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

Preface

The Committee on Carcinogenicity of Chemicals in Food, Consumer Products and the Environment (COC) evaluates chemicals for their human carcinogenic potential 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.advisorybodies.doh.gov.uk/coc/index.htm).

During 2005, the Committee provided advice on a wide range of topics, including the chemicals furan, proquinazid, perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA), and the potential carcinogenicity of nanomaterials. It also completed its review of the potential chemical aetiology of childhood cancer, in particular, the possible association between childhood leukaemia and living near sources of traffic exhaust and petrol fumes.

The assessments of furan, proquinazid, PFOS and PFOA were undertaken in conjunction with its sister committee, the Committee on Mutagenicity of Chemicals in Food, Consumer Products and the Environment (COM). The assessment of nanomaterial toxicology was undertaken jointly with the COM and the Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (COT).

The COC gives advice on generic issues and on new developments in chemical carcinogenicity. The COC held a successful joint meeting with the COM in June on the use of target organ mutagenicity data in carcinogen risk assessment. During the course of the year, the COC also considered the development by the International Life Sciences Institute (ILSI) of the mode of action approach for chemical carcinogens to generate guidance on how to assess the relevance of an animal carcinogens to humans.

This was my last full year as chairman of the committee, as I stand down in March 2006. It has been a valuable and rewarding experience. I would like to record my thanks and appreciation of the work carried out by members of the committee during my 10 year tenure as chairman and of the support of the scientific and administrative secretariat. My successor as chairman is David Phillips, who is Professor of Environmental Carcinogenesis at the Institute of Cancer Research.

I wish him well and am sure he will lead the committee successfully through new challenges in the future.

Professor PG Blain CBE (Chairman)
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Carcinogenesis mode of action and human relevance framework

3.1 The International Programme on Chemical Safety (IPCS) has developed a framework for evaluating modes of action (MOA) of chemical carcinogens. The COC reviewed the IPCS MOA framework in 1999 and endorsed the proposed approach. Since then, the initial framework has been used by a working group under the sponsorship of the US Environmental Protection Agency (EPA) and ILSI Risk Science Institute (RSI) to develop guidance on how to assess the relevance of an animal carcinogen to humans, based on MOA data. This 'Human Relevance Framework' (HRF) is based on the premise that, once an MOA has been established for the tumourigenic effects of a chemical in experimental animals, a systematic analysis of the individual key events comprising that MOA should enable its relevance to humans to be determined.

3.2 At its April 2005 meeting, the COC considered a paper published in 2003 which describes the HRF and includes seven case studies. A further three evaluations of anonymised pesticides carried out by Defra's Pesticides Safety Directorate were also considered.

3.3 Members noted that the HRF approach extended the MOA approach by considering whether the key events in the MOA are plausible in humans, taking into account kinetic and dynamic factors. They also noted the advantages of collaborative working to enable a consensus view to be reached where there was both conflicting evidence and conflicting views in the published literature about the mechanism of carcinogenesis of a chemical. One particular value of the HRF approach was considered to be that data were presented in a structured format which allowed gaps in the data to be readily identified. It was hoped that completion of the case studies would provide generic evaluations, limiting the need for duplication of effort. The COC concluded that both the MOA and HRF approaches provided a logical framework in which to set the information needed when assessing the relevance of chemical induced animal tumours to humans.

Childhood cancer

Overview

3.4 The COC began a review of childhood cancer in 2004. It was asked to evaluate the published literature on whether there was an increased incidence of childhood cancer in the UK and to address whether evidence from epidemiological studies suggests a possible chemical aetiology. Based on the available epidemiological data, four childhood tumours were identified as being relevant for further consideration: CNS tumours; acute lymphocytic leukaemia (ALL); germ cell tumours (GCT) and neuroblastomas (NBT).

3.5 The COC obtained expert advice from the UK Childhood Cancer Group (UKCCRG) and the Newcastle Childhood Cancer Group on whether the reported increased incidences in these tumours was accounted for by better ascertainment. UKCCRG data confirmed an upward trend in the incidence of all four tumours. The increase in ALL was considered to be to some extent a true increase in incidence. However, chemical pollution was unlikely to be a significant risk factor. It was not clear if the upward trends in the incidence of childhood CNS tumours and germ cell tumours were real or due to better diagnosis. Advice was received that there is an increase in neuroblastomas but probably largely in benign tumours, under 1 year of age, and due to better diagnosis.

3.6 Overall, Members considered the evidence for a chemical aetiology for these tumours to be weak. The COC discussed the possible role of transplacental carcinogens and paternal exposure, and the significance of animal models in the risk assessment of childhood cancer. It was pointed out that any risk assessment modelling of transplacental carcinogenesis needed to separate genotoxic compounds and non-genotoxic compounds.

The possible association between childhood leukaemia and residence near sources of traffic exhaust and petrol fumes

3.7 The COC was asked by the Department of Health Air Pollution Unit to advise on three epidemiology studies which had investigated whether there was an association between residence near to petrol stations, garages, and road traffic exhaust fumes and the occurrence of childhood leukaemia. The COC subsequently conducted a full review of the epidemiological literature on this topic. It concluded that the available evidence provided insufficient evidence from which to conclude that there was an association between risk of childhood leukaemia and proximity to petrol stations, garages and road traffic. A full statement can be found at the end of this report.

Consideration of paper by Knox et al on "Childhood cancers and atmospheric carcinogens (Journal of Epidemiology and Community Health (2005), volume 59, pp 101-105)

3.8 The COC was asked to consider this paper by the Department of Health. The paper reports an investigation of the relation between major sources ("hotspots") of emissions of a range of pollutants, taken from Internet maps for 2001 complied from the National Atmospheric Emissions Inventory, and birth and death addresses of children dying from cancer between 1966 and 1980. It reports a positive association between deaths from cancer and hotspots for a number of air pollutants.

3.9 The COC noted a number of problems with the paper and concluded that they were unable to draw any conclusions from the study, because of flawed methodology and the inadequate information provided.

Furan

3.10 Furan is used as an industrial chemical or in the synthesis of commercial compounds. It is also present as a contaminant in air, tobacco smoke and many foods and beverages. The FSA sought the advice of the COT on the toxicity of furan during 2005 and the COT referred it to the COC for advice on carcinogenicity.

3.11 The COC noted that furan is rapidly and extensively absorbed and distributed following administration to rats. A key reactive metabolite is cis-2-butene-1,4-dial. Two NTP studies have indicated that furan increases the incidence of cholangiocarcinoma in rats, hepatocellular tumours in rats and mice, phaeochromocytoma of the adrenal gland in mice, and mononuclear cell leukaemia in rats. At least two possible modes of action for furan-induced liver changes have been described: altered cell proliferation and oxidative stress mechanisms.

3.12 The COC heard that the COM had concluded that furan should be regarded as an *in vitro* mutagen, but that there was insufficient evidence to reach a conclusion on the available *in vivo* mutagenicity data.

3.13 Recent work conducted by a group from the University of Birmingham in collaboration with AstraZeneca had examined the role of oxidative stress in the induction of cholangiocarcinoma in the rat. A representative from AstraZeneca gave a presentation of the findings to the COC. He outlined what is known about cholangiocarcinomas in humans, specifically, that many oncogenes, including p53, are up regulated. In rats, these tumours are induced by potent genotoxins and also by proposed non-genotoxic mechanisms (coumarin), when the same oncogenic changes are apparent. The 'furan' model of tumour formation is the best characterised. In this model, administration of furan results in characteristic morphological changes in the liver: intestinal metaplasia, diffuse centrilobular toxicity and a distinct focal response in the portal tracts. Notably, only particular lobes are affected. Administration of furan for one month is sufficient to cause tumours by one year. Areas expressing the enzyme CYP2E1 correlate with areas of necrosis and p53 activation, although it is noted that CYP2E1 is down regulated on continued administration. In areas where the periportal damage occurs, ductal cells develop as early as day 3, although only in the target lobes. This lesion spreads, becomes surrounded by basement membrane, and is infiltrated by intestinal metaplasia and capilliarisation of the damaged area. If furan administration is discontinued, the properties of intestinal metaplasia are maintained. High, cytotoxic doses are required to elicit this response.

3.14 Members proposed the following mode of action: the cytotoxic intermediate generated by CYP2E1 was stimulating the regenerative response via oval cell proliferation and/or biliary progenitor cells. The intestinal infiltration seen may be a secondary response. Phenotypic changes were evidently being induced by the chronic inflammatory response, although it was not clear how furan was activating the mechanism leading to the response. It was unfortunate that no data were available on the response at low doses i.e. below those used in the NTP study, where cholangiocarcinoma occurred at all dose levels.

3.15 In conclusion, the COC considered that it was reasonable to hypothesise that there was a threshold for the mechanism for induction of cholangiocarcinoma but this needed further investigation before a decision could be made. It was suggested that the following were required: carcinogenicity studies with lower doses of furan, *in vivo* mutagenicity data, and information on metabolite production and the contribution of metabolites to an oxidative stress mechanism. The COC noted that the mode of action for cholangiocarcinoma may not be applicable to the other tumours induced by furan but commented that the strain of rat used in the NTP study was known to have a high incidence of mononuclear cell leukaemia and that the phaeochromocytomas in the mouse may arise from a neuroendocrinological mechanism.

Perfluorooctane sulfonate (PFOS) and Perfluorooctanoic acid (PFOA)

3.16 Perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) are multifluorinated chemicals based on an eight carbon linear molecule (some branched chain and lower alkane fluorinated carbon impurities may be present at low levels). PFOS and PFOA have unusual physico-chemical properties, forming microdispersion micelles in aqueous systems. PFOS and related compounds are used as in industrial and household applications. PFOA is used in industrial applications and is also produced by

environmental degradation of some PFOS precursors and fluorotelomer alcohols. The PFOS salt in most widespread use is ammonium perfluorooctanoate (APFO). PFOS and PFOA are persistent and bioaccumulative and could potentially enter the food chain. The FSA sought the advice of the COT on the toxicity of furan during 2005 and the COT sought the advice of the COC on the carcinogenicity of these chemicals.

PFOS

3.17 The COC considered the one dietary carcinogenicity study on PFOS, in Sprague-Dawley rats. Non-neoplastic effects reported in the liver included increased absolute and relative liver weight, hepatocellular cystic degeneration and hepatocellular hypertrophy (often associated with vacuolation), with a NOAEL of 2 parts PFOS per million parts diet (2 ppm). No signs of hepatotoxicity were evident 52 weeks after cessation of administration of the highest dose for 52 weeks. The incidence of hepatocellular adenomas was significantly increased at the 20 ppm level in both male and female rats. There was a single hepatocellular carcinoma in the female high dose group. The incidence of thyroid follicular cell adenoma was significantly increased in the male high dose recovery group, but not in the male and female high dose groups fed PFOS for 104 weeks. The study had investigated treatment-related effects on hepatic palmitoyl-CoA oxidase activity, but the COC considered that the time points at which activity was measured were not optimal for this endpoint.

3.18 The COC also reviewed the results of a dietary carcinogenicity study in Sprague Dawley rats in which *N*-ethylperfluorooctanesulfonamido ethanol (*N*-EtFOSE) was administered at dose levels of 3, 30 and 100 ppm in the diet for 104 weeks. This compound has been shown to degrade to PFOS. No significant treatment-related effects were observed at 2 years. However, survival in all groups, including the controls, was relatively poor. There was evidence of hepatocellular hypertrophy in high dose males and females. The incidence of hepatocellular adenomas was slightly higher in high dose male and female groups than in controls. A single hepatocellular carcinoma was observed in a high dose female.

3.19 Two limited epidemiological studies (a retrospective mortality study and an 'episodes of care' analysis) had been conducted in occupationally exposed populations. Cohorts were relatively small and also relatively young. The COC noted that, in the retrospective cohort mortality study, standardised mortality ratios were below one for all causes of death and all malignant neoplasms when analyses were restricted to workers with at least one year of employment and high exposure to PFOS. There was a statistically significant increase in deaths from malignant neoplasms of the bladder in males with over 5 years in high exposure jobs. Members questioned the adequacy of exposure assessment by using job categories and noted that there had been potential exposure of the workers to benzidine, a known bladder carcinogen. The COC advised that, overall, it was not possible to draw definite conclusions from this study. The episode of care analysis was considered unusual in design and uninformative.

3.20 In conclusion, the COC concluded that there was equivocal evidence for carcinogenicity in the animal studies, limited to hepatocellular adenoma, and that the NOAEL was 5 ppm. It further concluded that a threshold approach could be used for the risk assessment of PFOS. The COC noted that a mode of action involving peroxisome proliferation had been proposed but was not convinced that adequate evidence had been provided to support this.

PFOA

3.21 The carcinogenicity of PFOA has been investigated in two dietary exposure studies in Sprague-Dawley rats. In the first, ammonium perfluorooctanoate (APFO) was administered at levels of 0, 30, or 300 ppm in the diet for 104 weeks. Dose-related non-neoplastic liver effects included megalocytosis, cystoid degeneration and portal mononuclear infiltration. A significant increase in the incidence of mammary fibroadenoma in female rats was considered by the study authors not to be related to treatment on the basis of comparisons with the incidence in historical control data. Similarly, a dose-related increase in the incidence of Leydig cell adenomas was not significant compared to the historical control incidence. The COC noted that there were at least two viral infections in the rats used in this study and considered that this limited its value. In the second study, a single high dietary dose of PFOA (300 ppm) was administered for 24 months. Increased incidences of hepatocellular adenoma, Leydig cell adenoma and pancreatic acinar cell adenoma were reported. Serum estradiol concentrations were significantly increased in treated animals.

3.22 The COC noted that a hypothesis had been put forward which proposed that PFOA induces liver, Leydig cell and pancreatic acinar cell tumours via PPAR-alpha activation and that, therefore, these tumours are unlikely to be induced in humans. However, Members noted that PFOA showed indications of oestrogenicity, possibly via aromatase induction, and thus it was not possible to be certain that all toxic effects were mediated via peroxisome proliferation. They were informed of some recently retrieved research which indicated that PFOA had effects on liver weight in PPAR-alpha null mice. The committee agreed with a proposal that the observed increases in serum estradiol levels suggested a mode of action for the Leydig cell tumours. However, it considered that it was not possible to propose modes of action for the liver and pancreatic tumours observed in the second study. However, the committee concluded that it would be acceptable to use a threshold approach for the risk assessment of PFOA i.e. to select an appropriate NOAEL for a precursor event for the most sensitive tumour and to apply an uncertainty factor of 100.

3.23 In two linked, retrospective cohort studies of mortality in an occupationally exposed population, small increases were reported in death from cancer of the large intestine and from cancer of the prostate in employees with over 1 year definite exposure to PFOA. The COC considered that none of the effects reported were significant for the risk assessment of PFOA.

Proquinazid

3.24 Proquinazid (6-iodo-2-propoxy-3-propyl-3H-quinazoli-4-one) is a novel fungicide under consideration by the Advisory Committee on Pesticides (ACP). It is intended for use in agriculture and viticulture, providing control of powdery mildew in cereals and grapes. The ACP sought advice from the COC and COM on an increased incidence of cholangiocarcinoma in female rats in a carcinogenicity study with proquinazid.

3.25 At its meeting on 14 July 2005, the COC heard a presentation from representatives of the data holders, Du Pont, on the interpretation of the cholangiocarcinomas. Du Pont representatives noted that proquinazid was not genotoxic, that cholangiocarcinomas of the "intestinal" type occurred only in female rats fed 600 ppm or more at the end of the two-year study, the tumour type was linked to

chronic hepatocellular injury with "cholangiofibrosis" and there was no risk of the lesion occurring in the absence of severe hepatic toxicity. A number of representative sections were shown to COC members. It was noted that the lesions termed "cholangiocarcinoma" were quite unlike the usual pattern seen in human cholangiocarcinoma. COC members supported the view that the lesions were not neoplastic but were a florid inflammatory response to severe acute and chronic hepatocellular damage.

3.26 The COC concluded that it was uncertain that the lesions termed "cholangiocarcinomas" were truly neoplastic but accepted the conventionality, used by the study pathologist in the report of the carcinogenicity study, of classifying them as neoplastic. Members considered that a satisfactory non-genotoxic Mode of Action explanation had been submitted. The COC agreed that a threshold approach to risk assessment could be used and agreed that there was a satisfactory margin of safety between the NOAEL for putative cholangiocarcinoma and the Acceptable Daily Intake proposed by the Pesticide Safety Directorate. A full statement of the committees' assessment of proquinazid is attached at the end of this report.

Review of the quantitative relationship between alcohol consumption and squamous cell carcinoma

3.27 The COC reviewed the relationship between alcohol and oesophageal cancer in 1995 as part of the review of alcohol and cancer. Several studies indicated that there was a quantitative relationship between alcohol intake and squamous cell carcinoma (SCC) of the oesophagus but a threshold level could not be defined. Therefore, the COC had estimated levels of alcohol intake for which there was convincing evidence of an increased risk of SCC. In 2004, a paper on oesophageal cancer had suggested that further consideration of alcohol-induced SCC was necessary. Following consultation, it was decided that a review should be conducted to review the post 1995 data on the quantitative relationship between alcohol and SCC. The COC also reviewed data on the risk in smokers who drink heavily and the possible existence of any susceptible groups.

3.28 Twenty-four new epidemiological studies were retrieved for review by the COC. It was noted that the data suffer from the same limitations as were noted in 1995 including a lack of data in women, poor study design, and imprecise estimates of alcohol intake. The studies rarely considered confounding by other risk factors, such as dietary intake, lack of vitamins and, possibly, Barrett's oesophagus. Also, members did not consider that the studies were easily comparable and suggested that it may only be necessary to consider the meta-analysis data, as these give the best estimates. Nevertheless, it was considered that they strengthened the overall picture. An increased risk was apparent at intakes above 30 g alcohol per day. However, the COC could not identify a level of consumption below which there is no increase in risk, due to both lack of data and the intrinsic weakness of the data (in particular, the fact that the 'non-drinkers' group may include light drinkers).

3.29 The COC considered that the data provided evidence of potentially susceptible groups. It noted the multiplicative effects of alcohol and smoking on the incidence of SCC and that smoking increased the risk of SCC at even moderate doses of alcohol. However, gender and ethnic differences were considered possibly to reflect temporal differences in drinking habits rather than a real difference in susceptibility.

Single or short term exposure to carcinogens

3.30 Carcinogenicity is usually evaluated in the context of long term exposure to a chemical. In carcinogenicity studies, animals are subject to medium to long-term exposure to a test substance with the aim of establishing whether it has any carcinogenic potential. This information, together with any available epidemiological or other data, is used to characterise the risk to humans from prolonged exposure to the chemical. In the case of genotoxic carcinogens it is assumed that there is no threshold and that any level of exposure carries a theoretical risk. The risk from non-genotoxic carcinogens is characterised using a NOAEL and uncertainty factor to estimate a level of exposure at which no risk is anticipated.

3.31 It may be necessary to provide advice on whether there is a risk associated with acute exposure to a carcinogen, e.g. in the case of a chemical accident or a food contamination incident. This would entail the characterisation of cancer risk in later life following a single or short-term exposure some time earlier. At its horizon scanning exercise in 2004, the COC agreed to review this issue and it did so at its April 2005 meeting.

3.32 In 1999, Calabrese and Blain published information about a database of data from approximately 5500 studies which assess whether a single dose of a chemical (or physical) agent can cause tumour development in animals, without exogenous promotional stimuli. The database contained information on approximately 800 chemicals, of which 426 caused a positive response. The authors claim that it reveals that a single dose of an agent can cause tumours to develop in males and females of numerous animal models in all principal age groups. The COC reviewed this paper and an overview of studies relating to limited exposure (less than one year) to occupational carcinogens, also cited in the paper.

3.33 Calabrese and Blain had described the period of less than one year as relevant to the identification of potential single/short-term exposure to carcinogens but the COC considered that there was no rationale for this criterion. Members considered that exposure to single doses of certain cytotoxic medicines or genotoxic carcinogens, or possibly asbestos, might be associated with an increased risk of cancer. It was possible that the persistence of a carcinogen after exposure was a potentially important factor in the assessment of risk associated with a single exposure: beryllium was cited as an example of a persistent carcinogen. The COC cautioned that care was needed when interpreting the data cited in the Calabrese and Blain paper; for example, single parenteral exposure of experimental animals to chemicals in pellets might be associated with carcinogenicity in animals but such data were unlikely to be relevant to single exposures of humans to carcinogens. It considered that there was no evidence to support the view proposed by Calabrese and Blain that risks associated with single exposures to carcinogens varied with life stages. It was suggested that a more detailed review may be required of the information cited in the Calabrese and Blain database.

Advice to the European Food Safety Authority (EFSA)

3.34 The committee submitted a response as part of the consultation on the 'Draft Opinion of the EFSA Scientific Committee on a Harmonised Approach for Risk Assessment of Compounds which are both Genotoxic and Carcinogenic'.

Horizon scanning

3.35 The COC undertakes "horizon scanning" exercises at regular intervals to identify new and emerging issues which have the potential to impact on public health. At the 2005 exercise, Members considered that the following topics should be considered further.

Age-related differences in risk of carcinogenesis

3.36 Conventional animal cancer studies start treatment with the test chemical in early adulthood – usually 6-8 weeks of age in rats and mice. However, after reviewing an extensive set of nonconventional animal carcinogenicity study results, the US Environmental Protection Agency (EPA) concluded in 2003 that there was appreciable evidence that juvenile exposures to genotoxic carcinogens conferred greater risks per day of dosing than do exposures during adulthood. The EPA proposed additional uncertainty factors for individuals aged under 2 years and aged 2-15 years. Recently, an improved analysis of the data for 9 chemicals suggested that the proposed additional factors may not be appropriate in all cases. This improved analysis proposed that the estimated population mean risk from lifetime exposures to a generic genotoxic carcinogen is about 2.8 forld larger than expected from adult-only exposure. The COC decided that it should review this work, and the topic should have high priority. The project should revisit the information which was examined when the committee reviewed the EPA guidelines for cancer risk assessment but should also look independently at the original data. It was noted that there is considerable discussion at present as to whether animal carcinogenicity data applied to children.

3.37 The COC also agreed to review data on *in-utero* exposures to carcinogens. This topic should have high priority.

Trends in cancer incidence

3.38 Members noted that the incidence of both testicular cancer and of non-Hodgkin's lymphoma were increasing and considered that the possible chemical aetiology of both should be reviewed. It was considered that these topics should both be given high priority. The committee also noted that the incidence of upper outer quadrant breast cancer was increasing and that there is interest in the media in the proposal that this cancer may be caused by carcinogens, e.g. parabens, absorbed through the armpit. The secretariat was asked to come forward with a proposal for work on breast cancer. This topic was also deemed to be high priority.

Comparative risk assessment

3.39 The committee supported a proposal to explore whether it was possible to compare risks from environmental carcinogens with risks from other activities. It also agreed with the view of the COM that more should be done on how to get the work of the committees across to the public. Both topics were deemed to be high priority.

Hormesis

3.40 Members agreed that the topic of hormesis should be discussed further, including a review of recent publications. This topic was considered to be of medium priority.

Paper by Jacobs (2005) on prediction of carcinogenicity results from the results of short-term studies

3.41 It has been proposed that the results of 2-year carcinogenicity studies could be predicted from certain short-term data, structural analyses or omics information. The committee considered a paper which concluded that such data are not currently sufficient to accurately and reliably predict the outcome of long-term carcinogenicity studies. It suggested that the committee should keep a watching brief on this issue.

Computational systems biology

3.42 It was also agreed that the committee should consider whether computational systems biology could be applied to its work. This topic was deemed medium priority.

Ongoing reviews

Aspartame

3.43 The COC provided *ad hoc* advice to the FSA on the results of a rat carcinogenicity study with aspartame published by the European Ramazzini Foundation. The committee expressed a number of reservations about the study and noted that it was due to be formally evaluated by EFSA.

Statements of the COC

Review of the possible associations between childhood leukaemia and residence near sources of traffic exhaust and petrol fumes

Background to review

1. Vehicle-related air pollution has increased considerably over the last 45 years, due to the increased numbers of vehicles on the roads (IEH, 1999). Traffic exhaust contains a complex mixture of many chemicals, of which several are known or suspected to be carcinogens. These include benzene, 1,3-butadiene and diesel-particulate matter. Diesel engine exhaust has been classified by IARC as Group 2A (i.e. probable human carcinogen) and gasoline† engine exhaust as Group 2B (i.e. possible human carcinogen) (IARC, 1989). The nature of traffic exhaust has, however, changed over the years following legislation about the composition of fuel.

2. Evaporative fuel emissions also contribute to vehicle-related air pollution. High airborne concentrations of fuel vapour are present in petrol stations and garages. Given that benzene is a component of both petrol/diesel exhaust and petrol vapour and is well documented as a cause of leukaemia in adults‡, there has been some concern as to whether residence near petrol stations/garages could be associated with an increased incidence of childhood leukaemia (petrol stations/garages are unlikely to generate sufficient traffic on their own to cause a substantial increase in pollutants from vehicle exhaust).

3. The Committee was asked by the Department of Health Air Pollution Unit to review three recent epidemiological studies which report an association between residence near to petrol stations and garages and/or road traffic exhaust fumes and the occurrence of childhood leukaemia. The Committee considered these studies (http://www.advisorybodies.doh.gov.uk/pdfs/cc0437.pdf) at the November 2004 meeting, but were unable to reach definitive conclusions on whether the studies indicated a causative link. The Committee therefore conducted a full review of the epidemiology literature on this issue, including studies reporting on benzene exposure and leukaemia in children.

Information reviewed

Childhood leukaemia: current perspectives on incidence trends and aetiology

1. The Committee first reviewed the incidence trends and potential chemical causes of childhood leukaemia in 2004, as part of a horizon scanning exercise to examine evidence for a possible role for chemicals in childhood cancer (http://www.advisorybodies.doh.gov.uk/pdfs/cc0431.pdf).

† Gasoline is the US term for petrol

‡ Benzene has been reported to cause Acute Myelogenous Leukaemia in ocupationally-exposed adults

2. Of the two main types of leukaemia that occur in children§, Acute Lymphoblastic Leukaemia (ALL) is the most common. There is evidence suggesting that the incidence of childhood ALL in the UK is increasing but members questioned whether this increase was real or an artefact resulting from better ascertainment of cases. An expert opinion was sought from the UK Childhood Cancer Research Group (UKCCRG) and the Newcastle Childhood Cancer Group who advised that the increase in ALL was, at least in part, real.

3. Only two non-hereditary risk factors are clearly associated with the development of leukaemia in children: intrauterine exposure to diagnostic X-rays and postnatal exposure to therapeutic doses of ionising radiation. In recent years, the use of these medical procedures in pregnant women and children has been extremely rare, and so it is likely that these aetiological factors now account for only a small percentage of leukaemia cases. The Committee noted that there is strong evidence for the involvement of non-chemical factors in the aetiology of childhood leukaemia (IARC, 1999). However, this does not preclude the possibility that there may be chemical risk factors, as childhood leukaemia is a biologically and clinically diverse disease and is likely to arise via a number of aetiological pathways (Greaves & Alexander, 1993).

Exposure to vehicular emissions as a possible cause of childhood leukaemia

4. The Committee considered that, because benzene is a well established leukaemogen in adults, benzene in vehicle exhaust and/or fuel vapour could represent a potential risk factor in the development of childhood leukaemia. Both vehicle exhaust and fuel vapour also contain several other carcinogenic compounds**, although there are differences in the profile of these carcinogenic constituents (and thus the carcinogenic potential). The Committee decided that there are differences in the hazards presented by these two types of emissions and, therefore, considered that information on exposure to fuel vapour should be discussed separately from information on exposure to vehicle exhaust.

(a) Exposure to fuel vapour

Exposure to fuel vapour arises largely from the evaporation of fuel from the tanks and engines of parked vehicles. Petrol evaporates more readily than diesel because diesel has a lower vapour pressure. Therefore, there are only a limited number of studies examining the risk of inhalation exposure to diesel vapour.

The Committee reviewed a draft report by the Oil Companies' European Organisation for Environment, Health and Safety (CONCAWE) on the risk assessment of inhalation exposure to 'gasoline††' (petrol) and benzene vapour by the general public (CONCAWE, 2004). Members commented on CONCAWE's main findings in relation to childhood exposure as described below.

§ Acute leukaemia tends to affect younger people with symptoms developing rapidly. The most common types are Acute Lymphoblastic Leukaemia (ALL) and Acute Myeloid Leukaemia (AML; also termed Acute Non-Lymphoblastic leukaemia (ANLL)). Chronic leukaemia tends to occur in older people and has a slow progression rate.

** Most tailpipe emissions consist of many hydrocarbons, including benzene, which itself is likely to derive from a mixture of unburnt benzene and benzene formed from the combustion of other aromatic petrol components.

†† CONCAWE referred to petrol as gasoline in their report.

1. CONCAWE assessment of exposure to petrol vapour

CONCAWE prepared separate risk assessments for three child-relevant scenarios:

- visiting service stations
- travelling in cars
- residing indoors at locations near petrol vapour sources

The report calculated typical and reasonable worst case (RWC) airborne concentrations (mg/m^3) and average daily doses (ADD) (mg/kg/day) for inhalation exposure to petrol for each of the above scenarios as shown in Table 1 below. (See http://www.advisorybodies.doh.gov.uk/pdfs/cc058.pdf for rationale)

Scenario	Airborne Concentration (mg/m^3)		Estimated Inhalation Intake (mg/kg/day)	
	Typical	RWC	Typical	RWC
Visiting service stations	2.7	3.83	0.0005	0.0006
Adults*	2.7	3.83	0.0011	0.0015
Children**				
Travelling in cars	3.15	3.94	0.04	0.06
Adults	3.15	3.94	0.12	0.15
Children				
Staying indoors (residence)	2.07	7.16	0.25	0.87
Adults	2.07	7.16	1.66	5.71
Children				

**Body weight used for adults = 70 kg*
***Body weight used for children = 9 kg*

Table 1. Summary of the exposure results for petrol vapour inhalation for several consumer scenarios (CONCAWE, 2004)

The Committee noted that children had considerably higher estimated inhalation intakes than adults. It was further noted that children staying in their homes received the highest estimated airborne exposures to petrol vapour (largely due to the amount of time spent indoors) with an ADD (RWC) value approximately ten-fold greater than that estimated for a service station attendant working a full shift (data not shown). However, it was further noted that much of the difference was due to variations in the duration of exposure represented by the three scenarios. The Committee accepted that these estimates were very conservative‡‡ and so were likely to have overestimated true exposures.

2. CONCAWE assessment of exposure to benzene vapour

The Committee also considered data from the CONCAWE report, as presented in Table 2, on airborne concentrations and estimated ADD for inhalation exposure to benzene vapour. Similar trends to those seen in the petrol vapour exposure assessment were apparent, i.e. indoor rather than outdoor exposures to benzene from petrol fumes appear to be of more significance. Again, the duration of exposure represented by the three scenarios accounted for much of the difference in intake between them.

Scenario	Airborne Concentration (μg/m³)		Estimated Inhalation Intake (μg/kg/day)	
	Typical	RWC	Typical	RWC
Visiting service stations	12	17	2.0E-03	2.9E-03
Adults*	12	17	4.7E-03	6.7E-03
Children**				
Travelling in cars	14	18	0.2	0.3
Adults	14	18	0.5	0.7
Children				
Staying indoors (residence)	10.7	26	1	3
Adults	10.7	26	9	20
Children				

**Body weight used for adults = 70 kg*
***Body weight used for children = 9 kg*

NB. The current levels of exposure to benzene from gasoline are likely to be lower than those estimated above. This is because the data was obtained from studies conducted prior to the 2000 European EU directive 98/70/EC, (in which levels of benzene in gasoline was reduced from 5% to 1%)

Table 2. Summary of the exposure results for benzene vapour inhalation for several consumer scenarios (CONCAWE, 2004)

(Figures have been converted into μg/m³ and μg/kg/day for ease of reference)

‡‡ Many of the underlying assumptions used to derive these estimates were based on the regulatory requirements of the risk assessment process. For example, CONCAWE used guidance provided in the EU Existing Substances Risk Assessment Technical Guidance Document to convert air concentrations of chemicals into daily doses. CONCAWE states that these estimates represent industry obligations in the process rather than the organisation's view of the current situation.

A substantial amount of information was available to the Committee from the published literature on levels of benzene inside and outside the home. This enabled the Committee to obtain a greater understanding of the issues relating to inhalation exposure to benzene vapour, and to generate a more considered view on the above exposure assessment.

i) Work by the Building Research Establishment showed that the mean indoor benzene concentrations in 174 homes within the Avon area (UK) were higher than outdoor levels, especially in those households with an attached garage (Duarte-Davidson et al., 2001). Furthermore, benzene concentrations in rooms directly above an integral garage have been reported to be 2.5 times the ambient air standard (Mann et al., 2001). See http://www.advisorybodies.doh.gov.uk/pdfs/cc058.pdf for further details.

ii) Studies providing data on mean air concentrations of benzene outdoors showed that UK levels were consistent with levels in the US and Canada. These levels varied depending on the proximity of parked or moving traffic – the highest levels of benzene were documented to be within the cars [up to 2527.2 $\mu g/m^3$ (780 ppb)§§] and may even reach the US Occupational Exposure Limit of 3240 $\mu g/m^3$ (1000 ppb), although Wolff (1992) reports that the typical range is in the order 32.4 – 64.8 $\mu g/m^3$ (10-20 ppb). Table 3 provides a summary of the range of benzene levels detected inside and outside the home.

iii) Members noted that there were a number of national and international initiatives intended to reduce population exposure to benzene. In a personal communication to the Committee, CONCAWE identified four important technological developments designed to reduce current levels of benzene emissions from automobiles (see http://www.advisorybodies.doh.gov.uk/pdfs/cc058.pdf). Air Quality Objectives in the UK are for an annual running mean standard*** for benzene of 5 $\mu g/m^3$ (1.54 ppb) in England and Wales, and 3.25††† $\mu g/m^3$ (1 ppb) for Scotland and Northern Ireland by 31 December 2010 (http://www.ehsni.gov.uk/pubs/publications/AQS_addendum_web.pdf). In Europe, the ambient air limit value‡‡‡ for benzene is 5 $\mu g/m^3$ (to be met by 1 January 2010, as set by the European Community Directive 2000/69/EC) (http://europa.eu.int/comm/environment/air/pdf/ppbenzene.pdf).

§§ 1 ppb = 1 part by volume, in one thousand million i.e. 1 in 10^9; 1 ppb of benzene = 3.24 $\mu g/m^3$ at 20ºC and 1013 millibars (http://www.defra.gov.uk/environment/airquality/aqs/benzene/4.htm)

*** the annual running mean standard refers to a value calculated from data collated for the 12 months prior to the quoted date

††† 3.246 ¤g/m3 rounded to nearest 2 decimal places

‡‡‡ the air limit value is a legislative standard, which takes cost and benefit into account and provides a date for when the legislation must be effected. The long term policy aim for England and Wales is to achieve an Air Quality Objective of 1 ppb annual mean concentration.

	[Benzene]/(μg/m^3)
Air Quality Objective	5
Outdoor*†	1 – 8
Indoor	4 – 40
Outdoor (20m busy road)*	10 – 45
Outdoor (kerbside)*	118
Cars (inside)*	32 – 2527
US Occupational Limit	3240

** It would be difficult to determine the contribution of evaporative emissions or vehicle exhaust to the benzene levels detected.*

† Levels of benzene in outdoor air during winter can be three times the level detected in summer.

Table 3. Ambient air concentrations of benzene (taken from Duarte-Davidson et al., 2001; Mann et al., 2001 and Wolff, 1992)

iv) The Committee was also reminded of the findings of the Working Group on Benzene (1998), which conducted a similar assessment of the potential risks from benzene exposure to the general population as part of a process to deal with "Ambient Air Quality Assessment and Management". These also suggested that children received much higher intakes of benzene than adults.

On the basis of the available information on levels of evaporative emissions inside and outside the home, the Committee concluded that exposures of children to benzene and petrol vapour inside the home could be greater than outside the home and that the significance of indoor exposures to petrol vapour and benzene should be considered further.

(a) Exposure to vehicle exhaust

One of the major challenges in epidemiological studies investigating the effects of exposure to vehicle exhaust is quantifying the exposure. This is because vehicle exhaust is a chemically complex mixture and its components derive from multiple sources (see Table 4 for a list of compounds present in vehicle exhaust). It was noted by the Committee that most epidemiological studies examining the effects of vehicular emissions have focussed on the combustion of fuel rather than on fuel evaporation. Therefore, the subject of exposure to vehicle exhaust is discussed further in the following section.

Gas phase	Particulate phase***
Acrolein	Heterocyclics and derivatives
Ammonia	Hydrocarbons (C14-C35) and derivatives
	Inorganic sulphates and nitrates
Benzene	Metals (e.g., lead and platinum)
*1,3-Butadiene**	
Formaldehyde	**Polycyclic aromatic hydrocarbons and derivatives**
Formic acid	
Heterocyclics and derivatives**	
Hydrocarbons (C1-C18) and derivatives**	
Hydrogen cyanide	
Hydrogen sulphide	
Methane	
Methanol	
Nitric acid	
Nitrous acid	
Oxides of nitrogen	
*Polycyclic aromatic hydrocarbons and derivatives**	
Sulphur dioxide	
Toluene	

(Source: IARC, 1989)

* compounds in italics have been classified by IARC as either carcinogenic to humans, probably carcinogenic to humans or possibly carcinogenic to humans (Groups I, IIA or IIB, respectively)

** derivatives include acids, alcohols, aldehydes, anhydrides, esters, ketones, nitriles, quinines, sulphonates and halogenated and nitrated compounds and multifunctional derivatives

*** diesel exhaust particulates are reasonably anticipated to be human carcinogens according to the National Toxicology Program 10th Report on Carcinogens

NB. Nitroarenes are also present in engine exhaust and are produced in large amounts from diesel engines. They are formed when nitric acid reacts with PAHs to form nitrated PAHs.

Table 4. Some compounds and classes of compounds in vehicle engine exhaust (IARC, 1989)

Epidemiological studies of exposure to traffic-derived pollutants and childhood leukaemia

The Committee was provided with information from 17 studies (sixteen§§§ published as peer-reviewed papers), which have investigated the possible association between proximity of residence to road traffic exhaust fumes, and/or petrol stations and garages, and either childhood cancer or childhood leukaemia. Table 5 provides a summary of the 17 studies reviewed by the Committee. Three of the studies were from the UK and only nine specifically investigated childhood leukaemia http://www.advisorybodies.doh.gov.uk/pdfs/cc058.pdf. Two studies examined exposures to vehicle evaporative emissions; vehicle exhaust was the predominant chemical exposure evaluated.

> Ten of the 17 studies reported some positive significant associations, although three authors concluded that their positive findings were only suggestive of an association due to the qualitative limitations of the study.

§§§ The unpublished results of a study by Urayama et al. (2004) were presented as a poster at an international conference on 'Children with Leukaemia' in London (2004).

Evaluation of epidemiological evidence

The Committee reviewed the methodology used in the studies to help formulate its conclusions and proposed that, based on the quality of the study designs, the actual number from which conclusions could be drawn was much lower than 17. Only two studies were considered to have adopted an adequate method for the assessment of exposure to petrol fumes and exhaust (i.e. Raaschou-Nielsen et al., 2001, and Reynolds et al., 2002). Both of these studies reported null results. However, Members considered it would be inappropriate to dismiss completely the 10 studies reporting positive findings.

Most of the studies were population-based, case-control studies where cases were identified from cancer registries. One study identified cases and controls from hospitals (Steffen et al., 2004) and five were geographical studies. Controls for population based studies were mostly selected from registers of the general population or via random digit dialling (RDD) – the latter method was considered to be a source of selection bias. In Steffen et al. (2004), controls were selected from children in the same hospital receiving treatment for acute pathologies such as trauma or orthopaedic diseases. The majority of studies matched controls for age, sex and area (for more information on control selection see http://www.advisorybodies.doh.gov.uk/pdfs/cc058.pdf).

In general, researchers based exposure assessments on objective measurements such as residence, traffic counts and/or measurements of concentrations of air pollutants, which are not affected by recall bias. However, Steffen et al. (2004) cited possible recall bias in that the mother of cases may have over-declared previous exposures to sources of hydrocarbons; Crosignani et al. (2004) suggested that the method used by his group to sample controls could be a source of selection bias.

Few of the 17 studies obtained comprehensive information on the child's or parent's past exposures to chemicals and other potential confounders.

Members considered a suggestion that a meta-analysis be carried out on the studies but concluded that the methodologies used in the studies were too divergent for this to be feasible.

Members made the following observations and suggestions:

(a) Sample size

With the exception of Reynolds et al. (2003), most of the papers reporting a positive association were based on much smaller sample sizes (the average number of leukaemia cases = 394) than those reporting a null association (average no.= 861). The two negative studies with adequate exposure assessment referred to above Raaschou-Nielsen et al. (2001) and Reynolds et al. (2002) were noted to be among the largest.

(b) Exposure Assessment

Most of the studies used area-based measures as proxies for traffic-exhaust exposures (See http://www.advisorybodies.doh.gov.uk/pdfs/cc058.pdf for further details). The Committee suggested that, although the methods currently used to assess exposure to petrol fumes/exhaust have generally improved with time, there is still potential for further development/refinement. However, useful information can be obtained from existing studies provided it is clear what the exposure metric represents. It was suggested that the distance-weighted traffic density metric was a useful approach although it should be made more specific for each pollutant to give greater utility in the ensuing risk estimation (http://www.advisorybodies.doh.gov.uk/pdfs/cc058.pdf).

(c) Case-control distance comparison

The Committee also considered the distance between the source of traffic exhaust/petrol fumes and the index**** child's house. The impact of main roads on air quality is not considered to be detectable above local background air pollution more than 100-200m away. Therefore, those studies in which concentrations of pollutants were measured or estimated in homes that were located more than 200m away from traffic roads or petrol stations and garages were considered to provide little useful relevant information.

(d) Age-related factors and residential history

The Committee noted that most studies of traffic and childhood leukaemia examined exposure potential based on a child's residence at either diagnosis or birth. Only two investigations obtained information on residence of the parent prior to birth, usually due to limitations in the recorded information available at the time, although the Committee noted that the study by Raaschou-Nielsen et al (2001) collated data spanning a child's complete residential history and encompassed an exposure window from 9 months before birth through to the child's date of diagnosis. The Committee suggested that, ideally, future studies should take into account a child's complete residential history and not just residence at diagnosis, although it was recognised that this would be difficult.

(e) Genetic susceptibility

Finally, the Committee considered the question of genetic susceptibility to possible traffic fumes/fuel vapour related childhood leukaemia, in the context of the study by Urayama, 2004. This investigated the combined effects of genetic polymorphisms and traffic exposure on risk of childhood leukaemia and found an association between leukaemia and traffic only in those with the CYP1A1 m2 allele. Interestingly, without accounting for individual genetic susceptibility, the authors found no association between risk of childhood leukaemia and traffic density. The Committee noted that the study was not in line with the COC criteria for the design of gene-environment studies because it lacked a prior hypothesis and hence the power of the study was lower than that assumed in the analysis.

**** child diagnosed with cancer of interest

Conclusion

The Committee concluded that the available evidence provided insufficient evidence from which to conclude that there was an association between risk of childhood leukaemia and proximity to petrol stations, garages and road traffic.

The Committee considered that further studies to examine the possibility of an association between residence near traffic exhaust/petrol fumes and leukaemia would be valuable. The Committee concluded that there are differences in hazard between vehicle exhaust and evaporative emissions. Therefore, it considered that information on exposure to fuel vapour should always be discussed separately from exposure to vehicle exhaust. It requested that indoor exposures of children to petrol vapour and to benzene should be considered in a separate review.

It was recommended that future studies should also investigate leukaemia on a subtype basis (ALL, ANLL) rather than leukaemia as a whole, and any evaluation of genetic susceptibility should include the examination of a comprehensive range of Phase I and II enzymes involved in the metabolism of compounds deriving from traffic.

COC/05/S2
October 2005

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Mutagenicity and Carcinogenicity of Proquinazid (Cholangiocarcinoma in the rat)

Background

1. Proquinazid (6-iodo-2-propoxy-3-propyl-3*H*-quinazolin-4-one) is a new fungicide intended for use in agriculture and viticulture providing control of powdery mildew in cereals and grapes. It is a novel class of fungicide acting by inhibiting the development of the appressorial germ tube. An application for first inclusion of proquinazid in Annex 1 of 91/414/EEC and for UK provisional approval (PPPR), in the product 'Proquinazid 200g/L EC', formulated as an emulsifiable concentrate containing 200g/L proquinazid was discussed at the 311th meeting of the Advisory Committee on Pesticides on 13 January 2005. The ACP deferred making a decision pending advice from the COC and COM with regard to the occurrence of cholangiocarcinoma in the rat carcinogenicity bioassay. The COC and COM have not been asked to review any other tumour reported in rodent carcinogenicity bioassays with proquinazid.

2. Proquinazid has slight solubility in water but is soluble in a range of organic solvents (Log K_{ow} is 5.5 at 25^{0}C). It is slightly volatile. A number of batches were produced and used in the toxicology tests between 1997 and 2001 ranging from 96.4% w/w up to 97.9% w/w. Proquinazid is extensively absorbed from the gastrointestinal tract of rats and widely distributed in both single and repeated dose oral studies. It is noted that levels of radiolabel in bone marrow were reported to be equivalent to plasma and blood. Absorbed proquinazid was extensively metabolised and excreted via the bile and urine. Metabolism comprised predominantly of phenyl ring hydroxylation and hydroxylation at the propyl and propoxy side chains, as well as hydrolysis of some side chains. Major excretory metabolites included conjugates of these metabolites. One minor de-iodinated metabolite was identified.

Introduction to COM/COC review

3. The COM considered proquinazid at its meeting of the 26 May 2005. The COM had access to the individual study reports[1-7], the evaluation prepared by PSD for the ACP[8] and some additional information from the data holder provided in a power point presentation document. The data holder made a presentation to COM.[9-10]

4. The COC considered proquinazid at its meeting of the 14 July 2005. The COC had access to the summary evaluation prepared by PSD for the ACP[8], the draft COM conclusions on proquinazid, detailed information on survival and pathology from the rat and mouse carcinogenicity bioassays[11,12] information from a one year study in dogs[13], the report of the Scientific Advisory Panel review of liver pathology from the rat and mouse carcinogenicity bioassays commissioned by the data holder[14], a mode of action assessment document for intestinal-type cholangiocarcinomas in the two year feeding study with proquinazid submitted by the data holder[15], and information from an internal memorandum on the hepatic pathology in the 2 year feeding study with proquinazid in rats[16]. The data holder made a presentation to COC, which included photomicrographs of a number of histological sections of liver tissue from control and proquinazid treated rats.[17] (An information paper on the pathogenesis of cholangiocarcinoma in humans and rats was drafted by the secretariat http://www.advisorybodies.doh.gov.uk/pdfs/cc0510.pdf)

COM review of Mutagenicity (26 May 2005)

Presentation by data holder

5. The COM heard a presentation from DuPont on the mutagenicity of proquinazid. The purpose of the presentation was to provide DuPont's rationale on the adequacy of the genetic toxicology data base for proquinazid and to specifically discuss the results of the mammalian cell *in-vitro* chromosome aberration assay in human lymphocytes and the *in-vitro* mammalian cell gene *hprt* mutation assay in CHO cells. DuPont sought the committee's agreement that proquinazid is not genotoxic and further testing is unnecessary.

6. DuPont was aware of minor limitations in the *in-vitro* chromosomal assay and *in-vitro* gene mutation assay in mammalian cells. A battery of seven genotoxicity studies had been conducted with technical grade material during the period 1996-1998 (in-life dates of studies). The studies complied with the EU directive 91/414/EEC, the COM guidance of 1989, and OECD test guidelines (current at the time of testing). Two batches of technical material were evaluated (which had been produced by different manufacturing methods, the earlier batch DPX-KQ926-45 was 97% pure and the latter batch DPX-KQ926-75 was 97.9% pure). Tests undertaken with the 45 batch included the Ames test, the *in-vitro* chromosomal aberration assay, the *in-vitro* gene mutation assay in CHO cells (*hprt*) , the *in-vitro* UDS assay using primary rat hepatocytes, and an *in-vivo* bone marrow micronucleus (MN) assay. Tests undertaken with the 75 batch included an Ames test and an *in-vivo* bone marrow MN assay.

7. The presentation then focused on the two *in-vitro* mutagenicity studies where limitations had been identified. The design of and results obtained in the *in-vitro* chromosomal mutation assay in human lymphocytes were reviewed. The negative results and adequate performance of the positive control trials were noted. Some additional historical positive control data were presented (additional slide not in information tabled for members). COM members asked for further information on these additional positive control data. The information was intended to show the lower limit of positive control responses in the test facility both before and after the in-life phase of the *in-vitro* chromosomal aberration assay. The data were consistent with those already provided to members and were generated from a number of tests with cyclophosphamide and MMC. DuPont noted that the study complied with an early proposed revision of the OECD guideline (ca 1996) and included a delayed harvest and that marked cytotoxicity was seen at the 24 h and 48 h harvests. In addition the consistent negative findings in this assay were supported by negative findings in two *in-vivo* bone marrow MN assays in mice in which oral dose levels up to 2g/kg bw had been tested with the occurrence of clinical signs of toxicity and/or mortality in mice and evidence of bone marrow exposure in the rat metabolism study.

8. The design of and results obtained in the *in-vitro* mammalian cell gene mutation assay (CHO/*hprt*) were reviewed. Two independent assays had been conducted for each treatment and a third trial had been performed with activation due to a lack of toxicity in the second trial. The study was a qualitative assessment of mutagenic potential. A positive response was obtained if the mutation frequency was › 40 x 10^6 cells at two or more consecutive concentrations. Plating of 10^6 cells was sufficient to detect a positive response. There was no evidence for a positive response in the assay. Mutation frequencies obtained with the test material were within the negative historical control range and there was a lack of reproducible dose-dependent increases. Positive controls produced marked increases in mutation

frequency in all trials and it was noted that there was comparatively little variance in the positive control data between trials using the same treatment condition. DuPont had commented that all negative control mutation frequencies were within the range of historical controls for the test laboratory, the positive controls exhibited consistent responses despite the fluctuation in negative control frequencies and the negative findings were supported by a lack of genotoxicity in the battery of other *in-vitro* and *in-vivo* genotoxicity studies.

9. DuPont concluded that proquinazid does not pose a mutagenic concern. There were negative findings in a battery of seven *in-vitro* and *in-vivo* studies. There was no result to suggest a positive or weak positive response. Neither proquinazid, nor metabolites formed in rats, contain structural alerts for DNA reactivity. The studies were conducted to the prevailing guidelines at the time. The limitations noted by PSD with regard to the *in-vitro* chromosomal aberration assay and the mammalian cell gene mutation assay (CHO/hprt) did not impact on the overall genotoxic assessment of proquinazid. Thus further testing was unnecessary to conclude that proquinazid is not genotoxic.

COM consideration of presentation and mutagenicity data

10. The COM held a detailed discussion with the representatives of DuPont regarding the mutagenicity data and in particular the adequacy and conduct of the *in-vitro* chromosomal aberration assay in human lymphocytes and the *in-vitro* mammalian *hprt* gene mutation assay in CHO cells. The Committee agreed that as the COM and COC had been asked to evaluate the potential mechanism of cholangiocarcinomas seen in the rat long-term carcinogenicity bioassay with proquinazid, there was a need to have a high level of confidence in the mutagenicity data on proquinazid. Whilst members agreed that there was no convincing evidence for positive results in any of the tests, it was not possible to conclude that the *in-vitro* cytogenetic assay in human lymphocytes and the *in-vitro hprt* mammalian gene mutation assay in CHO cells were negative in view of the limitations. Members accepted that some reassurance was gained from the *in-vivo* bone marrow micronuclei assays in mice.

11. The COM discussed and reached the following conclusions with regard to the mutagenicity data on proquinazid.

Bacterial reverse mutation assay with batch DPX-KQ926-75 (97.9% pure). Date 18/11/98[1]

12. Members agreed that this study had been adequately conducted and no further information was required.

Bacterial reverse mutation assay with batch DPX-KQ926-45 (97% pure). Date 28/2/97[2]

13. Members agreed that this study had been adequately conducted and no further information was required.

In-vitro mammalian cytogenicity test with batch DPX-KQ926-45 (97% pure). Date 26/2/97[3]

14. Members agreed that some aspects of the *in-vitro* chromosome aberration assay in human lymphocytes had been conducted in excess of requirements but considered that a prolonged exposure assay was required so that cells were exposed over the whole cell cycle. Members did not consider that the test had adequately covered this aspect or that the potential for aneugenicity had been examined. Members observed that the dose selection had resulted in high levels of cytotoxicity and that the positive control response reported (towards the lower limit of a very wide range, ca 6-80% cells with aberrations) limited the value of this assay. The Committee agreed that it would be advisable to request a continuous 20 h exposure in a mammalian cell chromosomal aberration assay (in human lymphocytes) in the absence of exogenous metabolic activation. This would ensure that all stages of the cell cycle were exposed. It would be appropriate to include estimation of polyploidy as an indicator of potential for aneugenicity. The study should be conducted to internationally accepted standards. The Committee was aware of the need to adequately explain the mode of action of the cholangiocarcinomas reported in the long-term bioassay of proquinazid in rats and agreed that this information would help to complete the *in-vitro* mutagenicity evaluation of proquinazid which was an important part of the carcinogenicity risk assessment.

In-vitro gene mutation in mammalian cells with batch DPX-KQ926-45. (97% pure). Date 22/5/97[4]

15. Members commented that the data from the *in-vitro* mammalian cell *hprt* gene mutation assay in CHO cells were consistent with the conclusions COM had previously published on practical difficulties in undertaking this assay (http://www.advisorybodies.doh.gov.uk/com/mut033.htm). There was considerable inter-trial variance in mutation frequency in the proquinazid assay and the finding of very low mutation frequencies in some trials (<0.6 x 10^6 cells) limited the conclusions that could be drawn. Thus it was noted that the mutation frequency in the third trial in the acetone control and test material experiments was low at all dose levels. Members concluded that the data were not interpretable. The Committee agreed that a mouse lymphoma assay conducted to internationally accepted standards should be requested. The Committee was aware of the need to adequately explain the mode of action of the cholangiocarcinomas reported in the long-term bioassay of proquinazid in rats and agreed that this information was essential to complete the *in-vitro* mutagenicity evaluation of proquinazid which was an important part of the carcinogenicity risk assessment.

In-vitro unscheduled DNA synthesis with batch DPX-KQ926-45 (97% pure). Date 21/5/97. Minor editorial revision to report 22/2/99[5]

16. Members agreed that this study had been adequately conducted and no further information was required.

In-vivo bone marrow micronucleus in mice with batch DPX-KQ926-75 (97.9% pure) Date 14/1/99[6]

In-vivo bone marrow micronucleus in mice with batch DPX-KQ926-45 (97% pure) Date 22/2/99[7]

17. Members agreed that the data provided were indicative of negative findings in these assays. Members agreed the evidence for small increases in percentage micronucleated polychromatic erythrocytes seen at some doses most probably represented fluctuation in the background incidence of micronuclei. Members accepted the evidence of toxicity in these studies and information from the toxicological and toxicokinetic studies with proquinazid suggested that the bone-marrow would have been exposed to proquinazid and metabolites in these two studies.

COM conclusions from May 2005 meeting

18. Members considered that the company had provided some relevant information and comments but had not been convinced that the *in-vitro* mutagenicity test package was adequate. Members agreed that a key element of any proposal regarding mechanism of proquinazid induced cholangiocarcinoma in the rat would require an evaluation of mutagenicity and hence it was important to complete the mutagenicity test package.

19. The Committee considered that a mouse lymphoma assay should be conducted and that it was advisable to also conduct a continuous 20 h exposure in a mammalian cell chromosomal aberration assay (in human lymphocytes) in the absence of exogenous metabolic activation. Both studies should be conducted to internationally accepted standards. These studies were required to provide full information on the mutagenicity evaluation of proquinazid.

COM post meeting consideration of additional mouse lymphoma assay

20. The data holder submitted on 12 July 2005 the results of a new mouse lymphoma assay undertaken with proquinazid.[18] The assay included an exposure of 3 hour (in presence and absence of exogenous metabolic activation) and a continuous 24 h exposure in the absence of metabolic activation. The results suggested that proquinazid was not mutagenic in this assay. A full report was not available during the postal consultation with COM members. The Committee considered the available data from the new mouse lymphoma assay by postal consultation and agreed there was no evidence for a mutagenic effect. It was also agreed that the full report should be considered by COM when available. The final report was submitted to COM members by postal consultation on the 22 August 2005. Members agreed that the submitted mouse lymphoma assay was acceptable and gave negative results.[19]

COC evaluation of cholangiocarcinoma in the rat (14 July 2005)

21. The COM heard a presentation from DuPont on the interpretation of cholangiocarcinoma reported in female rats fed a diet containing proquinazid as part of a long term carcinogenicity bioassay.[15,16] In brief, groups of 80 male and 80 female Cr1:CD®(SD) BR rats were fed diets containing 0, 10, 30, 300, 600 (females), 1000 (males), 1200 (females) or 2000 ppm (males) for 104 weeks. Groups of five animals/sex were subject to interim haematology, clinical chemistry necropsy and histopathological evaluation at 1 week and 1 year of feeding. Survival at termination was increased at dose levels of 600 ppm and above. A statistically significant increase in intestinal-type cholangiocarcinoma was reported in female rats (8/60 at 600 ppm and 12/61 at 1200ppm, cf none in concurrent control).[11]

22. The representatives from DuPont were accompanied by Dr P Greaves (University of Leicester) who had been a member of a Scientific Advisory Panel (sponsored by DuPont). The Scientific Advisory Panel had reviewed the liver slides from the rat and mouse carcinogenicity bioassays. COC members questioned the representatives from DuPont and Dr Greaves during the presentation.

23. The Scientific Advisory Panel (SAP), had reviewed aspects of the liver pathology and a report had been finalised in November 2003. Dr Greaves had reviewed the slides again recently before the submission for the COC consideration was compiled.[16] The SAP considered that the lesions seen in the female rats were not indicative of a carcinogenic risk for humans. The Panel's rationale had been that proquinazid was not genotoxic, that cholangiocarcinomas of the "intestinal" type occurred only in female rats fed 600 ppm or more at the end of the two-year study, the tumour type was linked to chronic hepatocellular injury with "cholangiofibrosis" and there was no risk of the lesion occurring in the absence of severe hepatic toxicity. A number of representative sections were shown to COC members. Features of the histopathology of the lesions seen at 1200 ppm included: cellular degeneration, inflammatory infiltrate, extensive necrosis, giant nuclei and fibrotic changes termed "cholangiofibrosis" by the original reporting pathologists. The cells within the lesions showed goblet cell differentiation and were therefore termed "intestinal-type". The cholangiofibrosis was not necessarily related to the portal tract and the lesions termed "cholangiocarcinoma" were quite unlike the usual pattern seen in human cholangiocarcinoma. COC members supported the view that the lesions were not neoplastic but were a florid inflammatory response to severe acute and chronic hepatocellular damage with marked reactive epithelial changes including cytoplasmic alterations, nuclear pleomorphism, and intestinal metaplasia. COC pathologists noted that there was little evidence of increased mitotic activity in the slides shown and aberrant mitoses were not a feature.

24. Dr Greaves confirmed that the SAP had reviewed virtually all the lesions diagnosed as cholangiocarcinomas and he had reviewed a proportion of them again recently. In answer to specific questioning, he agreed that, if chronic exposure to proquinazid was sufficiently high to induce similar severe liver toxicity in humans, there was a risk that similar lesions to that identified in rats could occur. He noted that liver toxicity was seen in female rats receiving 300 ppm proquinazid but was less severe than at 600 ppm. There had been individual variation in severity at 600 ppm. In answer to another question, DuPont representatives noted that the pigment seen in the liver sections had been shown to be PAS positive and was therefore likely to be lipofuschin as expected in the setting of cell degeneration and increased turnover. There was minimal deposition of stainable iron.

25. The Committee raised a question as to whether the role of oval cells in liver regeneration, as proposed in the MOA[15], is hypothetical or is more generally accepted." It was agreed that oval cell proliferation was seen in rodents when a regenerative stimulus was supplied but hepatocyte proliferation inhibited, for example by 2AAF.

COC consideration and conclusions

26. The committee concluded that it was uncertain that the lesions termed "cholangiocarcinomas" were truly neoplastic but accepted the conventionality of the term used by the study pathologist. However, it was noted that the pathology seen with proquinazid lacked the expected characteristics of that caused by a genotoxic carcinogen.

27. Members agreed that the MOA proposed by DuPont for the putative cholangiocarcinomas was plausible. They noted that the lesions were only seen at dose levels which caused severe liver toxicity and which exceeded the MTD. They further agreed that the lesions were relevant to humans in that they were a potential hazard. However, whether they were a risk in humans depended on the level of exposure.

28. Members agreed that the No Observed Adverse Effect Level (NOAEL) for putative cholangiocarcinoma was 16 mg/kg bw/day (300 ppm in female rats). They were informed that PSD had proposed an acceptable daily intake for proquinazid of 0.01 mg/kg bw, based on the overall No Observed Adverse Effect Level of 1.2 mg/kg bw/day derived from all the toxicology data on proquinazid and a safety factor of 100. Members confirmed that this would give an adequate margin of safety (1600) for cholangiocarcinoma.

Overall discussion of COM/COC on proquinazid

29. The COM has reviewed the available mutagenicity data on proquinazid (26/5/05). All of the studies were considered acceptable with the exception of the *in-vitro* mammalian mutagenicity assay in CHO cells (*hprt* locus) and it was also noted that an extended exposure of human lymphocytes in the absence of exogenous metabolic activation had not been undertaken as part of the assay for chromosomal aberrations. The COM was aware of a recently submitted *in-vitro* mouse lymphoma assay and agreed by postal consultation that the data were suggestive of a negative response (results submitted 12/7/05).[18] The final report was submitted to COM members by postal consultation on the 22 August 2005. Members agreed that the submitted mouse lymphoma assay was acceptable and gave negative results. The COM agreed that it would not be necessary to complete the *in-vitro* mutagenicity test package by undertaking an extended exposure treatment of human lymphocytes for chromosomal aberrations in view of the totality of negative *in-vitro* and *in-vivo* mutagenicity data available on proquinazid if the MOA assessment for the cholangiocarcinoma which had been submitted to COC was deemed acceptable.

30. The COC considered the available carcinogenicity data and additional information on histopathology of the liver from the carcinogenicity bioassay in rats on 14/7/05. Members were unconvinced that the lesions reported as cholangiocarcinomas were truly neoplastic, but accepted that the study pathologists' diagnosis could be used for risk assessment. The COC agreed that a threshold approach to risk assessment could be used and agreed that there was a satisfactory margin of safety between the NOAEL for putative cholangiocarcinoma and the ADI proposed by PSD.

Overall conclusions of COM/COC on proquinazid

31. The COM concluded that the mutagenicity data submitted provided evidence that proquinazid is not an *in-vitro* or *in-vivo* mutagen. There were limitations in the adequacy of the submitted *in-vitro* gene mutation assay in mammalian cells (CHO, *hprt*) and the submitted *in-vitro* assay in human lymphocytes for chromosomal aberrations. The data holder submitted negative results from a mouse lymphoma assay which included both short-term (3h) and extended exposure (24 h) treatments after the COM meeting (26/5/05). The full report of this study would need to be considered by COM when available. The final report was submitted to COM members by postal consultation on the 22 August 2005.

Members agreed that the submitted mouse lymphoma assay was acceptable and gave negative results. The COM agreed that the advice for a further *in-vitro* study to examine chromosomal aberrations in human lymphocytes study would not be a requirement in view of the totality of negative mutagenicity data on proquinazid and if the MOA assessment for the cholangiocarcinoma which had been submitted to COC was deemed acceptable (see para 32).

32. The COC concluded that it was uncertain that the lesions termed "cholangiocarcinomas" were truly neoplastic but accepted the conventionality, used by the study pathologist in the report of the carcinogenicity bioassay of proquinazid in rats, of classifying them as neoplastic. A satisfactory non-genotoxic Mode of Action explanation for the cholangiocarcinomas in rats had been submitted. The COC agreed that a threshold approach could be used for the risk assessment of cholangiocarcinoma reported in the rat.

COM/05/S4, COC/05/S1
September 2005

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Declaration of members' interests during the period of this report

	Personal Interest		Non Personal Interest	
MEMBER	COMPANY	INTEREST	COMPANY	INTEREST
Professor P G Blain (Chairman)	NONE	NONE	NONE	NONE
Dr C Allen	NONE	NONE	NONE	NONE
Professor A Boobis	Abbey Life 223 Abbey National 875 Barclays Bank 112 BG Group 94 BT Group 110 Centrica 99 HBOS 80 Marks and Spencer 102 National Grid Transco 37 Scottish Power OSI Pharmaceuticals	Shareholder Shareholder Shareholder Shareholder Shareholder Shareholder Shareholder Shareholder Shareholder Consultancy	Servier	Research support
Dr P Carthew	Provalis Unilever	Shareholder Salary	NONE	NONE
Professor P B Farmer	Abbey National Bradford & Bingley Celltech Foreign & Colonial Friends Provident Health Effects Institute Torotrak	Shareholder Shareholder Shareholder Shareholder Shareholder Research Committee Member Shareholder	American Chemistry Council CEFIC	Research Support Research Support
Professor D Forman	Barclays Friends Provident HBOS Woolwich	Shareholder Shareholder Shareholder Shareholder	NONE	NONE
Mrs R Glazebrook	Dr Foster Ltd BT Group Lloyds TSB National Grid	Salary Shareholder Shareholder Shareholder	NONE	NONE
Professor D Harrison	Medical Solutions Qunitiles Scottish Medicine	Shareholder Consultant Consultant	NONE	NONE
Ms D Howel	NONE	NONE	NONE	NONE
Dr S J Kennedy	Unilever	Shareholder	NONE	NONE

	Personal Interest		Non Personal Interest	
MEMBER	COMPANY	INTEREST	COMPANY	INTEREST
Professor D Phillips	Aviva Banco Santander BG Group Bradford & Bingley Centrica Lattice Group National Grid Takeda	Shareholder Shareholder Shareholder Shareholder Shareholder Shareholder Shareholder Consultancy	NONE	NONE
Dr R Roberts	AstraZeneca P&O	Salary Shareholder Shareholder	NONE	NONE
Professor D Shuker	NONE	NONE	NONE	NONE
Dr N Wallis	Pfizer	Salary Shareholder	NONE	NONE