Committee on Carcinogenicity of Chemicals in Food, Consumer Products and the Environment

Preface

The Committee on Carcinogenicity (COC) evaluates chemicals for their human carcinogenic potential at the request of the Department of Health and Food Standards Agency and other Government Departments including the Regulatory Authorities. All details concerning membership, agendas, minutes and statements are published on the Internet.

During the year 2002 the Committee has provided advice on a wide range of chemicals including malathion (a pesticide) and polycyclic aromatic hydrocarbons (contaminants which may be present in air and food). The COC also discussed an approach to using estimates of the upper bound risk estimate for carcinogenic air pollutants at environmental levels of exposure. The COC agreed the approach provided it was based on good quality epidemiological data and that risk estimates were not quoted as if they were real estimates of risk but were used in the consideration of risk management options.

The Committee discussed its procedures in the light of the new code of practice for Scientific Advisory Committees published by the Office of Science and Technology (OST). The Committee adheres to most of the recommendations and agreed to publish more of the substantive background papers to discussions at the earliest opportunity. The COC devised a template showing its methods of working and expertise which is easy to follow.

The Committee also discussed the use of uncertainty factors in its evaluations, the minimum duration of carcinogenicity tests in animals and the use of short term tests in the future. The Committee finished a major piece of work on the investigation of interaction between environment and genotype in the induction of cancer by chemicals. A detailed statement and a lay statement have been published.

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Malathion

3.1 Malathion is an organophosphorous insecticide. It has been marketed in the UK for use in agriculture and horticulture since 1956. There were three products with approvals for use in agriculture and horticulture, home garden and use in pigeon lofts at the time when this review was initiated in January 2002. A number of products containing malathion are also licensed as human medicines for use in the control of head lice.

3.2 The Advisory Committee on Pesticides is reviewing the available toxicological information on malathion as part of its ongoing review of organophosphorous compounds. The ACP asked for advice from COM and COC on mutagenicity and carcinogenicity at its 289th meeting on 17 January 2002. The Chairs of COM and COC agreed that a joint statement was required in view of the need for a full review of all mutagenicity and carcinogenicity data. There were inconsistent results in mutagenicity studies (both *in-vitro* and *in-vivo*) and there was evidence for the mutagenic activity of some impurities which might be present in some batches of technical malathion.

3.3 There was some limited evidence for tumourigenicity in rats at high oral doses given via the diet which adversely affected growth and survival of the animals. This included the occurrence of benign nasal tumours in a few animals given high oral doses of technical grade malathion in the diet and benign liver tumours in female rats at the highest dose level. An increased incidence of benign liver tumours was reported in male and female B6C3F1 mice at high dietary levels which were associated with reduced weight gain.

3.4 The COC reviewed the available carcinogenicity data on malathion which included in confidence reports (provided by the Pesticide Data Holder regarding two studies in rats one in mice) and published reports of long-term bioassays in rats and mice at its 27 June 2002 meeting. Three long-term bioassays using malaoxon (the principle metabolite of malathion and also present in technical grade malathion as an impurity) were available which included two in rats and one in mice. In addition the Committee also considered in confidence reports of Peer Reviews of the histology slides from the 1993-96 malathion bioassay in F344 rats, the 1992-94 bioassay in B6C3F1 mice and some additional supplemental information for the 1992-1994 bioassay in B6C3F1 mice and the 1993-1996 bioassay of malaoxon in F344 rats provided by the Pesticide Data holder.

3.5 A number of additional follow up reports from the contract laboratory concerning the 1993-96 bioassay of malathion in F344 rats were also reviewed. In addition the Pesticide Data Holder submitted a response to questions from COC secretariat which provided an overall summary of the histology of the nasal tissue in animals with tumours and additional evaluation of the historical control data on nasal tumours in F344 rats and possible mechanisms for nasal tumours induced in F344 rats fed high doses of technical grade malathion. A published Peer Review of a number of the older carcinogenicity bioassays was also available. The COC also considered expert reports from the EPA and a Scientific Advisory Panel established by EPA to review malathion.

3.6 The Committee reached the following overall conclusion.

3.7 "The COC agreed that technical grade malathion had been tested in four long-term dietary bioassays in rats and two long-term dietary bioassays in mice. The most recent studies undertaken in F344 rats (1993-96) and in B6C3F1 mice (1992-94) were adequate for the evaluation of carcinogenicity. There is evidence for tumourigenicity in the nasal tissue and liver (females only) of F344 to rats fed malathion. The nasal tumours were associated with severe ongoing inflammation, which is most likely involved in the mechanism of tumourigenesis. There was evidence for liver tumours in female F344 rats and male and female B6C3F1 mice. The weight of evidence suggested that these liver tumours were induced through a non-genotoxic mechanism and were not relevant to human health."

3.8 Malathion was also considered at the COM meetings of 25 April and 10 October. A full statement from COC and COM is in preparation.

Polycyclic Aromatic Hydrocarbons: Advice on Dibenzo (a,l)pyrene

3.9 Polycyclic aromatic hydrocarbons (PAHs) are a large group of highly lipophilic chemicals that are present ubiquitously in the environment as pollutants. Many of them are generated as by-products of the combustion of organic material and they occur in particulate and/or vapour phases. Humans are widely exposed to low levels of mixed PAHs in air, food and drinking water. Higher levels of atmospheric exposure are encountered by workers employed in industries such as aluminium production, coal gasification, coke production and iron and steel founding. Cigarette smoke is also a major source of PAHs.

3.10 The COM and COC were asked by DoE and MAFF for a scheme to evaluate and rank 25 selected PAHs which could be used as a basis for further monitoring and/or surveillance. When COC started this work in 1994 it was originally intended to use a ranking system based on 'toxic equivalency factors' with benzo(a)pyrene as the comparator substance. The data were, however, inadequate for some of the listed PAHs and a simple 5 category system was devised:

(Group A) There is a high level of concern about a carcinogenic hazard for humans because the compound is an *in vivo* mutagen and/or a multi-site carcinogen in more than one species.

(Group B) There is concern about a carcinogenic hazard for humans, but the data are incomplete or the mechanism is unclear.

(Group C) The compound is a non-genotoxic carcinogen. (This category may contain compounds with an equal amount of evidence for carcinogenic hazard as compounds in categories A or B, but these are placed in a separate category because subsequent management may be different). In practice none of the 25 PAHs considered fell into this group.

(Group D) The data are inadequate for assessment.

(Group E) There is no concern about carcinogenic hazard, ie the compound is non-genotoxic and non-carcinogenic or the mechanism of carcinogenesis is not relevant to humans.

3.11 Specific information regarding the classification of the 25 PAHs considered in 1994/5 can be found in the 1995 Annual report.

3.12 The COC consideration of dibenzo(a,l)pyrene is given below.

3.13 The COC agreed that the *in-vitro* mutagenicity tests and information on *in-vivo* DNA adduct formation was consistent with dibenzo(a,l)pyrene being an *in-vivo* mutagen. Members also agreed that dibenzo(a,l)pyrene was carcinogenic in mice and rats. Dermal application to mice produced skin tumours (squamous cell carcinomas) and tumours at a number of sites (such as lungs, spleen and lymphomas) and intraperitoneal administration to rats produced lung tumours. Intramammary instillation in rats resulted in mammary tumours. Dibenzo(a,l)pyrene also acted as an initiator in mouse skin carcinogenicity promotion assays. The COC therefore considered that dibenzo(a,l)pyrene should be assigned to group A of its hazard ranking scheme for PAHs.

3.14 Regarding carcinogenic potency, the committee agreed that dibenzo(a,l)pyrene was a very potent genotoxic carcinogen and that potency varied depending on factors such as species, route of administration, dose and site of tumour produced. From the available data where a comparison could be made, members considered that the dibenzo(a,l)pyrene carcinogenic potency was likely to be in the range of 10-100 times more potent than benzo(a)pyrene depending on the test system used.

3.15 A full statement on dibenzo(a,l)pyrene is in preparation. Further consideration of the relative potency of dibenzo (a,l)pyrene compared to other PAHs is underway.

Quantification of risks associated with carcinogenic air pollutants

3.16 In the air pollution area, the non-cancer health effects of these pollutants are quantified for cost-benefit analysis using dose-response functions from epidemiological studies of environmental exposure to air pollutants. The Department of Health had been asked whether the benefits (ie reduction in cancer incidence), which could be attributed to lowering levels of carcinogenic air pollutants below current standards, could be quantified. Simple linear extrapolation using WHO unit risk factors were used to highlight relevant issues for discussion. The Committee was asked for its views on possible approaches (eg risk estimation and relative ranking) to quantification of effects of carcinogenic air pollutants at the March 2002 meeting.

3.17 At the November 2002 meeting, Members considered a proposal to use the upper bound estimate from the one-hit model to set upper bounds of risk at low levels of exposure on the basis of data from human epidemiology studies. Members were advised that the intention was to use this very conservative approach to advise on the practicality of risk management options for air pollutants and there was no intention to publish risk estimates based on this approach. The primary objective would

be to assess the cost of reducing levels of air pollution to the exposures associated with the upper bound estimate of risk based on the one hit model. The COC was content with the approach provided that it was limited to chemicals for which there was good cancer epidemiology data and that data were used only as a guide when considering risk management options. Members felt it important to restate that extrapolation of risk estimates below the observed range was very problematic, as no model was completely satisfactory.

Review of Committee Procedures

OST Code of Practise for Scientific Advisory Committees

3.18 A copy of the new "Code of Practice" for Scientific Advisory Committees published by the Office of Science and Technology (OST) on 19th December 2001 was provided to members for information and comment. Many of the issues were considered at the last (November 2001) COC meeting when the Committee reviewed the Government's response to the BSE enquiry. Members agreed that most of the COC procedures conform to the new code of practice and where this is not the case steps are being taken to comply. The Committee noted that substantantive background papers would be published (excluding those containing commercial in-confidence data). Due to the highly technical nature of the work it would be difficult for papers to be truly comprehensible to the non-specialist, but it was hoped that the 'what's new section' of the COC internet site and lay summaries would help in this regard. Additionally, overview lay summaries should accompany some statements and a glossary of technical terms could help with public understanding. The COC would also contribute to a joint COT/COC/COM glossary. With respect to dealing with dissenting views members agreed that it should be made clear in the minutes and statements when decisions were not unanimous.

COC Template

3.19 The COC agreed a template diagram which provided an overview of how COC undertakes risk assessment of carcinogens and the interaction of COC with its sister Committees (COT and COM) and with Government Departments, Regulatory Agencies and the Chief Medical Officer Professor Sir Liam Donaldson. For ease of reference this template is reproduced at the end of this section of the Annual Report.

Test Strategies and Evaluation

IGHRC paper on uncertainty factors

3.20 The Interdepartmental Group on Health Risks from Chemicals (IGHRC) is developing cross-Government guidance on the handling of uncertainty in the toxicological hazard aspects of human health risk assessment. IGHRC intends to produce a document setting out a harmonised framework for UK Government departments, agencies and their advisory committees on how to address the uncertainties in toxicological hazard aspects of risk assessment including the derivation and application of uncertainty factors. The Committee heard a short presentation from Professor Iain Purchase (Chair of Executive Committee of IGHRC).

3.21 The document provided a review of the approaches used in chemical risk assessment in the UK. The draft document reflected the current position in relation to the assumption of absence of a threshold for genotoxic carcinogens as laid out in the 1991 COC *Guidelines for the Evaluation of Chemicals for Carcinogenicty* (see section on ongoing work). It was noted that the COM had recently reaffirmed its position that no threshold could be assumed for *in-vivo* mutagens in the absence of compound specific mechanistic data to suggest otherwise. (http://www.doh.gov.uk/comivm.htm.) The draft IGHRC document indicated that for carcinogenicity believed to arise through a non-genotoxic mechanism of action, a conventional approach using estimation of a No Observed Adverse Effect Level (NOAEL) and application of uncertainty factors could be adopted. It was also recognised that in some cases an extra uncertainty factor has been applied to non-genotoxic carcinogens because of concern over the severity (and irreversible nature) of the effect or uncertainties in the mechanism of carcinogenicity.

3.22 The COC confirmed that the approach set out in the document was acceptable. It was pointed out that in some instances there would be a limited range of risk management options and this could influence the approach to risk assessment used, but not the outcome of any risk assessment. Members commented that the Bench Mark Dose (BMD) approach made use of the data from all dose levels and avoided uncertainties in trying to set a NOAEL. However the BMD required more doses at levels expected to result in some toxic effects and might result in greater animal usage. In respect of risk assessment of chemical carcinogens, members cautioned against a "numerical" approach to the use of data from long term bioassays in animals in risk assessment. It was noted that the interpretation of long-term cancer bioassays was influenced by, often subtle, interpretation of histology.

Minimum duration of carcinogenicity studies in rats

3.23 The proper conduct of carcinogenicity studies in rats is an important part of the evaluation and prediction of potential human carcinogens. Significant reductions in the number of control rats surviving to termination have been widely reported in the scientific literature. This is a matter of concern since inadequate carcinogenicity studies could be important in decisions regarding the identification of potential human carcinogens and in particular the failure to identify such compounds. In addition there is a possibility that inadequate studies could be rejected by regulatory agencies with the consequent need for use of further animals to obtain a valid result. For a negative result from a rat carcinogenicity bioassay to be considered acceptable, survival at 24 months should be 50% or greater in all groups.

3.24 The Committee had reviewed the evidence for application of dietary restriction techniques in 2000 and a statement was published (http://www.doh.gov.uk/longevity.htm). It was concluded that the available information supported the view reached by the COC in its guidelines published in 1991 that dietary restriction in carcinogenicity studies should be applied with caution and is the responsibility of the toxicologist undertaking the study. The COC had agreed that the subject of dietary restriction should be reviewed when more information is available. Some investigators have also proposed that reducing the duration of carcinogenicity bioassays undertaken in rats would have the desired effect of terminating studies before survival was reduced to below 50% in tests groups and that such bioassays

would also be adequate. The Committee reviewed two published papers which had evaluated published data on the duration of carcinogenicity studies. These two papers came to contrasting views (see statement published at end of this Annual Report for overview). The Committee was asked to provide generic advice on the desirability of reducing the minimum duration of carcinogenicity studies in rats from 2 years as currently stated in international guidelines for the conduct of such studies.

3.25 The COC concluded that there was insufficient evidence from the new publications to recommend a change to the international guidelines for the conduct of long term carcinogenicity bioassays. The current guideline is that for a negative result to be acceptable in a rat carcinogenicity bioassay, survival should be at least 50% in all groups at 24 months. The Committee reaffirmed that it was the responsibility of the study director to use rat strains that would ensure adequate survival at 24 months. The COC statement is included at the end of this report.

Short term tests for carcinogenicity (ILSI/HESI research programme on alternative cancer models)

3.26 The International Conference on the Harmonisation of Technical Requirements for the Registration of Pharmaceuticals for Human Use (ICH) has agreed that the required bioassay data may be derived from only one species, i.e. the rat. This would be supported by appropriate mutagenicity and pharmacokinetic data and also information that could come from newly proposed short-term *in-vivo* test models for assessment of potential carcinogenic activity in mice (in particular heterozygous p53+/- deficient Tg.AC model, and ras H2 models). Although these recommendations apply only to human medicines, any decision could have significant implications for other categories of chemical (e.g. food additives, pesticides, industrial chemicals etc). The key public health issue is whether the proposed short term tests in transgenic mice are appropriate adjuncts to the rat carcinogenicity bioassay in the identification of chemical carcinogens.

3.27 The COC has as part of its remit to advise on issues relating to chemical carcinogenesis and to present recommendations for testing strategy. The Committee was asked by the Department of Health in 1997 to consider the available literature on the proposed short-term *in-vivo* tests for assessment of carcinogenic activity in mice, and specifically, on the transgenic mice models (heterozygous p53 +/- deficient, Tg.AC model, and ras H2 model). The conclusions reached by the Committee can be found in full in the Annual report for 1997. Overall the COC agreed that much of the current research effort had been placed on the evaluation of the three short-term animal model tests systems reviewed but little interest had been devoted to the underlying mechanistic basis for these tests and also to the most appropriate transgenic animal model for screening for potential human carcinogens. The Committee agreed that many transgenic models were likely to be developed over the next few years which might be applicable to specific areas of interest, such as the identification of tumour promoters. This was an area to keep under review.

3.28 The International Life Sciences Institute (ILSI) and the Health and Environmental Science Institute (HESI) have co-ordinated a multinational research programme, from 1996 –2000, on the use of alternative cancer models. The research involved input from over 50 industrial, governmental (USA,

Denmark, Netherlands and Japan) and academic laboratories and cost around $35m. The data from the project along with a number of evaluation papers from independent experts and abstracts of additional work were published as a supplement to volume 29 of Toxicologic Pathology in November 2001 pp1-351.

3.29 The COC acknowledged the considerable administrative and practical problems that had confronted ILSI/HESI in co-ordinating this work. It was considered that the programme had provided a large amount of information on the evaluation of performance of these assays but the data were not sufficient to validate the use of any of the assays for regulatory testing.

3.30 The COC agreed an overall conclusion that none of the models used in the ILSI/HESI Alternative Cancer Test programme were suitable as a replacement for the mouse carcinogenicity bioassay (the primary purpose for the development of these models) and that further research should look to identify models with a greater relevance to mechanisms of carcinogenicity in humans. Of the animal models assessed there was evidence that p53+/- transgenic mouse model could identify some genotoxic carcinogens. There was insufficient data to suggest that the animal models under consideration (*RasH2, Tg.AC, Xpa, Xpa/P53+/- and p53+/-*) provide essentially similar results. The COC statement is included at the end of this report.

The investigation of interaction between genotype and chemicals in the environment on the induction of cancer

3.31 Many diseases (such as cancer) are thought to be due to a combination of heredity and other factors in the environment (such as lifestyle, diet and to a lesser extent exposure to chemicals in the environment). The DNA sequence of an individual (his or her genotype) may be one factor which contributes to whether a person who is exposed to chemical carcinogens (e.g. from tobacco smoke) may develop cancer. The Human Genome Project is showing that there are a great many small differences between individuals in their DNA sequences.

3.32 The Committee was asked by the Department of Health to review the available information on the interaction between genotype and exposure to chemicals in the environment and the induction of cancer. The Committee was asked to provide advice on the methods of epidemiological research used in this area and the approaches to identifying genes of interest for such studies. Of particular importance is the evaluation and significance of data from relevant studies in cancer risk assessment.

3.33 The Committee reviewed the methods used to investigate possible interactions between genotype, exposure to chemicals and occurrence of cancer. The types of study, which all involved investigating genotype and exposures to chemicals in humans, could be separated into two types, i. gene characterisation studies, which aim to investigate the nature and strength of interactions and ii. gene discovery studies, which are intended to screen for genes which might be of importance for future gene characterisations studies.

3.34 The Committee agreed that the available data had so far failed to show any consistent and strong interaction between genotype and chemically-induced cancer.

3.35 Key conclusions reached are highlighted below. The COC statements (including a lay statement) are appended at the end of this report.

- The most appropriate study designs for gene characterisation investigations will vary according to study purpose. Many of the currently available studies are either too limited in size or relied on *post hoc* analyses to highlight selected results. It is essential that such studies should involve *a priori* hypotheses.

- The rapid development of DNA sequencing techniques means that many gene discovery studies will become available in the future.

- Before the results of genotype-environment interaction studies can be used in risk assessment it is necessary to establish whether there is a reasonable case to infer that the genotype-environment interaction is associated with a real and important increased frequency of cancer A tiered approach has been recommended.

- It was unlikely that the interactions studied to date (which mainly concerned genes responsible for the metabolism of chemicals) were of importance to public health.

- There is little value in using genetic screening to identify individuals with particular genotypes of interest for carcinogenesis induced by environmental chemicals.

- The possibility could not be excluded that important genotype-environment interactions involved in chemically induced cancers would be identified in the future.

Ongoing Reviews

Alcohol and Breast Cancer

3.36 The Committee heard a presentation by researchers from the Department of Epidemiology and Public Health, Imperial College of Science Technology and Medicine on the finalised results of a formal systematic review (meta-analysis) of the association between drinking alcohol and breast cancer. The COC also considered an important paper from the Collaborative Group on Hormonal Factors in Breast Cancer recently published in the *British Journal of Cancer* (2002, vol 87, 1234-1245) at its meeting of 22 November 2002. A number of questions have been forwarded to the authors. The Committee will further consider this topic at its March 2003 meeting.

Glossary of Terms for COT/COC/COM

3.37 A document is in preparation.

Prostate Cancer

3.38 There is evidence for an increase in the number of diagnosed cases of prostrate cancer. The COC are to consider a review of the literature on the aetiology of prostrate cancer. A review paper is in preparation for the March 2003 meeting.

Revision of COC guidelines

3.39 The COC guidelines are used by Government Department as the basis for risk assessment of chemical carcinogens. The current guidelines were published in 1991. The Committee agreed to update its guidance on approaches to risk assessment in the light of developments over the last decade.

Statements of the COC

Statement on ILSI/HESI research programme on alternative cancer models

The minimum duration of carcinogenicity studies in rats: review of two selected papers published in 2000

Statement on the investigation of interaction between genotype and chemicals in the environment on the induction of cancer

Statement on ILSI/HESI research programme on alternative cancer models

Introduction

1. The International Conference on the Harmonisation of Technical Requirements for the Registration of Pharmaceuticals for human use (ICH) has agreed that the required bioassay data may be derived from only one species, i.e. the rat[1] This would be supported by appropriate mutagenicity and pharmacokinetic data and also information that could come from newly proposed short-term *in-vivo* test models for assessment of potential carcinogenic activity in mice (in particular heterozygous p53+/- deficient Tg.AC model, and ras H2 models). Although these recommendations apply only to human medicines, any decision could have significant implications for other categories of chemical (e.g. food additives, pesticides, industrial chemicals etc). The key public health issue is whether the proposed short term tests in transgenic mice are appropriate adjuncts to the rat carcinogenicity bioassay in the identification of chemical carcinogens.

2. The COC has as part of its remit to advise on issues relating to chemical carcinogenesis and to present recommendations for testing strategy. The Committee was asked by the Department of Health in 1998 to consider the available literature from three research groups (namely NTP/NIEHS, ILSI/HESI and CIEA*) on the proposed short-term *in-vivo* tests for assessment of carcinogenic activity in mice, and specifically, on the transgenic mice models (heterozygous p53 +/- deficient, Tg.AC model, and ras H2 model). The Committee reached the following conclusions in 1998, which were published.[2]

    i) The Committee recognises that the three transgenic models considered in this paper (p53+/- , Tg.AC, ras H2) appear to be highly sensitive to carcinogens but questions whether data from such tests would add much to the information which can be derived from a well conducted *in-vivo* evaluation of mutagenic potential.

    ii) Dose-response data from tests in transgenic animals might be useful but at present there is no way of interpreting these data and extrapolating them to humans.

    iii) The Committee considers that the further development and validation of short-term *in-vivo* models to evaluate non-genotoxic carcinogenesis and tumour promoters may be valuable. However, there is likely to be less scope for the use of the proposed short-term animal models for other categories of chemicals, such as pesticides and industrial chemicals, where the available supporting information, such as the results of metabolism studies, is likely to be more limited. Hence the development of short-term carcinogenicity tests for these chemicals needs to be considered carefully.

    iv) The Committee concludes that, in view of the lack of appropriate validation data, it would not be appropriate to use the data from short-term transgenic bioassays considered in this statement to support regulatory decisions at the present time.

* NTP National Toxicology Program U.S.A. NIEHS National Institute for Environmental Health Sciences U.S.A. ILSI International Life Sciences Institute. HESI Health and Environmental Sciences Institute U.S.A. CIEA Central Institute for Experimental Animals, Japan.

Introduction to ILSI/HESI Programme in Alternative Cancer Models

3. The International Life Sciences Institute (ILSI) and the Health and Environmental Science Institute (HESI) have co-ordinated a multinational research programme, from 1996 –2000, on the use of alternative cancer models. The research involved input from over 50 industrial, governmental (USA, Denmark, Netherlands and Japan) and academic laboratories and cost around $35m.[3]

4. The data from the project along with a number of evaluation papers from independent experts and abstracts of additional work were published as a supplement to volume 29 of Toxicologic Pathology in November 2001 pp1-351. These papers were distributed first in draft form then as a final version to Regulatory Authorities and Advisory Committees, world wide, for comment. ILSI/HESI have co-ordinated a collaborative research project using 21 chemicals, including six known human carcinogens (three genotoxins, one immunosuppressant and two hormonal carcinogens), 12 rodent specific carcinogens (presumed on the basis of epidemiology and /or mechanism of action data) and three non-carcinogens. Chemical selection was targeted predominantly at non-genotoxic carcinogens in view of the need to examine specific mechanisms of chemical carcinogenicity in the animal models under consideration. In addition appropriate data were already available on a number of genotoxic carcinogens in some of these animal models. All chemicals used were readily accessible to test laboratories and certain core data were available; i.e. 2 year bioassay data in 2 species, established toxicology database, data on human exposure and effects.

5. The research programme was overseen by a Steering Committee of scientists drawn from academia and from pharmaceutical companies. The models under consideration were: p53+/-, ras H2+/- , Tg.AC, Xpa-/- , Xpa-/-/p53+/- double knockout, neonatal mouse, and Syrian Hamster Embryo (SHE) assay.

6. The protocols used were based on existing knowledge for each model. Positive control chemicals were used to demonstrate that each testing laboratory could undertake and report a positive assay for the model under test. Participating laboratories volunteered to act as compound co-ordinators, identifying sources of supply, co-ordinating the characterisation of chemicals and analytical methods for toxicokinetic studies. They also provided advice on the evaluation of 4-week dose range funding studies. It is noted that in practice the high dose level used equated to the Maximum Tolerated Dose (MTD). Assay Working Groups (AWGs) were formed for each assay, initially to refine protocols and to make recommendations on dose levels but eventually provided considerable assistance in resolving practical issues which arose during the research programme. AWGs also acted to collate data and to as a focal point for review of data and the application of the evaluation criteria. A Pathology Subcommittee and Statistics Subcommittee of the Alternative Cancer Test Committee were established to help set consistent criteria for evaluating studies.[4] The AWG acted as peer-review for data assessment before the results of studies were entered into the ILSI Alternatives to Carcinogenicity Testing Database. The database will eventually be made publicly available. A workshop was held 1-3 November 2000 in Leesburg, Virginia, USA to review the data from the research programmes.

7. The Committee's assessment was based predominantly on pre-publication reports submitted to the June 2001 COC and a brief consideration of the published results. The Committee's comments focused on the proposal that the alternative cancer tests models under consideration could be used as replacements to a long-term carcinogenicity bioassay in the mouse. The Committee made a number of general comments on the strategy used by ILSI/HESI before considering the results of each model. The Committee agreed to consult the COM for additional advice on the conduct of the Syrian Hamster Embryo cell transformation assay.

General Comments on ILSI/HESI strategy

8. Members welcomed the opportunity to comment on the pre-publication papers and raw data from the AWGs. Members acknowledged the considerable administrative and practical problems that had confronted ILSI/HESI in co-ordinating this work. It was considered that the programme had provided a large amount of information on the evaluation of performance of these assays but the data were not sufficient to validate the use of any of the assays for regulatory testing. Members asked for a number of comments to be forwarded to the ILSI/HESI Alternative Cancer Test Committee for inclusion in the peer review process.

9. The Committee noted that one of the aims in the selection of test chemicals had been to expand the available data set to included non-genotoxic carcinogens as data were already available on a range of genotoxic carcinogens. A key aim was to examine the ability of the individual alternative cancer models to detect human carcinogens. The carcinogens selected by ILSI/HESI were considered to act by a range of mechanisms including immunosuppression, enzyme induction, cell proliferation, and receptor mediated. Members agreed the rationale proposed by the investigators but commented that the categorisation of some of the carcinogens based on mechanisms in rodents and epidemiology data was debatable. However, it was agreed that the categorisation as suggested by ILSI/HESI would be used in this statement.

10. The Committee agreed that it was important to have the results of tests for all of the 21 chemicals selected using all of the assays. Thus it was agreed that a good level of testing had been achieved with perhaps the exceptions being for some rodent carcinogens in the Tg.AC, Xpa, Xpa/P53 and neonatal mouse models. Members also considered that the inconsistent response of some positive control chemicals in some of the assays confounded the evaluation of the data. With regard to the test methods, Members agreed the rationale of using 3 dose levels and a transgenic control, but noted that there would be only a minimal reduction in animal usage if it proved necessary to also undertake additional concurrent studies with non-transgenic animals in order to provide adequate results for regulatory assessments of chemicals. Members also commented that the duration of testing required in the *Xpa* assay (39 weeks) and the duration of observation required in the neonatal mouse tests (1 year) were such that these two assays could not be called "short-term" assays.

Comments on Alternative Cancer Tests

11. The Committee then discussed the results from each of the assays included in the ILSI/HESI programme.

12. **With regard to the p53+/-** transgenic mouse model, Members confirmed their previous conclusion that there was a rationale for assuming that this model could identify genotoxic carcinogens. All 21 chemicals selected by ILSI/HESI had been tested.[5,6] The Committee agreed that there were a number of queries regarding the results of some of the tests undertaken to be resolved before definite conclusions on assay performance could be reached. Members noted that a negative result had been obtained with phenacetin whereas a positive result should have been obtained. Members considered the positive result reported for cyclosporin but noted that there was little difference between the tumourigenicity observed in P53 +/- transgenic mice compared to wild type mice. Inconsistent results had been obtained with diethylstilbestrol and oestradiol whereas positive results should have been obtained. Members commented that the inclusion of hyperplasia as a positive result was not justified and overall diethylstilbestrol had, in their view, given a negative response in this assay. Equivocal responses had been found with chloroform and DEHP whereas negative responses should have been obtained. Members noted that there were inconsistencies between laboratories with regard to the performance of *p*-cresidine as a positive control in one study (negative result obtained) and the inadequate results obtained with benzene in one study. These data suggested a possible lack of reproducibility of the assay. Members confirmed their previous conclusion that the p53+/- mouse model could identify some genotoxic carcinogens.

13. **With regard to the Tg.AC** transgenic mouse model, Members confirmed their previous conclusion that there is a mechanistic rationale which could potentially support the use of this model to identify chemical carcinogens and potentially tumour promoters. It was noted that 14 out of the 21 chemicals selected by ILSI/HESI had been tested, and that data for only 6 out of the 13 rodent specific carcinogens had been presented.[7,8] The incomplete testing with this model therefore limited the conclusions which could be reached from the ILSI/HESI project. The Tg.AC transgenic mouse model identified positive results for 5 out of the 6 human carcinogens tested (including those acting by genotoxic, immunosuppressant and hormonal mechanisms) when data for dermal and oral tests were considered together. However there were inconsistencies in the current trial such that the genotoxic carcinogens cyclophosphamide and mephalan gave equivocal results when tested dermally but positive results when tested by oral administration. Cyclosporin, diethylstilboestrol and oestradiol gave positive results in dermal tests and equivocal (cyclosporin) or negative results in oral tests. A negative result was obtained for phenacetin in both oral and dermal tests. With regard to the rodent specific carcinogens tested, the positive response with topically applied clofibrate and equivocal response with Wy-14, 643 needed further explanation. Taking all of the available data on the Tg.AC transgenic mouse model, Members agreed that further explanation of the results for glycidol (false negative) and resorcinol (false positive) were required before the utility of his model could be further considered. It was noted that the problems with non-responder phenotype reported in earlier studies with the Tg.AC transgenic mouse model had been overcome. However, Members were concerned that the rate of

spontaneous tumours was significantly higher in the ILSI/HESI sponsored studies than in previous investigations using Tg.AC mice. Members were also concerned, for animal welfare reasons, at the sensitivity of these mice to audio induced seizures but were reassured to note in practice that such reactions were very rare. Members agreed that the available data on the Tg.AC transgenic mouse model showed that there were problems in consistently identifying human carcinogens which needed to be resolved. This suggested a need for further optimisation of methods, an understanding of the mechanisms underpinning differences between dermal and oral tests with the same chemical and a greater database before the performance of the model could be evaluated.

14. **With regard to the Xpa-/- and Xpa-/- p53+/-** transgenic mice models, Members observed that selection of the Xpa gene was only of relevance to the identification of bulky genotoxic carcinogens and possibly cross linking agents. Members agreed that there was no mechanistic rationale for producing a transgenic animal model with which was deficient for Xpa and heterozygous for p53 gene other than maximising the predisposition to detection of specific categories of genotoxic carcinogen such as cross linking agents. It was noted that 13 out of the 21 chemicals selected by ILSI/HESI had been tested in the Xpa transgenic mouse model.[9,10] Negative results had been obtained with phenacetin and oestradiol in Xpa mice but this was not unexpected given the specificity of the transgenic modification used in this particular assay. The positive results obtained in the Xpa mouse for Wy-14,643 needed further explanation. An inconclusive result had been obtained for clofibrate. Members also noted that there was significant interlaboratory variation in results for the positive control chemical p-cresidine with a negative result reported for one laboratory. Fewer results were available for the Xpa/p53+/- transgenic mouse model, with results for only 10 out of the 21 ILSI/HESI selected chemicals available. Members noted that oestradiol had given a positive result in Xpa/p53+/- transgenic mice in contrast to the negative result with Xpa-/- and agreed that an explanation for the difference in results would be valuable. It was also noted that the peroxisome proliferators Clofibrate and Wy-14,643 had not been tested in Xpa-/- p53+/- which might have given some insight into the unexpected results with these two chemicals reported for Xpa-/- transgenic mouse model. Overall few conclusions could be drawn from such limited data with these two models. The Committee felt that a valid rationale for developing these two particular transgenic animal models for short-term testing of potential carcinogenicity had not been proposed.

15. **With regard to the *rasH2+/-*** transgenic mouse model, members reiterated their previous conclusion that there was uncertainty about the relevance of this model, which entailed the integration of multiple copies of the c-Ha-ras gene into the CB6F1mouse in respect of the relevance of the model to the carcinogenic process in humans. Data were available for 20 out of 21 test chemicals selected by ILSI/HESI.[11,12] The study with Wy-14,643 was ongoing at the time of publication. Members noted that the immunosuppressant cyclosporin A and the hormonal human carcinogen oestradiol were negative in this model. Members considered that further explanation of the positive results with the peroxisome proliferators clofibrate and DEHP was required. It was noted that the papers supplied by ILSI contained the postulation that overexpression of the ras transgene followed by mutation of the transgene was the probable mechanism of carcinogenicity. Overall the Committee agreed that very little weight could be attached to results from this particular transgenic animal model given the proposed mechanism of carcinogenicity.

16. **With regard to the neonatal mouse model,** Members recalled the conclusion reached in 1998 that there was no evidence to support the use of either the neonatal rat or mouse bioassays as a part of regulatory testing strategies.[13] The new information from the ILSI programme, where 13 out of the 21 selected test chemicals had been tested in neonatal mice, supported this view.[14,15] Five out of 6 human carcinogens had been tested and a positive response had only been documented for cyclophosphamide and for oestradiol (in one out of three studies). Members reiterated their animal welfare concerns about the evidence of considerable animal mortality during these experiments. Overall there was no rationale for including the neonatal mouse model in carcinogenicity testing strategies.

17. With regard to the available data from the Syrian Hamster Embryo test[16], the COC noted that full consideration of these data would be given by the COM and a separate statement published in due course.

Conclusion

18. The COC agreed an overall conclusion that none of the models used in the ILSI/HESI Alternative Cancer Test programme were suitable as a replacement for the mouse carcinogenicity bioassay (the primary purpose for the development of these models) and that further research should look to identify models with a greater relevance to mechanisms of carcinogenicity in humans. Of the animal models assessed there was evidence that p53+/- transgenic mouse model could identify some genotoxic carcinogens. There was insufficient data to suggest that the animal models under consideration (RasH2, Tg.AC, Xpa, Xpa/P53+/- and p53+/-) provide essentially similar results. (A separate statement from the COM on the ILSI/HESI evaluation of the Syrian Hamster Embryo test would be published in due course).

April 2002

COC/02/S3

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The minimum duration of carcinogenicity studies in rats: review of two selected papers published in 2000

Introduction

1. The proper conduct of carcinogenicity studies in rats is an important part of the evaluation and prediction of potential human carcinogens. Significant reductions in the number of control rats surviving to termination have been widely reported in the scientific literature.[1-5] This is a matter of concern since inadequate carcinogenicity studies could be important in decisions regarding the identification of potential human carcinogens and in particular the failure to identify such compounds. In addition there is a possibility that inadequate studies could be rejected by regulatory agencies with the consequent need for use of further animals to obtain a valid result. For a negative result from a rat carcinogenicity bioassay to be considered acceptable, survival at 24 months should be 50% or greater in all groups.[6,7]

2. The Committee reviewed the evidence for application of dietary restriction techniques in 2000 and a statement was published (COC/00/S3). It was concluded that the available information supports the view reached by the COC in its guidelines published in 1991 that dietary restriction in carcinogenicity studies should be applied with caution and is the responsibility of the toxicologist undertaking the study. The COC agreed that the subject of dietary restriction should be reviewed when more information is available.

3. Some investigators have also proposed that reducing the duration of carcinogenicity bioassays undertaken in rats would have the desired effect of terminating studies before survival was reduced to below 50% in tests groups.[8] The Committee reviewed two published papers which had evaluated published data on the duration of carcinogenicity studies. These two papers (see paras 4 and 5 below) came to contrasting views. The Committee was asked to provide generic advice on the desirability of reducing he minimum duration of carcinogenicity studies in rats.

Review of two selected investigations

Davis et al, 2000[8]

4. Davis et al studied IARC chemical Monographs (Vols 1-70) to determine the time of onset to 'treatment-related' tumorigenicity in long-term rodent studies for chemicals classified by the IARC as showing evidence of carcinogenicity in animals. The chemicals were categorised as producing tumours at ‹12m, 12-18m, or ›18m. The analysis excluded studies on metals and their salts, studies on particulates, studies by parental routes of administration that resulted in tumours only at the site of exposure, and studies that did not approximate to the current standard long term rodent carcinogenicity bioassay e.g. transplacental or multigeneration studies, initiator-promoter studies, lung tumour assays in 'Strain A' mice and studies in new born animals. Davis et al considered that from a total of 210 chemicals, overall, evidence of treatment related tumorigenicity was first apparent within 12 months for 66% of the chemicals and that studies longer than 18 months were necessary for 7%. All IARC Group 1 chemicals

were detected in animals within 18 months and most within 12 months. Most of the tumour types that required more than 18 months for detection were considered by Davis et al to be of "dubious" relevance to human risk assessment On this basis Davis et al concluded that termination of rodent carcinogenicity studies at 18 months or earlier was justified, and would greatly reduce the complications that arise in interpreting findings in aged animals.

Kodell et al, 2000[9]

5. Data from bioassay studies in rats using selected pharmaceuticals were used to formulate biologically based dose-response models of carcinogenesis based on the 2-stage clonal expansion model. These dose response models, which were chosen to represent 6 variations of the initiation-promotion-completion cancer model were employed to generate a large number of representative bioassay data sets using Monte Carlo simulations. The six variations of the model were based on data:

Model Variation	Data on which model variation was based
initiator only	anonymous drug 1 and pancreas adenoma in females
completer only	anonymous drug 1 and mammary adenocarcinoma in females
initator + completer	anonymous drug 1 and mammary adenocarcinoma in males
initator + promoter	anonymous drug 2 and pancreas acinar cell carcinoma in males
promoter + completer	anonymous drug 3 and thyroid follicular cell adenoma in males
promoter only	selenium sulphide and liver hepatocellular carcinoma in females

For a variety of tumour dose-response trends, tumour lethality and competing risk-survival rates, the power of age-adjusted statistical tests to assess the significance of carcinogenic potential was evaluated at 18 and 21 months and compared to the power at the normal 24 month termination time. Kodell et al results showed that termination at 18 months would reduce statistical power to an unacceptable level for all 6 variations of the 2-stage clonal expansion model, with the pure-completer models being most adversely affected.

COC Discussion

6. The committee agreed that some rat strains, namely, Sprague-Dawley (in certain labs) have inadequate survival at 24 months. Members noted the argument put forward by Davis et al that the pathology associated with old age might mask important cancer pathology in animals terminated at 24 months. Davis et al had also argued that it is possible that an earlier onset of the incidence of a common spontaneous tumour type could be detected at 18 months and missed at 24 months. However, members considered that in 24-month studies, autopsy of the dead animals and analysis of tumour incidence in deceedents would pick this up.

7. The committee considered that a single study would not be looked at in isolation and that consideration of the mechanism of an effect was crucial in the overall evaluation. Members were also concerned about modifying an already imperfect lifetime model, and agreed that possible dietary methods of extending life span, such as by caloric restriction, needed to be considered on a case-by-case basis with regard to laboratory historical control data on tumour incidence.

8. The committee did not agree with the conclusions drawn by Davis et al that carcinogens detected after 18 months were unlikely to be relevant to human health assessment. Members were concerned that such shortened studies might not be sufficiently sensitive to detect some human carcinogens. The Committee agreed that the approach taken by Kodell et al to the modelling of carcinogen dose-response was satisfactory.

COC Conclusion

9. The COC concluded that there was insufficient evidence to recommend a change to the international guidelines for the conduct of long term carcinogenicity bioassays, that for a negative result to be acceptable in a rat carcinogenicity bioassay, survival should be at least 50% in all groups at 24 months. The Committee reaffirmed that it was the responsibility of the study director to use rat strains that would ensure adequate survival at 24 months.

March 2002

COC/02/S2

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Statement on the investigation of interaction between genotype and chemicals in the environment on the induction of cancer

Background to review

1. The Committee was asked by the Department of Health to review the available information on the interaction between genotype and exposure to chemicals in the environment and the induction of cancer. The Committee was asked to provide advice on the methods of epidemiological research used in this area, the approaches to identifying genes of interest for such studies and the evaluation and significance of these data for cancer risk assessment.

2. The Committee was aware of the major technological advances in rapid DNA sequencing which had been published by the Human Genome Project (HGP) (http://www.ornl.gov/hgmis/project/.html) and the Environmental Genome Project (EGP) (http://www.niehs.nih.gov/envgenom/). [See Introduction to review and glossary for explanation of abbreviations and terms used in this statement] A draft scaffold sequence for the human genome was published in February 2001 and it has been proposed by HGP that a complete high quality DNA reference sequence will be available by 2003. These projects have as their major goal, the diagnosis, prediction and intervention in diseases where there is a genetic contribution to the cause of disease. However the EGP is focused on the role of genes implicated in cellular responses to environmental chemicals. Ambitious projects have been set up by EGP, for example, to identify Single Nucleotide Polymorphisms (SNPs) for up to 30,000 genes, new statistical methods to aid in the evaluation of the interaction effects of carcinogen metabolism and bioinformatics tools to assist in the evaluation of the large amounts of data generated from epidemiological studies. These, and other developments[1-3] are likely to lead to a rapid increase in the published information on the interaction between genotype and exposure to chemicals in the induction of specific cancers. It was considered timely to examine the questions raised by the Department of Health in order to draw conclusions on what advice could be given.

3. The Committee considered that it was necessary to set out a discussion of the key terms in the text of the statement. The Committee agreed that a concise "non-technical" summary was also required which should provide a glossary of key terms. Members considered it appropriate to discuss the critical areas of the review, particularly the design of epidemiology studies for genotype-environment interactions for specific cancers, the identification of genes of interest and risk assessment, before providing advice and suggestions for further research. The Committee was provided with a set of detailed papers drafted by the DH Toxicology Unit at Imperial College of Science, Technology and Medicine for use in their discussions.[4-7] The relevant papers will be published on the COC website (www.doh..gov.uk/coc.htm).

Introduction to review

Introduction to the review

*Background to terminology**

**(see HGP and EGP internet sites (para 2 for addresses) and refs 4-7)*

4. The human genome comprises all the genetic material (i.e sequence of DNA) in the 23 pairs of chromosomes present in all somatic nucleated cells in the body. Within the genome, the gene is the fundamental physical and functional unit of heredity. A gene is an ordered sequence of DNA located in a particular position on a particular chromosome that encodes for a specific functional product(s) (i.e. a sequence of RNA which may be translated to give a protein(s) which, with any subsequent necessary posttranslational modification, gives the functional protein). One key development arising from the expansion in DNA resequencing work described in paragraph 2 of this statement has been the recognition that the human genome does vary considerably between individuals (i.e it is subject to considerable interindividual variability). Thus it is estimated that approximately 1 in every 300-500 base pairs will differ between any two individuals. Variation in the DNA sequence of a particular gene between individuals comprising a single nucleotide difference is called a Single Nucleotide Polymorphism (SNP). The variations in the genome between individual ranging from SNPs, differences in small sequences of DNA, up to whole chromosomes are collectively referred to as "genotypic variation". During its discussions the Committee was principally concerned with SNPs. These may have no impact on the function of the encoded gene products and are called "non-functional but in some cases SNPs do result in variation between individuals in the function, e.g. qualitative and/or quantitative changes in protein function. This is referred to as phenotypic variation". The phenotype of an individual is defined as the observable physical biochemical or physiological characteristics of that individual.

5. Within the genome, SNPs can be found in the coding region of a gene; i.e. functional DNA, (cSNPs), in potential regulatory sequences, i.e. peri-genic regions (pSNPs) or in intervening stretches of DNA with no apparent function (intergenic DNA; iSNPs). The term genetic polymorphism is often used to indicate phenotypic variation and as such is frequently used in association with genetically-determined variations in the metabolising capacity for chemicals. The changes in DNA sequence responsible for metabolic polymorphisms are often SNPs. It is now easier and more pragmatic to identify the genotype in large numbers of individuals by DNA sequencing rather than to elucidate phenotype (i.e. measure the expression and function of genes) and thus it is possible that fewer studies of phenotype will be undertaken. However it is the phenotypic expression of genes that is most likely to be important with regard to the interaction between a gene and an environmental chemical in the induction of specific cancers.

6. The development in DNA sequencing techniques has allowed for the rapid and easy identification of SNPs, and hence closer examination of whether there is an interaction between the occurrence of a particular SNP in an individual and chemical exposure that is associated with adverse health effects such as cancer. Many epidemiological studies have investigated associations between cancer incidence and polymorphisms of the enzymes responsible for the metabolism of chemical carcinogens since many carcinogens require metabolic activation. It is therefore logical to suggest that variation in metabolism of these chemicals will accord with changes in risk of cancer development. The Committee reviewed

several examples, e.g. *N*-acetyltransferase 2 (NAT 2) and exposure to tobacco smoke associated with bladder cancer, and glutathione-S-tranferase M1 (GSTM1) and exposure to tobacco smoke associated with lung cancer.[7] However the Committee felt it was important also to consider target genes other than those associated with the metabolism of chemicals. A discussion paper was therefore drafted on this topic.[6]

7. The term "penetrance" is used in this statement to describe the frequency with which carriers (e.g. of a particular genotype) develop cancer, i.e. the ratio of carriers who develop cancer compared to all carriers. Inherited cancer genes are considered to be "high penetrance" if affected individuals have a high probability of getting cancer. An example is the breast cancer susceptibility gene BRCA1, where the lifetime cumulative risk of cancer in individuals carrying specific mutations within this gene has been estimated as approximately 90%. However "high penetrant" genes are usually rare , i.e their prevalence in the population is low. The genotypes under consideration in this statement are considered to be of low penetrance, i.e. the increase in risk of cancer is very low. However their prevalence in the population can be very high (e.g. 40-50% of the population as with N-acetyltransferase 2 slow acetylator (NAT2) allele polymorphism and GSTM1 null polymorphism). The Committee noted that the penetrance and prevalence of genotypes that were of importance to carcinogenesis induced by chemicals could vary and thus this should be considered in strategies to identify genes for research.

8. The use of the term "interaction" has been considered in detail.[8-10] There are two ways in which this term has been used in the scientific literature: either to describe a biological model of interaction between two or more factors in the aetiology of disease or to describe the statistical concept of interaction which describes the patterns of disease risks. Thus for genotype-environment interactions, a biologically significant effect infers that there is evidence for or there is a presumed (as yet unknown) biological consequence arising from the function of a particular gene variant and exposure to chemical(s) on the risk of cancer. The degree of statistical interaction can be measured in two ways, depending on whether it is the differences (i.e additive scale) or ratios of risks (i.e multiplicative scale) that are of interest. An illustrative numerical example is given in the Annex at Table 1 based on the lifetime risks of lung cancer. Further explanation of the example is given below in paragraphs 30-31 that concern risk assessment.

Discussion of critical areas to be considered

9. Members noted the rapid increase in the number of publications on genotype-environment interactions and in particular those concerning the potential impact of metabolic polymorphisms. They were also aware of the suggestion that genetic screening could be used to identify individuals carrying a particular genotype or to identify chemicals to which individuals should avoid exposure. The Committee therefore agreed there were two questions which needed to be addressed during the review namely;

    i) The extent to which subgroups of the population can be identified, who because they have a particular genotype, are at greater risk of developing cancer, when exposed to particular chemicals.

ii) Is it appropriate or desirable to use genetic screening to identify individuals with a particular genotype of importance to chemically induced cancers.

10. Members agreed that in order to consider these two questions, it would be important to review the epidemiological methods used and to comment on the significance and potential value of the results from these studies for risk assessment. An integral part of this consideration would be to provide advice on the numbers of individuals required in such studies. This review would also provide advice on gene selection, and the formulation of hypotheses for future epidemiological investigations.

11. Members agreed that a further critical area for review involved the discussion of the nature of the interactions between genotype and exposure to chemicals resulting in an increased risk of cancer. The objective was to define criteria which could help to assess whether an interaction existed between a particular genotype and exposure to chemicals leading to an increase in the frequency of cancer that was significant for public health. This will assist in differentiating between genotype-environment interaction associated with increased risk of cancer, and those which are chance findings, and therefore not relevant to risk assessment.

12. It would then be important to define the data necessary to assess the potential impact of interactions between genotype and exposure to chemicals and if possible to estimate potential numbers of cancer cases that might be involved. It would also be important to provide advice on the prospects and desirability in regard to the suggestion for genetic screening. In this regard, the Committee was of the view that a number of critical genotype-environment interactions have yet to be discovered.

13. The Committee agreed that any conclusions should be prefaced with a discussion of the uncertainties in the assessment.

The Assessment of Genotype-Environment interaction studies[4,5]

14. The recognition that many cancer susceptibility genes are likely to be of low penetrance has led to the evolution of two major study designs for the assessment of gene-environment interactions.

    i) epidemiological studies of candidate susceptibility genes (gene characterisation studies) and,

    ii genetic association studies (gene discovery studies).

15. In the first, the influence of known polymorphisms (or SNPs) on cancer risk is determined, usually in case-control or cohort studies, whilst in the second, cases and controls are genetically screened in an attempt to identify a clear difference in one or more gene loci. Most studies of the interaction between genotype and exposure to chemicals to date have involved the first design, but the increasing availability of dense SNP (single nucleotide polymorphism) maps and the technology to perform large numbers of genotyping tests is making the second design much more feasible.

Gene characterisation

16. Gene characterisation studies should involve the *a priori* selection of candidate genes to be included in the study protocol before the investigation is initiated. A number of different study designs had been used including case-control (with a variety of methods for choosing controls), cohort and case-only. Members noted that the use of case-only designs was relatively recent and could provide an estimate of the strength of interaction between genotype and exposure to chemicals but such studies assumed independence between the effects of genotype and exposure to chemicals. Members felt that overall many of the available genotype-environment interaction studies suffered from flaws in design and/or interpretation, reducing their potential value in cancer risk assessment. The Committee considered that apart from the limitations often found in epidemiological studies such as measurement error, bias and confounding, a key concern for many published studies was the absence of clearly stating the *a priori* hypotheses to be tested before undertaking the epidemiological investigation. The reliance of many research groups on *post hoc* analyses of sub-groups after data had been generated could yield biased statistical analysis of the multiple comparisons common in such studies. Members considered that the *a priori* hypotheses under investigation should be clearly stated in publications, perhaps even lodged with a third party before the analysis. The Committee reviewed study designs used for case-control gene characterisation studies and agreed that good study design would require careful selection of cases and controls from the same population, adequate exposure assessment, appropriate analysis strategy, and power calculations of necessary study size (given assumptions on penetrance, relative risk of disease and prevalence of susceptible genotype).

17. The Committee reviewed some model calculations for a case-control study design based on published approaches to the consideration of study size. The calculations assumed that genotype and chemical exposure had independent effects on cancer risk, there was no matching of cases and controls, and a mulitplicative interaction was of interest, a baseline cancer rate of 0.001 and the odds ratio (OR) for cancer in non-susceptible subjects from exposure was 1.5.

18. These calculations provide evidence to show that many of the currently published case-control studies are of insufficient size to identify moderate interactions between genotype and exposure to chemicals in the induction of cancer.

Table 1. Number of subjects required in case-control studies

Proportion of susceptible genotype in population	Strength of interaction to be detected	Number of subjects (equal number of cases and controls)
0.5 (50%)	2x	2,215
	5x	485
0.2	2x	3,891
	5x	1,017
0.05	2x	13,902
	5x	3,949

Two-tailed test of null hypothesis, $P < 0.05$; power, 0.8. (Calculations were performed using the "Power" program described by García-Closas and Lubin (1999), American Journal of Epidemiology, **131**, 552-566.

Gene Discovery

19. Until recently, gene discovery designs have not been used widely in genotype-environment studies of cancer. This is because of the impracticalities involved in screening the very large numbers of subjects and alleles that would be necessary for the detection of genes (i.e. sequence variants such as SNPs) of low penetrance. However, as indicated above, rapid advances in both knowledge and technology are making such study designs more feasible, and several groups have commenced or are about to commence such studies.

20. The Committee considered that there were at least four broad categories of gene for which it was reasonable to hypothesise that genotype-environment interactions might be of importance with regard to cancer.[6]

Table 2 Categories of gene associated with Genotype/Environment interactions

Category of gene	Examples
Increased metabolic activation and/or reduced detoxication, elimination.	Cytochrome P450 isozymes (e.g CYP1A1 and CYP2E1). Glutathione S-transferases (e.g GSTM1 and GSTT1). N-acetyltransferases (e.g NAT1 and NAT2). P-glycoprotein transporters.
Reduced capacity for DNA repair.	Very few studies to date have examined DNA repair capacity, suggestions include base and nucleotide excision repair genes.
Immune surveillance.	No data available in respect of immune surveillance, suggestions include human leukocyte antigen complex (HLA).
Increased potential for cell proliferation and survival resulting from alterations in control of cell cycle and apoptosis.	No data available. Suggestions for cell cycle control include cyclinD1 and HRAS1 and for apoptosis Bcl-2.

21. Members were aware that there was a large number of publications which had reported investigations of genetic polymorphisms of enzymes of metabolic activation and detoxication (e.g. cytochrome P450 dependent monooxygenases, glutathione-S-transferases and *N*-acetyltransferases) and some studies had included investigations of the combined effect of two or more metabolic polymorphisms for these enzymes. Comparatively few studies had investigated variants of the other categories of genes identified by the Committee, and the extent to which these might interact with environmental chemicals was unknown.

22. The Committee considered that it was difficult to know how to prioritise the search for gene variants with increased risks for environmentally induced cancers as this could plausibly involve many thousands of such variants. However, members believed that the benefits of improving and developing technology could result in this exercise being practical and useful in the future. Members noted that the Environmental Genome Project had identified similar categories of genes for inclusion in the first phase of its project on gene discovery.

23. Members agreed that as understanding of the pathways and genes involved in the biological processes critical to cancer development increases, the number of candidate genes within those pathways that may be relevant to study for interaction with environment would increase rapidly.

Criteria for assessing interactions between genotype and environment in the aetiology of cancer[7]

24. The Bradford-Hill criteria for causality[11] have been used in the past to investigate single risk factors (environmental or genetic) by both this Committee and the WHO International Agency for Research on Cancer (IARC) reviewed studies of selected metabolic polymorphisms and susceptibility to cancer. Whilst there was no formal attempt to establish causality, the conclusions reached were based on the Bradford-Hill criteria.[12]

25. In contrast to investigations of single factors, the Committee agreed that, consideration of genotype-environment interactions referred to the assessment of whether the occurrence of a particular genotype and exposure to chemicals was associated with an increased frequency of cancer that was of significance for public health . The Committee agreed that an assessment of genotype-environment interactions should ideally require information on the gene variants(s) under consideration, the mechanism of carcinogenicity of the chemical under consideration and evidence to link all of this information together to form a reasoned case.

26. Members acknowledged, however, that it was likely that future investigations would examine the potential role of several hundreds or thousands of genes simultaneously and felt that, for the assessment of genotype-environment interaction studies, initial emphasis would be placed on the strength and consistency of the association. This would require demonstration of consistency in both gene discovery and characterisation studies and preferably by several different methods in adequately conducted gene characterisation studies. There would also need to be a plausible rationale for the mechanism of carcinogenicity for the chemical under consideration. This assessment should ideally include information on phenotype, but it is recognised that such information may not always be available.

27. Members also highlighted the potential problem of random co-inheritance (i.e. linkage disequilibrium), where alleles of one gene (associated with increased risk) are inherited with specific alleles of adjacent genes (unrelated to risk) giving the false impression that these latter genes were also causally associated with increased risk. Therefore, in the absence of knowledge of which genes are co-inherited, it would be important to have some understanding of the mechanism of carcinogenesis of an environmental chemical before any final conclusions could be reached.

28. Thus the Committee agreed that a tiered approach to the assessment of genotype-environment interactions was required as outlined in paras 24-27 It would only be possible to undertake a quantitative risk assessment if there was compelling evidence that a true interaction existed.

Risk Assessment[7]

Significance of genotype-environment interactions for public health

29. The Committee agreed that a full assessment of the significance of genotype-environment interactions with regard to chemically induced cancer required considerable information to be available. Thus ideally data on the prevalence of chemical exposure, prevalence of susceptible genotype and the cancer incidence rate in those exposed with and without the genotype and in those non-exposed with and without the genotype of interest. However, some useful measures of the size of an interaction and its impact can be estimated if relative risks (or odds ratios) are available instead of incidence rates (See Annex). In many instances such data would not be available and thus any evaluation would be based on incomplete data.

30. The Committee reviewed a worked example where appropriate data were available, namely lung cancer, smoking and GSTM1 polymorphism. The rationale for choosing this example was that the particular cancer is common, there is good agreement regarding the exposed attributable fraction for lung cancer associated with smoking (cf 90%) and the polymorphism chosen was common (i.e. 50% of population).

31. The results of the model calculations are given in Tables 1 and 2 in the Annex at the end of this statement. Any measure of *population impact* needs to take into account the prevalence of both the high-risk genotype and the environmental exposure, as well as the risks of disease in each exposure combination. One approach would be to simply use this information to work out the numbers of cancer cases who would be predicted to occur in each exposure subgroup (as outline in para 29, exposed with and without the genotype and non-exposed with and without the genotype of interest) and hence the population impact. Another approach would be to use the *population attributable fraction* (PAF). For a single risk factor this is usually considered to be the fraction for exposure to a single factor of disease in a population that might be avoided if the exposure had not occurred (or by eliminating that exposure). The model calculation estimates the PAF for all potential exposure subgroups. It is also possible to calculate the *exposed attributable fraction* of disease which provides information on the fraction in the exposed subgroups which might be avoided by eliminating exposure. The results shown in the Annex Table 1 suggest that for the example of GSTM1 polymorphism and lung cancer there is a slight benefit to the population impact (in reduction of numbers of individuals with lung cancer) in targeting smokers with GSTM1 but only if effective intervention is feasible. This has important implications when reviewing the practicalities of screening (see para 34 below).

32. Members were aware that to date most studies had investigated the interaction between metabolic polymorphism (i.e variation in the metabolising capacity for chemicals) and cancer.[11] The majority of studies (using either case-control or cohort methods) report modest increases in relative risk in exposed individuals with the susceptibility genotype. Without information on the factors outlined in paragraph 29 above, it would be difficult to derive conclusions on the significance for public health of the genotype-exposure interaction. The Committee concluded that the available data on metabolic polymorphisms had failed to demonstrate any consistent strong association between any one

gene-environment interaction and cancer risk and therefore the interactions studied to date were likely to be of little importance for public health or risk assessment.[11] However, this did not exclude the possibility that genotype-environment interactions with a significant impact on cancer risk would be identified in the future.

33. The existence of an association between a genotype and chemical exposure in the induction of cancer (e.g. a phenotype that results in enhanced metabolic activation of the chemical) could provide supporting epidemiological evidence in the identification of human carcinogens. It would be important to demonstrate a plausible biological association between the mechanism of carcinogenesis and the genotype/phenotype measured.

Significance for genetic screening

34. A final measure of impact which can be derived is the Number Needed to Screen.[13] This combines together the prevalence of genotypes, the risks of cancer in each subgroup and the reduction in risks which could be achieved by screening identified individuals where effective intervention was possible. The Committee noted that the data for the example used in model calculations suggested that there was little value in screening for GSTM1 polymorphism. The Committee reaffirmed its view that when the environmental exposure is smoking, the only appropriate public health intervention was to aid all smokers in giving up smoking. The Committee reviewed some further published calculations which confirmed that it was impractical to screen for these low-penetrant genotypes in the general population.[13] The Committee noted that, at present, there is little value for risk assessment in screening for the genotypes identified to date in gene-environment interaction studies. This is because the number of individuals with the genotype of interest who would develop cancer would be small, whereas there would be large numbers of individuals with the genotype of interest who would not develop cancer. Members were also concerned that screening for such low penetrant genotypes was undesirable in that the information would not have any significant predictivity of individual risk of cancer. In addition, other risk factors for cancer such as diet and smoking were likely to be of much greater importance in determining individual risk. The Committee also noted that there were considerable ethical, legal and social issues to be considered with regard to any proposal for screening which were beyond the scope of this review.[14]

Discussion and conclusions

35. The Committee noted that there were considerable practical difficulties in assessing the significance for public health of the currently available genotype-environment interaction epidemiology studies of cancer. These related to the size and design of the investigations and the absence of clearly set out *a priori* hypotheses as an essential part of study design. Many of the studies published also had limited power to detect genotype-environment interactions. The Committee was aware of the rapid advances in DNA re-sequencing in the last few years which meant that many potential candidate genes and genotypes/SNPs for investigation in genotype-environment cancer studies were being identified and many more would be forthcoming. The Committee noted that several projects had been set up under the U.S Environmental Genome Project and other initiatives to address these issues.

36. The Committee agreed that there was a need to assess all the available information and to consider if there was compelling evidence that a true genotype-environment interaction existed before using the information in quantitative risk assessment. The Committee agreed that an assessment regarding a genotype-environment interaction should ideally require information on the gene variants(s) under consideration, the mechanism of carcinogenicity of the chemical under consideration and evidence to link all of this information together to form a reasoned case. It was necessary to consider the possibility of linkage disequilibrium. With regard to the assessment of genotype-environment interactions, initial weight should be placed on the strength and consistency of the association. There was also a need to provide a reasoned case linking the mechanism of carcinogenicity of the chemical with the genotype under consideration. This would involve some knowledge of the function of the gene in question. Members agreed it would be valuable to have full information on the phenotype including characterisation of the function of the gene product and information on chemical-phenotype interaction but acknowledged this might be a lengthy process. An interim assessment could be drawn on basic information on gene function.

37. The Committee concluded that the available data on metabolic polymorphisms had failed to demonstrate any consistent strong association between any one gene-environment interaction and cancer risk and therefore the interactions studied to date were likely to be of little importance for public health or risk assessment. However, this did not exclude the possibility that genotype-environment interactions with a significant impact on cancer risk would be identified in the future. The Committee discussed the likely scenarios under which genotype-environment interactions might be of significance for public health and also commented on the feasibility and desirability for genetic screening for low penetrance gene variants in genotype-environment interactions. It was acknowledged that future gene discovery studies might identify genotype-environment interactions involving gene variants of significant penetrance and prevalence for cancer and thus the literature on this subject should be kept under review.

38. The Committee agreed the following overall conclusions.

    i) The most appropriate study designs for gene characterisation investigations will vary according to study purpose. Many of the currently available studies are either too limited in size or relied on *post hoc* analyses to highlight selected results. Ideally, studies should include information on phenotypic variation, but it is unlikely that such data would be available for all candidate genes selected for investigation. It is essential that such studies should involve *a priori* hypotheses. There is an argument that such hypotheses should be lodged with a third party before epidemiological investigations are undertaken.

    ii) The rapid development of DNA sequencing techniques means that many gene discovery studies will become available in the future. There is currently no clear rationale for gene selection for gene discovery studies, other than to state broad categories of genes that could be prioritised for consideration (such as metabolic activation, DNA repair and immune surveillance, cell proliferation and cell cycle control).

iii) Before the results of genotype-environment interaction studies can be used in risk assessment (either for the identification of susceptible populations or identification of human carcinogens), it is necessary to establish whether there is a reasonable case to infer that the genotype-environment interaction is associated with a real and important increased frequency of cancer A tiered approach has been recommended. Initially the strength and consistency of evidence from the epidemiological studies should be considered. In addition information to establish if there is a credible link between the mechanism of carcinogenicity for the chemical and the function of the gene and genotype under investigation should be considered. This assessment should ideally include information on phenotype, but it is recognised that such information may not always be available.

iv) The Committee concluded that the available data on metabolic polymorphisms had failed to demonstrate any consistent strong association between any one gene-environment interaction and cancer risk and therefore the interactions studied to date were likely to be of little importance for public health or risk assessment.

v) There is little value in using genetic screening to identify individuals with particular genotypes of interest for carcinogenesis induced by environmental chemicals.

vi) The possibility cannot be excluded that genotype-environment interactions involving gene variants of significant penetrance and prevalence might be identified through gene discovery investigations in the future or that combinations of genotypes might result in significantly greater interaction with chemicals in the induction of cancer.

vii) The Committee recommended that it was important to keep this subject under review particularly in the light of expected developments arising from the Environmental Genome Project based in the U.S.A. and other initiatives in this area.

June 2002

COC/02/S4

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ANNEX

Calculating and interpreting genotype-environment interaction: an example using lung cancer, smoking and GSTM1 polymorphisms

The statistical definition of a genotype-environment interaction is that the effect of genotype on disease risk varies with the level of exposure to an environmental factor, or vice versa. The degree of statistical interaction can be measured in two ways, depending on whether it is the *differences* or *ratios* of risks that are of interest. For simplicity, it is assumed that the variables measuring disease, exposure and genotype are all dichotomous. An illustrative numerical example is given in Table 1 based on the lifetime risks of lung cancer. The environmental factor is cigarette smoking (+ = Yes — = No), and the genotype of interest is the GSTM1 polymorphism (+ = null — = wild). The lifetime risk of lung cancer in non-smokers with the low-risk genotype was assumed to be 1.2%, and the lung cancer risks in the other subgroups were plausible estimates from the literature.

Table 1 Lung cancer risks in each subgroup

Environmental factor (Smoking)	Genetic factor (GSTM1)	Lifetime lung cancer risk (%)	Relative risk
+	+	R++ 16.0	RR++ 16/1.2 = 13.33
+	-	R+- 12.0	RR+- 12/1.2 = 10
-	+	R-+ 1.6	RR-+ 1.6/1.2 = 1.33
-	-	R-- 1.2	Reference subgroup
Whole population		4.55	

Measures of genotype-environment interaction

If *differences* in lung cancer risk are of interest, the *Additive* measure of interaction contrasts the difference between the risks of those with the high and low risk genotype who are exposed to the environmental factor ($R_{++} - R_{+-}$), to the same difference for those unexposed to the environmental factor (R_{-+} — R_{--}).

i.e. There is no Additive interaction if $(R_{++} - R_{+-}) = (R_{-+} - R_{--})$
or equivalently, in terms of relative risks, if $(RR_{++} - RR_{+-}) = (RR_{-+} - 1)$

A measure of *Additive* interaction is therefore $(RR_{++} - RR_{+-} - RR_{-+} + 1)$ and a value of 0 denotes no additive interaction[1].

In Table 1 $(RR_{++} - RR_{+-}) - (RR_{-+} - 1) = (13.33 - 10 - 1.33 + 1) = 3.0$, so there is some interaction on an additive scale, since there is a larger difference between the cancer risks for the null and wild genotypes for smokers than for non-smokers.

If the *ratios* of the risks are of interest, the *Multiplicative* measure of interaction contrasts the ratio of the risks between those with the high and low risk genotype who are exposed to the environmental factor ($R_{++} \div RR_{+-}$), to the same ratio for those unexposed to the environmental factor ($R_{-+} \div R_{--}$).

i.e. there is no Multiplicative interaction if $(R_{++} \div R_{+-}) = (R_{-+} \div R_{--})$
or equivalently in terms of relative risks $RR_{++} = (RR_{+-} \times RR_{-+})$

A measure of *Multiplicative* interaction is therefore $RR_{++} \div (RR_{+-} \times RR_{-+})$, and a value of 1 denotes no multiplicative interaction[1].

In Table 1 $RR_{++} \div (RR_{+-} \times RR_{-+}) = 13.33 \div (10 \times 1.33) = 1$, so there is no interaction on a multiplicative scale, since the cancer risk increases by the same ratio between the null and wild genotypes in smokers and non-smokers.

NB If the data come from case-control studies, then the absolute disease risks will not be available. However, relative risks (RR) can be estimated by odds ratios (OR) and the measures of genotype-environment interaction above can be estimated using the appropriate OR.

Population impact of genotype-environment interaction

These measures of genotype-environment interaction give an idea of the size and type of any interaction between two factors, but don't permit an assessment of the impact of the interaction on the whole population or selected subgroups. A variety of such measures are described below. Any measure of population impact needs to take into account the prevalence of both the high-risk genotype and the environmental exposure, as well as the risks of disease in each genotype-exposure combination. In this example, the prevalence of smoking was taken to be 25%, while that of the null genotype was 50%, and they were assumed to occur independently.

Table 2 Measures of population impact of genotype-environment interaction

Environmental factor (Smoking)	Genetic factor (GSTM1)	Exposure prevalence (%)	Cases in population of 50 million (000s)	Population attributable fraction (%)	Exposed attributable fraction (%)
+	+	P++ 12.5	1000	40.7	92.5
+	-	P+- 12.5	750	29.7	90.0
-	+	P-+ 37.5	300	3.3	25.0
-	-	P-- 37.5	225	–	–
Total population		100	2275		

One approach would be to assess the population impact simply by calculating the numbers of lung cancer cases that would be predicted to occur in each subgroup: multiplying together the absolute cancer risks in each genotype-exposure subgroup by the appropriate prevalence. Table 2 shows the predicted numbers of subjects in a population of 50 million, who would get lung cancer at some point in their lifetime, for each genotype-exposure subgroup. It can be seen that of the 2.275 million cases expected in the whole population, the highest numbers of cases occur in the GSTM1-null & smokers subgroup, followed by the GSTM1-wild &smokers subgroup. The lack of multiplicative interaction between GSTM1 and smoking has increased the risk of lung cancer for the GSTM1 -null genotype by 33.3% in both smokers and non-smokers. However, the addition interaction means that given the 10-fold extra risk for smokers, the GSTM1-null genotype has had a more noticeable impact on the expected number of lung cancer cases among smokers than non-smokers: an extra 75 thousand cases amongst non-smokers against an extra 250 thousand cases amongst smokers. Note that this approach requires knowledge of the absolute risks, rather than just relative risks.

Another approach would be to use the *population* attributable fraction. The population attributable fraction (PAF) for exposure to a single factor is often interpreted as the fraction of disease in a population that might have been avoided if the exposure had not taken place (or, making some strong assumptions, if the exposure could be eliminated). For a single factor it can be calculated as

(Risk in whole population – Risk in unexposed subgroup)(Risk in whole population

If smoking is the exposure of interest, the lung cancer risk in non-smokers is the average of that for non-smokers with both genotypes (since they have equal prevalence). So, using the information in Table 1, the population attributable fraction for smoking is (4.55 – 1.4) ÷4.55 = 0.692 (69.2%). So 69.2% of the cases of lung cancer could have been avoided if cigarette smoking had not occurred.

The PAF is often used in a public health context to help decide which exposures to target. If it can be assumed that a number of different exposures all cause lung cancer, and there is an intervention to prevent their effect (e.g. eliminating an exposure or prophylactic treatment) then efforts may be directed towards whichever of exposures have the largest PAF. However, this approach is less useful if the intervention is not fully effective.

The PAF can be extended when there is more than one exposure category. This could be ordered categories of the same exposure factor (e.g. None, Low, Medium & High) or, as in our example, a combination of two factors (e.g. Unexposed & Low-risk-genotype, Exposed only, High-Risk-Genotype only, and High-Risk-Genotype & Exposed). The aim is to measure the effect on the population if the exposure-genotype combination in one subgroup had not occurred, using the doubly unexposed group (i.e. unexposed and low-risk genotype) as a reference group.

Population attributable fraction (PAF) = $P_i \times (RR_i - 1) \div (1 + \Sigma P_i \times (RR_i - 1))$ [2]
– the subscript i refers to each of the three exposure combinations (++, +- & -+)

Using the information in Tables 1&2, for ++ subgroup
PAF = 0.125x (13.33 –1) ÷(1 + 0.125x(13.33 –1) + 0.125x(10-1)+0.375x(1.33-1))
= 0.407 (40.7%)

Values of PAF for the other subgroups are given in Table 2. This shows that the largest population impact comes from the Exposed & High-Risk-genotype subgroup. So, if intervention were feasible, there would be more benefit to the population as a whole in targeting the smokers & GSTM1-null subgroup.

Rather than look at the effect on the whole population, another approach is to use the *exposed* attributable fraction (EAF). The exposed attributable fraction for a single factor is the fraction of disease amongst the exposed subgroup that might have been avoided if that exposure had not occurred (or its effects could be eliminated). PAF is used more widely, but the EAF is included here to show the distinction between them. For a single factor it can be calculated as

(Risk in exposed subgroup – Risk in unexposed subgroup)÷Risk in exposed subgroup

So, using the example in Table 1, the exposed attributable fraction for the single factor smoking (irrespective of genotype) is (14 – 1.4) ÷14 = 0.9 (90%). This approach can be also be used when there is more than one exposure category: in our example the Non-smoker & GSTM1-wild subgroup is used as the 'unexposed' subgroup. The results are shown in Table 2. The subgroup with the largest population impact with this measure is still the Smoker & GSTM1-null genotype, but using this measure,the impact is only slightly greater than that of the Smoker & GSTM1-wild genotype subgroup.

All three measures of population impact indicate that the Unexposed & High-risk-genotype subgroup has the largest impact (to a greater or lesser extent) in the numerical example. However, there are very real practical difficulties in acting on this. Firstly, to identify this subgroup, the population members with the high-risk genotype would have to be identified by genetic screening and their environmental exposure determined. Secondly, having identified this subgroup, it may be difficult or impossible to reduce their cancer risks down to those experienced by the Unexposed & Low-risk genotype subgroup. It may not be possible to remove the environmental exposure from those so identified (e.g. smoking cessation programs are only partially successful, and then annual risks of lung cancer in ex-smokers take some years to be reduced to those of never-smokers). The example used also has the feature that the high-risk genotype increases lung cancer risk on its own. Even if the effects of the environmental exposure were eliminated, that would still only reduce the risk to that of the Unexposed &High-Risk-genotype subgroup: there may or may not be interventions that could reduce their risk further (e.g. prophylactic treatment). Unless interventions to reduce cancer risk in a genetic subgroup exist, there is no point in genetic screening.

If an effective intervention is possible, then a final, more recent, measure of population impact is the Number Needed to Screen[3]. This combines together the prevalence of the genotypes, the risks of cancer in each subgroup and the reduction in risks which could be achieved if screening identified individuals where intervention was needed. To provide comparability with the other measures discussed above, assume that lung cancer risks in smokers could be reduced to those of non-smokers by some treatment or intervention (e.g. via smoking cessation program and/or chemoprevention). If we consider smokers with the *high*-risk genotype, they have a lifetime lung cancer risk of 16% that could be reduced to 1.6% if they were identified. This gives a number needed to treat (NNT) of 1/(0.16 – 0.016) = 6.9. However only 50% have the high-risk genotype, so we have to screen 6.9/0.5 = 13.8 to prevent one case – so the Number Needed to Screen (NNS) is 13.8. A similar argument applies to the smokers with *low*-risk genotype giving a NNS= 18.5. With these assumptions there is little benefit to screening, since there is little difference between NNS for the two genotypes. However, a more reasonable assumption might be that lifetime lung cancer risk in smokers could only be reduced to a fraction of current levels, rather than down to the level experienced by non-smokers. A recent paper [3] used the NNS approach when assuming it was possible to reduce lifetime lung cancer risks in smokers by 50% (rather than to the level of non-smokers), and also concluded that there was little advantage to screening for GSTM1.

In the numerical example used here, there is limited benefit in screening for the high-risk genotype for any measure of population impact. However, since smoking is a lifestyle choice that considerably increases the risk of lung cancer (and many other diseases), it is extremely unlikely in practice that genetic screening would be considered: the obvious approach would be target all smokers to reduce their smoking. Screening is more likely to be considered if the exposure is involuntary (e.g. exposure to an industrial chemical or family history of cancer). Even then, there are ethical, legal and social issues to be considered.[4] It should be noted that all these calculations are sensitive to changes in any of the estimates of risks, prevalence and interaction. Given the sample size requirements in genotype-environment investigations, it is rare to have precise estimates of all of these.

None of these measures of interaction or population impact can be interpreted in isolation. A recent paper has suggested a tabular layout that includes many of the measures discussed previously[5]. It would be helpful if there were consistent reporting of all the measures needed to interpret genotype environment interaction in future studies: it remains to be seen if this will happen.

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Lay summary

Statement on the interaction between genotype and chemicals in the environment on the induction of cancer in risk assessment

Introduction and Background Information

1. The Committee was asked by the Department of Health to review the available information on the interaction between genotype and exposure to chemicals in the environment and the induction of cancer. The Committee was asked to provide advice on the methods of epidemiological research used in this area and the approaches to identifying genes of interest for such studies. Of particular importance is the evaluation and significance of data from relevant studies in cancer risk assessment.

2. A short summary of the conclusions is given on pages 2 and 3 of this statement. A brief overview of relevant information which will help in understanding the reasons for undertaking this review and conclusions reached is given below and some additional information on the term used is given in the glossary apended to this statement.

   - Many diseases (such as cancer) are thought to be due to a combination of heredity and other factors in the environment (such as lifestyle, diet and to a lesser extent exposure to chemicals in the environment). The DNA sequence of an individual (his or her genotype) may be one factor which contributes to whether a person who is exposed to chemical carcinogens (e.g. from tobacco smoke) may develop cancer. (Most chemical carcinogens exert their effects after prolonged exposure, e.g. over several decades)

   - The information coming from the Human Genome Project (http://www.ornl.gov/hgmis/project/.html) and the Environmental Genome Project http://www.niehs.nih.gov/envgenom/ is helping scientists to gain an understanding of the differences between people in their DNA sequences (genes) and thus more information about possible chances of getting diseases (such as cancer). These projects are showing that there are a great many small differences between individuals in their DNA sequences.

   - There is a lot of knowledge available on how carcinogens can cause cancer (for example how chemicals can be metabolised in the body to form carcinogenic chemicals, see glossary for more information) . It is therefore possible to identify differences in DNA sequences between individuals (for example in genes controlling the metabolism of chemicals) which might affect susceptibility to cancer.

   - It is already known that a few genes (such as the breast cancer susceptibility gene BraC1) have a very strong association (link) with the occurrence of cancer; in this case breast cancer. However such genes are very rare. It is much more likely that a gene will increase the tendency to develop cancer in a weaker fashion, with a low proportion of carriers actually getting cancer. This review is about whether we can identify any combinations of exposure to chemicals and occurrence of a particular DNA sequence (genotype) that is associated with a higher risk of cancer compared to individuals who may be exposed to the same chemical but do not have the same DNA sequence. [The types of gene concerned (such as those which metabolise chemicals) have, on the available evidence, little or no direct association with cancer.]

Conclusions of Review

3. The Committee reviewed the methods used to investigate possible interactions between genotype, exposure to chemicals and occurrence of cancer. The types of study, which all involved investigating genotype and exposures to chemicals in humans, could be separated into two types. Gene characterisation studies which aim to investigate the nature and strength of interactions. Gene Discovery studies which are intended to screen for genes which might be of importance for future gene characterisations studies.

   - There are problems in using the results of many of the available studies because these have investigated too few individuals to allow legitimate conclusions to be made. Scientists/ epidemiologists conducting such studies may not have formulated clear reasons for doing the research before conducting the work. These studies often produce a large amount of information and it is possible that some of the associations reported (between genotype and chemical induced cancer) arose by coincidence simply because of the large number of analyses undertaken and could be considered as "chance findings". The Committee felt that the possibility of chance findings was highly likely in the future because such studies would provide information on many hundreds of genes at a time. The Committee felt that investigators should be asked to lodge the reasons for undertaking the research with a third party before the investigations were undertaken so that the possibility of the information being used for purposes that it was never intended could be avoided. This might help to clarify which genes were of most importance in each study.

   - The Committee agreed that the most appropriate way to assess the results from many of studies investigating the possible interaction between a particular genotype and exposure to chemicals on the occurrence of cancer should involve the following information. In many cases a provisional assessment would have to be made without full information on number (iii)

     i) Clear information on the mechanisms of carcinogenicity of the chemical under consideration

     ii) An assessment of the strength of the interaction and consistency of the information from epidemiological studies of genotype-environment interaction.

     iii) An assessment of the information on the function of the particular gene under consideration.

   - The Committee concluded that an assessment of likely numbers of individuals with a particular genotype at risk of developing cancer following exposure to chemicals needed a lot of information before such calculations could be undertaken. This included information on the type and extent of chemical exposure, the numbers of people with genotype in the whole population and in exposed individuals. It would also be necessary to know the incidence of cancer in exposed and non exposed individuals either with or without the particular genotype. It would therefore only be possible to undertake such an assessment in a very few cases given the information currently available.

- The Committee agreed that the available data had so far failed to show any consistent and strong interaction between genotype and chemical induced cancer. It was unlikely that the interactions studied to date (which mainly concerned genes responsible for the metabolism of chemicals) were of importance to public health.

- There is little value in using genetic screening to identify individuals with particular genotypes of interest for carcinogenesis induced by environmental.

- However the possibility could not be excluded that important genotype-environment interactions involved in chemically induced cancers would be identified in the future.

- The Committee recommended that it was important to keep this subject under review particularly in the light of expected developments from the Environmental Genome Project based in the U.S.A and other initiatives in this area.

June 2002

Glossary of important terms

Association: The finding that the occurrence of disease and a factor (such as exposure to a chemical) is greater than expected by chance.

Consistency: The association has been consistently identified in studies using different approaches and by different research groups and in different populations.

Cancer: A malignant neoplasm (commonly called a tumour) that grows progressively, invades local tissues and spreads to distant sites.

DNA sequence: The carrier of genetic information for all living organisms except some viruses. Most cells in humans contain 46 chromosomes, each consisting of two strands of DNA which make up genes (see definition given below). Each DNA strand consists of two interwound chains of linked nucleotides. The nucleotides are the chemical building blocks of genes.

Environmental Genome Project: (EGP) was initiated by the US National Institute of Environmental Health Sciences (NIEHS) in 1998.The mission of the EGP is to improve understanding of human genetic susceptibility to environmental exposures. The EGP supports the mission of NIEHS, which includes the goal of understanding how individuals differ in their susceptibility to environmental agents and how these susceptibilities change over time. The EGP has a well developed internet site from where it is possible to obtain a lot of information on the subject of genotype-environment interactions (http://www.niehs.nih.gov/envgenom/home.htm)

Gene: The functional unit of inheritance: a specific sequence along the DNA, which codes for a product which a specific function in the cell.

Gene characterisation Studies: Epidemiology studies designed to give information on the nature and strength of interaction between genotype and exposure to chemicals in induction of cancer. There are many different designs which can basically involve either investigation of cases (i.e. individuals who have got cancer) to examine if exposure to chemicals and genotype were risk factors or investigation of large groups of individuals where disease status (i.e. cancer) is unknown. In this instance it may be possible to follow a group of individuals to see who gets cancer or to use records to retrospectively assess the occurrence of cancer within a group. All of these approaches need good information on genotype status of individuals and information on exposures to chemicals.

Gene Discovery Studies: Studies designed to screen many hundreds (possibly thousands) of genes and particular gene variants to see if there is a potential association with a disease such as cancer. Such studies are becoming feasible due to rapidly advancing methods for sequencing DNA.

Genotype: The particular DNA sequence seen in an individual.

Genotype Environment Interaction: A biologically relevant effect of two or more factors contributing to the risk (likelihood) of getting a disease (e.g the effect of a particular genotype and exposure to chemicals in the induction of cancer). The degree of interaction can be measured to examine whether the risk of disease is the sum of the risks associated with individual factors or whether the risk is greater than the sum (e.g a mulitplication of the risks of disease associated with two or more factors).

Human Genome Project (US): Begun in 1990, the U.S. Human Genome Project is a 13-year effort coordinated by the US Department of Energy and the US National Institutes of Health. The project originally was planned to last 15 years, but effective resource and technological advances have accelerated the expected completion date to 2003. Visit http://www.hgmp.mrc.ac.uk/About/ Project goals are to

- *identify* all the approximately 30,000 genes in human DNA,
- *determine* the sequences of the 3 billion chemical base pairs that make up human DNA,
- *store* this information in databases,
- *improve* tools for data analysis,
- *transfer* related technologies to the private sector, and
- *address* the ethical, legal, and social issues (ELSI) that may arise from the project.

Several types of genome maps have already been completed, and a working draft of the entire human genome sequence was announced in June 2000, with analyses published in February 2001. An important feature of this project is the federal government's long-standing dedication to the transfer of technology to the private sector. By licensing technologies to private companies and awarding grants for innovative research, the project is catalysing the multibillion-dollar U.S. biotechnology industry and fostering the development of new medical applications.

Human Genome Mapping Project (UK): The UK Human Genome Mapping Project Resource Centre (HGMP-RC) provides access to leading-edge tools for research in the fields of genomics, genetics and functional genomics. The Research Division and the Bioinformatics Division are located on the Hinxton Genome Campus along with the Sanger Centre and the European Bioinformatics Institute. The Biology Services Division of the HGMP-RC is located on the site of the Babraham Institute, Babraham. The Mission of the UK Human Genome Mapping Project are;

- To provide both biological and data resources and services to the medical research community, with a special emphasis on those relevant to the Human Genome Programme.
- To facilitate genomic research by the provision of cost effective centralised collaborative and training facilities.
- To encourage users to share their data, information and resources.
- To encourage the transfer of technology from the academic to commercial/industrial applications.

Mechanisms of chemical carcinogenicity: There are a wide diversity of mechanisms by which chemicals may cause cancer. However a basic distinction between two types can be made. Chemicals that are mutagenic (in-vivo, i.e in whole animals) are persumed to be potential carcinogens. Other chemicals act by various mechanisms but are not mutagenic (e.g effects on hormones or inducing high levels of irritation/cytotoxicity).

Metabolism of carcinogens: It has been established that a number of chemicals which are carcinogenic act only after they have been metabolised to chemical structures which are mutagenic. Thus a key step in the carcinogenic mechanism of these chemicals is the metabolism.

2002 Membership of the Committee on Carcinogenicity of Chemicals in Food, Consumer Products and the Environment

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Declaration of COC Members' Interests during the period of this report

Member	Personal Interest		Non-Personal Interest	
	Company	Interest	Company	Interest
Prof P G Blain CBE (Chairman)	NONE	NONE	Unilever plc	Research Studentship
Prof C Cooper	HBOS	Share Holder	NONE	NONE
	Norwich Union	Share Holder		
Prof P B Farmer	Abbey National	Share Holder	NONE	NONE
	Bradford & Bingley	Share Holder		
	Celltech	Share Holder		
	Friends Provident	Share Holder		
	SBS Group	Share Holder		
	Torotrak	Share Holder		
Prof D Forman	Barclays	Share Holder	NONE	NONE
	Friends Provident	Share Holder		
	HBOS	Share Holder		
	Woolwich	Share Holder		
Prof D Harrison	Medical Solutions	Share Holder	NONE	NONE
	Quintiles	Consultant		
	Scottish Medicine	Consultant		
Ms D Howel	NONE	NONE	NONE	NONE
Dr S J Kennedy	Oxford GlycoSciences	Salary/ Share Holder	NONE	NONE
	Unilever	Share Holder		
Ms M Langley	BT Business Consolidating Services	Share Holder	NONE	NONE
	Ciebel	Director		
	CREE Research	Share Holder		
	Cyber Care	Share Holder		
	Eshelon	Share Holder		
	HBOS	Share Holder		
	MMO2	Share Holder		
	Quelcom	Share Holder		
	Wibex	Share Holder		

Member	Personal Interest		Non-Personal Interest	
	Company	Interest	Company	Interest
Prof D Phillips	Abbey National	Share Holder	NONE	NONE
	BG Group	Share Holder		
	Bradford & Bingley	Share Holder		
	Centrica	Share Holder		
	CGNU	Share Holder		
	Lattice Group	Share Holder		
	National Grid	Share Holder		
	Servier	Consultant		
Prof A G Renwick OBE	International Sweeteners Association	Consultant	American Chemistry Council	Research Support
	Novartis	Share Holder	Pfizer	Research Support
	Targacept	Share Holder	GlaxoSmithKline	Research Support
Dr R Roberts	AstraZeneca	Share Holder	NONE	NONE
	Aventis	Salary		
	P & O	Share Holder		
Prof D Shuker	NONE	NONE	NONE	NONE
Prof G T Williams	Abbey National	Share Holder	NONE	NONE
	AMP Ltd	Share Holder		
	Aphton Corporation	Consultant		
	Bradford & Bingley	Share Holder		
	CGNU	Share Holder		

Committee on Carcinogenicity of Chemicals in Food Consumer Products and the Environment

TEMPLATE FOR COC 2002

The template is designed to show the breadth of expertise available to the Committee and is intended to aide members in discussing future needs with regard to expertise necessary to fulfil the terms of reference of the COC. The compliment of COC is 13 members (10 specialists and one lay member), attendance of COM chair (ex-officio capacity) and one chair. A deputy chair has not been appointed at October 2002.