referred to as cells throughout the text and in the figures reported. In some instances where the membrane was not clearly visible, a micronucleus was scored based on proximity to the main nucleus. [This suggests that there were problems in distinguishing micronuclei from cell debris which would have been produced by the method used to isolate the primary bladder cells. In such studies the primary cell preparation needs to be cleansed from such debris before analysis; this was not done in this case]. Replicated cells were studied using BrdU and a labelling index determined. Changes in chromosome number were determined by fluorescence in-situ hybridization with a DNA probe for chromosome 4.

39) The results obtained with 2% 2-phenylphenol were compared to those obtained with 2% sodium chloride and also 2% sodium chloride plus 2% 2-phenylphenol in the diet.

40) A significant increase in frequency of micronuclei and in cell proliferation was seen in all treated groups. The effects seen were least with sodium chloride and greatest with 2-phenylphenol plus sodium chloride. No effects were observed on chromosome number in any group. The number of micronuclei per 2000 cells was about 5 in the controls, 15 in

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the animals treated with sodium chloride, 20 in animals treated with 2-phenylphenol, and 33 in those treated with 2-phenylphenol plus sodium chloride [data were only presented as a histogram]. Thus 2-phenylphenol produced essentially the same effect in this study as common salt.

41) In view of the limitations of this study, and the fact that the method is a novel assay, whose methodology has not been optimised or validated, it is suggested that no conclusion can be drawn regarding the mutagenicity of 2-phenylphenol.

Investigation of in-vivo genotoxicity using the COMET assay

42) The ability of 2-phenylphenol to induce DNA damage in-vivo in a range of tissues (stomach, liver, kidney, bladder, lung, brain and bone-marrow) has been investigated using a modified version of the COMET assay based on use of isolated nuclei⁽²³⁾. The COM have earlier [in the context of dichlorvos] expressed concerns at this technique used by Sasaki and colleagues.

43) In these studies a single very high dose level of 2-phenylphenol was used, namely 2000 mg/kg given orally by gavage in physiological saline. Tissue was obtained at 3, 8 and 24 hours post dose using 4 animals per group, homogenised, the nuclei obtained by centrifugation, placed on slides and examined by gel electrophoresis in 300mM sodium hydroxide to induce unwinding of the DNA. 50 nuclei were examined on one slide per organ. The length of the whole COMET at the diameter of the head were measured, with migration being calculated as the difference between length and diameter. It was recognised by the authors that cell death leads to DNA fragmentation and therefore positive responses should be considered with cytotoxicity data; however it was not possible to assess viability using this method since homogenisation disrupts the cell

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membrane. It was admitted that it was not possible to determine whether the observed damage was due to genotoxicity or secondary to cytotoxicity. [This was a major concern also of the COM when they considered the results of this group with another compound].

44) The results obtained indicated that activity was seen in the stomach, liver, kidney and lung at 3 hours, but only the stomach and kidney at 8 hours, and no tissues at 24 hours. It is very difficult to explain why activity should be seen in the liver at 3 hours following oral exposure, but not after 8 hours.

45) In view of the concerns about the methodology used in this assay and the somewhat odd time course seen with the activity, it is suggested that no conclusions can be drawn from this work with regard to the genotoxicity of 2-phenylphenol.

Summary

46) This section summarises the new data in the genotoxicity of 2-phenylphenol and considers the modifications necessary to the overall conclusions drawn in 1992 and 1997.

47) The key papers on which this is based are attached at Annex 2.

48) Additional data on the metabolism of 2-phenylphenol in the rat and the mouse confirms that at relatively low oral doses (of the order of 20mg/kg) the compound is metabolised and excreted in its urine as the sulphate, or, to a lesser extent, the glucuronate conjugate⁽⁸⁾. At higher doses conjugates of PHQ and PBQ were present, but very little non-conjugated material. Studies on the comparative metabolism in the rat and the mouse indicate only minor differences that would not be expected to explain the species specificity of the induction of the urinary bladder tumours in the male rat. It

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is possible that localised deconjugation of PBQ and PHQ glucuranide (or sulphate) may play a role at high dose levels in the male rat ⁽⁹⁾.

49) Further data are now available on the ability of 2-phenylphenol or its sodium salt to induce DNA adducts in the rat bladder in-vivo. The earlier studies had given conflicting results, with negative results following a very high acute exposure, but positive results in a subchronic study at 2% in the diet ^(7, 15).

50) The study considered by the COM in 1997, and criticised because of the insensitivity, has now been published, a rationale for the chosen methodology was given ⁽¹⁸⁾. More importantly a study to investigate both DNA and protein binding in the liver, kidney and bladder of male F344 rats has now been published using the highly sensitive AMS technique. An extensive dose response was investigated, (5 doses over range 15 – 1000mg/kg 2-phenylphenol). A clear dose-related and essentially linear response was seen with protein binding in the liver and kidney, but protein binding was only seen in the bladder at high dose levels (about 500mg/kg and above). There was no evidence for DNA binding at any dose level in any tissue.

51) The weight of evidence is now sufficient to conclude that 2-phenylphenol does not produce significant DNA binding in the male rat bladder.

52) The metabolic data indicate that conjugates of the quinone metabolites PHQ and PBQ can be formed at high dose levels and these can potentially give rise to oxidative DNA damage that may not be detected in the DNA binding studies. It is not possible to exclude this contributing in addition to the sustained cytotoxicity and compensatory hyperplasia, mechanistically in the induction of the bladder tumours. However such effects would not be expected to occur at low dose levels, and this is not incompatible with adopting a threshold based risk assessment.

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53) No conclusions can be drawn from the micronucleus assay in bladder and the COMET assay, in view of the difficulties in interpreting these studies, largely due to the inability to distinguish between genotoxicity and cytotoxicity^(22,23).

Draft Conclusions

54) The following draft conclusions with regard to the mutagenicity of 2-phenylphenol are proposed based on the 1992 conclusions, and revised in the light of the new data considered in this paper.

- i) Data from several assays to investigate the ability of 2-phenylphenol or its sodium salt to produce gene mutation in *Salmonella typhimurium* were consistently negative.
- ii) Positive results were obtained in in-vitro metaphase analysis studies in CHO cells and also in mouse lymphoma assays in the presence of an exogenous metabolic activation system. The induction of small colonies in the latter assay is consistent with the compound having clastogenic potential.
- iii) Phenylhydroquinone (PHQ) and phenylbenzoquinone (PBQ), which are metabolites of 2-phenylphenol after exposure to relatively high dose levels, have been shown to produce oxidative DNA damage and single strand DNA breaks in-vitro.
- iv) Negative results were obtained in bone marrow assays for clastogenicity in-vivo and also in germ cells (dominant lethal assay), indicating that any possible clastogenic potential was not expressed in the whole mammal.
- v) The weight of evidence from in-vivo studies to investigate DNA binding in the male rat bladder is negative, including a recent study using highly sensitive AMS techniques.
- vi) Although a contributory role of oxidative DNA damage cannot be excluded when considering the mechanisms of bladder tumour

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induction in the male rat, such effects would not be expected to occur at low dose levels

- vii) It is reasonable to adopt a threshold based risk assessment to 2-phenylphenol and its sodium salt

Secretariat

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