

Committee on _____
CARCINOGENICITY

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

**GUIDANCE ON A
STRATEGY FOR THE
RISK ASSESSMENT OF
CHEMICAL CARCINOGENS**

CHAIR

*Professor P.G. Blain CBE BmedSci PhD FRCP (London)
FRCP (Edin) FFOM Cbiol FIBiol*

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Preface

1. The Committee on Carcinogenicity of Chemicals in Food, Consumer Products and the Environment (COC) is an expert advisory committee whose members are appointed by the Chief Medical Officer for England and Chair of the Foods Standards Agency (FSA) following an appointment exercise involving public advertisement. The Committee comprises independent experts and lay members, who serve in their own capacity and observe a published code of practice including principles relating to the declaration of possible conflicting interests (<http://www.advisorybodies.doh.gov.uk/coc/index.htm>).
2. The remit of the committee is to advise on all aspects of the carcinogenicity of chemicals, as well as testing strategies and research, and the risk assessment of carcinogens, at the request of the Department of Health and the Food Standards Agency (FSA). Other Government Department and Agencies may also request advice. This includes the Department of Environment, Food and Rural Affairs (DEFRA), Department of Transport (DT), Department of Trade and Industry (DTI), Health and Safety Executive (HSE), Pesticides Safety Directorate (PSD – a DEFRA agency responsible for approval of pesticides), Veterinary Medicines Directorate (VMD - a DEFRA agency responsible for the licensing of veterinary drugs), Medicines and Healthcare products Regulatory Agency (MHRA – an executive agency of the Department of Health responsible for promoting public health and patient safety with respect to medicines, healthcare products and medical equipment), Home Office, and the devolved administrations (Scottish Executive, National Assembly for Wales, and Northern Ireland Executive). The Secretariat is provided by the Department of Health (who lead) and the Food Standards Agency.
3. The role of the COC is advisory and it has no regulatory status. Its advice may however be provided to an agency that does have such a role eg PSD for pesticides, VMD for veterinary medicines, HSE for occupational health and certain EU regulations on chemicals.
4. The COC first published guidelines for the evaluation of chemicals for carcinogenicity in 1982. These dealt in the main with the design, conduct and interpretation of long-term animal bioassays and provided guidance to the relevant government departments and agencies on best practice for testing at that time. The need for guidelines to be periodically updated, to reflect advances in development and validation of methods, was recognised and revised guidelines were published in 1991, which addressed the evaluation of chemicals as potential carcinogens. The strategy outlined in this document

concentrates on one section of the aforementioned 1991 guidelines, namely the risk assessment of chemical carcinogens, with reference to new approaches such as deriving minimal risk levels. However, the detailed approaches to the evaluation of epidemiological studies used in the risk assessment of carcinogens are to be considered later in a separate document.

5. The Committee believes that the approach outlined here should provide Government departments and regulatory agencies with a basis for risk assessment of chemical carcinogens. It is based on published literature retrieved up to June 2004.

Introduction

6. The Committee on Carcinogenicity of Chemicals in Food, Consumer Products and the Environment is an independent, expert advisory committee appointed by the Chief Medical Officer (CMO) for England and the Chair of the Food Standards Agency (FSA). The Committee advises the CMO, the FSA and through the CMO, the Government, on matters related to the carcinogenicity of chemicals. The COC also has a general remit to advise on important general principles or new scientific discoveries in connection with carcinogenic hazard (the inherent carcinogenic property of the substance) or risk (the likelihood of carcinogenic effects occurring after a given exposure) and to present recommendations for carcinogenicity testing. In practice, the bulk of the work of the Committee relates to assessing carcinogenic hazard.
7. The Committee last published guidance for the evaluation of chemicals for carcinogenic potential in 1991 (UK Department of Health, 1991). The emphasis of the 1991 guidelines was directed at difficulties that may be encountered in assessing potential human carcinogens for regulatory purposes it included sections concerning the design and interpretation of short-term tests for carcinogenicity and long-term bioassays for carcinogenicity, as well as epidemiology. Overall, the 1991 guidelines presented an overview of all aspects of carcinogen identification, including some consideration of quantitative risk assessment.
8. Since 1991, there have been developments in mathematical modelling, and the use of potency indices in risk assessment has been suggested. In addition, proposals have been presented for setting minimal risk levels, as well as a harmonised approach for evaluating the mode of action of carcinogens. The object of this guidance document is to review such areas and propose a generic approach to the risk assessment of carcinogens. It should also be noted that there have been considerable developments in the harmonisation of approaches to the assessment of carcinogens in the area of human medicines. The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) has published guidelines for the harmonisation of carcinogenicity testing requirements for human medicines (<http://www.fda.gov/cder/guidance/1854fnl.pdf>).
9. In this guidance document, the term hazard describes the intrinsic capacity of a chemical to cause an adverse effect on human health. Risk is the probability of that adverse health effect occurring. The level of risk will depend on particular circumstances, determined by the nature and degree of exposure to the chemical in question.

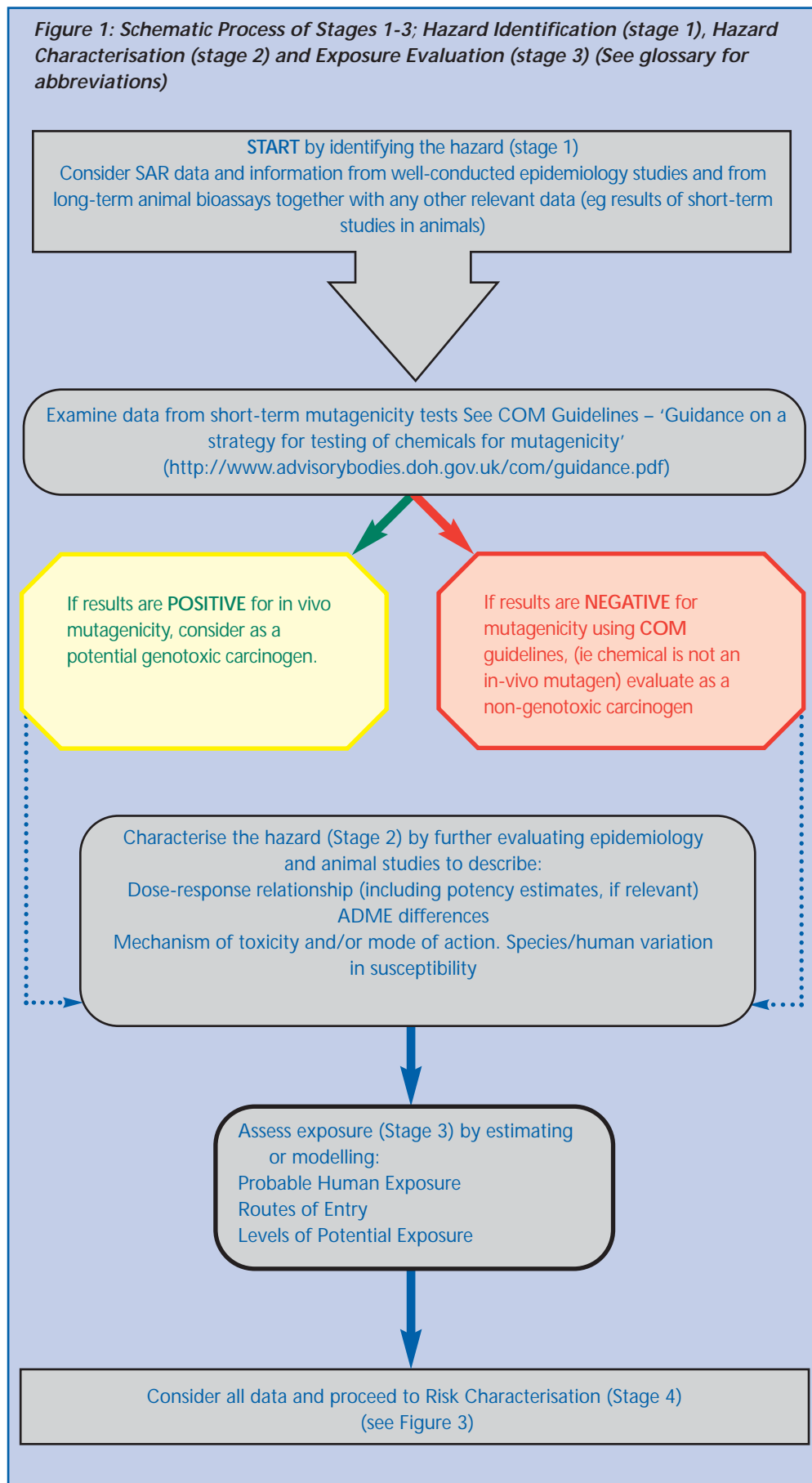
10. The Committee recommends a four stage evaluation strategy for the risk assessment process of carcinogenic hazard (see Table 1), which is essentially based on the widely adopted National Academy of Sciences risk assessment paradigm (US National Academy of Sciences, 1983). Initial identification of a carcinogenic hazard at Stage 1 is based upon a review of the toxicity data, the results of toxicity testing, and any knowledge of effects on human health. The characterisation of the hazard to humans at Stage 2 involves determination of the dose-response relationship, which can also include such factors as interspecies variation in susceptibility and mechanism/mode of carcinogenesis. Stage 3 involves estimating (or modelling) probable human exposure, routes of entry and levels of potential exposure. Issues and concerns relating to hazard identification, hazard characterisation and exposure evaluation (ie stages 1, 2 and 3) have been extensively reviewed elsewhere (Australian Department of Health and Ageing and Environmental Health (enHealth) Council, 2002; The European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC), 1996; and UK Department of Health, 1991). The final stage (stage 4) characterises the risk by summarising all the previous stages and outlining approaches to be taken for genotoxic and non-genotoxic carcinogens.

Table 1: Four stage evaluation strategy for the risk assessment process of carcinogenic hazard (further outlined in figure 1)

Stage	Risk Assessment Step
1	Hazard Identification
2	Hazard Characterisation
3	Exposure Assessment
4	Risk Characterisation

11. This document focuses on the principles of carcinogenic risk assessment and outlines the approaches to be used with genotoxic and non-genotoxic carcinogens, including new approaches such as estimating minimal risk levels. However, the detailed approaches to the evaluation of epidemiological studies used in the risk assessment of carcinogens are to be considered later in a separate document.

12. The Committee believes that biomarkers can provide valuable information for the risk assessment process. Within hazard identification (stage 1), biomarkers may provide specific evidence that a chemical has the potential to cause a carcinogenic effect. Biomarkers may also be used within exposure assessment (stage 3) to establish past, current or expected exposures to (and uptake of) actual or putative carcinogens in human populations. Biomarkers may additionally be used to quantitatively associate a dose or exposure with either a carcinogenic effect or the probability of a disease outcome (stage 2). In this way, biomarkers can represent a range of possible measurements from systemic exposure through to resulting causal events. The Committee is aware that problems, particularly for carcinogens, can arise in distinguishing between biomarkers that measure exposure and those that measure effect, although at the extremes the distinction may be clear. However, for the purposes of this document biomarkers will be broadly characterised as those of exposure and of effect.



Stage 1: Hazard Identification

13. Hazard identification involves the recognition of adverse effects that a substance has an inherent capacity to cause. With regard to the risk assessment of carcinogens, a distinction between genotoxic and non-genotoxic mechanism is critical, as this will influence subsequent stages of risk assessment (see Figure 1).
14. The Committee reaffirms its view as stated in the 1991 guidelines (UK Department of Health, 1991) that the most appropriate data to use for the positive identification of carcinogenic hazard are clear evidence from well conducted epidemiology studies. It was noted however that epidemiological studies may not have sufficient power to identify the absence of carcinogenic hazard.
15. Where appropriate epidemiological data are lacking, as is often the case, carcinogens may be identified through animal studies. Careful consideration is needed with respect to uncertainties within animal studies as well as for epidemiological studies, such as the validity of design and the interpretation of the data. Criteria for the technical adequacy of animal carcinogenicity studies have been published and should be used as guidance to judge acceptability of individual studies (OECD, 1998). The Committee considers that transgenic animal models, although an important future research area, have not yet been sufficiently validated for use in regulatory submissions to predict carcinogenic activity (COC statement/02/S3 – April 2002) (www.advisorybodies.doh.gov.uk/coc/ilsihesiaact.htm).
16. The Committee believes that structure activity relationship (SAR) analyses and models (Ashby and Tenant, 1994; UK Department of Health, 1991) may provide valuable initial information on carcinogenic potential for genotoxic carcinogens, especially when appropriate epidemiological and toxicological data are not available. However, employing SAR analyses as an alert for carcinogenicity can usually only be of benefit for establishing priorities of testing within a series of closely related chemical components or structural analogues.
17. When assessing the risks arising from a chemical carcinogen it is important to consider the mechanisms and in particular, whether a genotoxic mechanism is involved. The results from short-term mutagenicity tests should thus be included in any carcinogenicity assessment. The Committee on Mutagenicity has reviewed the risk assessment of mutagens and published a staged strategy for the identification of in vivo mutagens (UK Department of Health, 2000). Their conclusions concur with this view of the Committee on Carcinogenicity.

18. The Committee believes that it is prudent to assume that, in the absence of information to the contrary, genotoxic carcinogens have the potential to damage DNA at any level of exposure and that such damage may lead to tumour development. Thus, a compound identified as an *in vivo* mammalian mutagen should be regarded as being a potential genotoxic carcinogen for which it is assumed that there is no discernible threshold and any level of exposure carries some degree of carcinogenic risk.
19. Non-genotoxic carcinogens are identified by the absence of in-vivo mutagenic activity (see Figure 1 and UK Department of Health, 2000). Some information on their mode of action is necessary for an adequate consideration of such carcinogens. The IPCS (International Programme on Chemical Safety) have proposed a structured approach for the assessment of the overall weight of evidence for a postulated mode of action (MOA) (Sonich-Mullin *et al* 2001). More recently the Risk Sciences Institute of the International Life Sciences Institute have proposed a human relevance framework (HRF). The proposal extends the IPCS MOA approach by incorporating a systematic evaluation and comparison of animal and relevant human data. (Cohen SM *et al* 2003; 2004; Meek *et al* 2003)
20. The Committee believes that the IPCS and ILSI frameworks are of value in assessing carcinogenic risk. The human relevance framework (HRF) proposal developed by the Risk Sciences Institute of the International Life Sciences Institute provides a systematic approach to evaluating whether the key events in the mode of action of carcinogenic responses in experimental animals would be plausible in humans. The published report from the ILSI working group cites a number of tumourigenic responses in experimental animals that are generally regarded as irrelevant for humans such as $\alpha_2\mu$ -globulin-associated male rat kidney tumours (eg d-limonene) and inhibition of leutenising hormone surge-related rat mammary tumours (eg atrazine) (Cohen SM *et al* 2003).
21. Additional information on the potential use of biomarker studies to aid in the identification of carcinogens is given in paragraphs 52-57 below.

Stage 2: Hazard Characterisation

22. Hazard Characterisation involves a qualitative description of the nature of hazard together with a quantitative description of the dose-response relationship. The purpose of dose-response analysis is to investigate the magnitude of response (severity or incidence) within a dose range in animal bioassays or human epidemiology studies in order to assist in the estimation of response (and ultimately risk) due to exposure at environmentally relevant, often much lower, doses. The relationship between dose and response may be used to aid hazard characterisation by allowing a comparison of carcinogenic potency. However, other important factors that can effect this relationship and should be further considered are absorption, distribution, metabolism and excretion (ADME), mode of action and variability in susceptibility between species and within humans (see Figure 1). In particular, use of the dose-response relationship in the final assessment of risk will vary depending on whether or not a carcinogenic response occurs through a genotoxic mechanism (see Stage 4: Risk Characterisation).
23. The Committee believes that epidemiological studies provide the most appropriate data source for the quantitation of dose-response in the hazard characterisation process. There may be, however, difficulties with regard to measuring/estimating adequate and appropriate exposures within studies (see section on Exposure Evaluation). Dose-response, to estimate risk to exposed humans, can also be evaluated using data from animal studies, but the relevance of such evaluation needs to be assessed on a case-by-case basis due to uncertainties introduced when extrapolating between species. In general, dose-response analyses from animal studies are of most value in ranking potency within chemical groups, such as structurally related groups of putative genotoxic carcinogens. This is further explained in paragraphs 25-33 below. For substances identified as being non-genotoxic consideration should also be given to the assessment of precursor effects of such compounds and whether they may be a more appropriate way of identifying and representing the carcinogenic potency of the substance (Williams, 2001).
24. Absorption, distribution, metabolism and excretion (ADME) data can assist the extrapolation of animal data to human exposure situations. The Committee suggests that where extensive toxicokinetic data are available from both animal and human studies, this information may be used on a case by case basis to adjust doses and tissue exposure in animals to a human equivalent (eg by physiologically based pharmacokinetic (PBPK) modelling). The result of these types of studies would help clarify and describe as much as possible the variability in tissue exposure to be expected due to differences in species, sex, age, tissue and route of exposure.

Potency estimates

25. The Committee has considered a number of methods for the characterisation of hazard due to genotoxic carcinogens. These follow a ranking approach whereby chemical substances are classified with regard to tumourigenicity on the basis of potency. In this context, potency is ideally represented by the position and shape of the dose-effect or dose-response curve, but the value of a particular point on the curve is often used as a surrogate. The Committee recognises that where comparative data on tumourigenicity are lacking, it may be possible to use a surrogate measure of potency, such as specific DNA damage observed in target organs.
26. Relative Potency Estimates such as TD₅₀ and T25 may be used to compare carcinogenic potency for chemicals that are members of a particular class of genotoxic carcinogens. Potency Equivalence Factors have been suggested in circumstances where there is a good surrogate compound for comparison, eg PAH's (Collins, 1998). To date, there has been relatively little use of Potency Equivalence Factors for carcinogenicity.
27. The Tumour Incidence (TI) value (Bailer and Portier, 1993) and the TD_x approach (Nordic Council of Ministers, 1986) are two other examples of such approaches. The TI approach involves complex calculations whilst there are uncertainties in the TD_x approach compared to the relatively simple estimation of a 25% increase in tumour incidence (above background) as advocated in the in the T25 approach. This document will principally focus on the T25 and TD₅₀ approaches, which have been more widely advocated in setting potency estimates (Dybing *et al* 1997; Gold *et al* 1989). Potency estimation can be used as a pragmatic approach to ranking carcinogens (see paragraph 33 below).

The T25 approach

28. T25 is defined as the dose eliciting a 25% increase in the incidence of a specific tumour above the background level. This value has been used to estimate carcinogenic potency from the results of experimental animal studies. The estimation of T25 is dependent on the incidence of tumours at a selected site at a single dose level. The T25 is influenced by the quality of the bioassay information (eg design and evaluation of studies), and factors such as time to first tumour, the influence of toxicity on tumour induction and mortality and the approach taken regarding statistical analysis of tumour data. There may also be uncertainties regarding the application of the T25 for potency ranking particularly with regard to selection of the most sensitive site relevant for humans, the relevance of rodent tumours for humans and

different cancer susceptibilities between rodent species (ECETOC, 2002). The T25 has also been proposed for use in assessing risk and this is briefly described later (see paragraph 69).

29. The Committee considers that the use of the T25 in potency ranking of genotoxic carcinogens is an acceptable pragmatic approach, but that the parameter should not be over interpreted. The reasons for this are that there are a number of basic uncertainties, such as whether the relative ranking identified in the observed dose range would be maintained at low doses, and whether the relative potency in animal studies would be applicable to humans.
30. Currently, there is no basis to use the T25 to rank non-genotoxic carcinogens, for which tolerable exposure levels can be derived using an approach based on knowledge of mode of action, identification of no adverse effect level, and use of uncertainty factors.

The TD₅₀ Approach

31. The TD₅₀ concept (Peto *et al* 1994) is defined as the chronic dose-rate which would induce tumours in a given target site(s), in 50% of the test animals at the end of a standard lifespan for the species, provided that there were no tumours in control animals. However, since the tumour(s) of interest often do occur in control animals, the TD₅₀ is more precisely defined as the daily dose rate required to halve the probability of remaining without tumours at the end of a standard life span. TD₅₀ values have been estimated for chemicals listed in the Carcinogenic Potency Database developed by Gold and Zeigler (<http://potency.berkeley.edu/cpdb.html>) (Gold *et al* 1984, 1999)
32. The TD₅₀ concept is based on the assumption that there is linearity between dose and hazard until tumour onset, which may be complicated by premature deaths by causes other than tumour formation. The concept also depends on the assumption that tumour onset times are observable prior to mortality and as a result the approach relies heavily on observational methodology. Tumours that are discovered after death may cause confounding between mortality and tumour onset and would ultimately result in a biased TD₅₀ estimate. Alternatively, tumours that do not significantly alter survival and remain undiscovered until death would result in the TD₅₀ value relating to the 'rate of death with tumour', rather than the tumour incidence rate. This undermines the objective of the carcinogenicity study, which is to evaluate tumour incidence.

Summary of Potency Estimates

33. The Committee believes that potency estimates (such as T25 or TD₅₀) could have some pragmatic use in carcinogenic risk assessment as an aid to prioritising carcinogenic substances (eg for risk re-evaluation), but consider that such potency estimates are not adequate for quantifying cancer risks. Although potency estimates can rank chemicals within a particular group (such as structurally related groups of putative genotoxic chemicals), difficulties arise with respect to extrapolating from high to low dose and from animals to humans. Of the two approaches described in this document for estimating potency, the TD₅₀ has an advantage in that it accounts for effects of chemicals on survival, but requires specific software to undertake calculation. However, the T25 is quick and easy to calculate and there is evidence of a good correlation between rank order produced by TD₅₀ and T25 (Dybing, 1997)

Dose-Response Modelling

Mathematical models

34. Dose-response data from animal studies for either genotoxic or non-genotoxic carcinogens may be fitted using mathematical equations, as an attempt to produce numerical estimates of risk from human exposure. Many mathematical models have been developed for use in assessing carcinogenic risk (Edler *et al* 2002; Edler & Kopp-Schneider, 1998) but most are only loosely compatible with current understanding of mechanisms of chemical carcinogenesis and they have not been comprehensively validated. The models that are applied to carcinogenicity data attempt to define the probability range for the risk of developing a cancer at low dose by using mathematical processes to extrapolate below the available experimental points. The curves derived from the models are either fitted to the available data points and then extrapolated to low doses with appropriate confidence bands, or used to extrapolate from a 'point of departure' (eg ED05 or ED10) to lower doses. All of the models attempt to define the dose-response relationship on the basis of a particular extrapolation of the experimental data beyond the lowest data point.
35. Mathematical models are loosely categorised on the basis of their underlying statistical assumptions. These categories are termed stochastic (mechanistic), tolerance distribution (empirical) and time-to-tumour (See Table 2 overleaf).

Table 2: Summary of mathematical models

Mechanistic (stochastic)	Tolerance Distribution (empirical)	Time-to-Tumour
One Hit Multi Hit Armitage Doll Linearised Multi Stage Multiplicative Two Stage Model with Clonal Expansion	Weibull Logit Log Probit	Log Normal Distribution Weibull Distribution Hartly-Seilken MultiStage (adapted)

36. Stochastic (mechanistic) models are very roughly compatible with a broad range of experimental observations in carcinogenesis and are generally based on the concept that a carcinogenic response is the result of a random occurrence of one or more fundamental biological events. On this basis they can subsequently be used to model cancer risks at low doses (Crump 1994). Widely used models, such as the Linearised Multistage Model and the Two Stage Model with Clonal Expansion (also called the Moolgavkar-Venzon-Knudson (MVK) model), aim to describe the formation of carcinomas at the level of the cell and try to incorporate a biological theory of carcinogenesis (Moolgavkar *et al* 1999). The use of the linearised multistage model in particular for quantitative risk assessment has been strongly debated in the scientific literature (Crump, 1996; Lovell & Thomas 1996). More recently, the multiplicative model is emerging as a possible alternative to the previously mentioned models (Paulsson *et al* 2001)
37. Tolerance distribution (empirical) models are based solely on curve fitting methods. The concept of a tolerance distribution model is that a tumour is considered to occur at a particular dose if the individual's tolerance to the substance is exceeded at that dose. Hence, the models take into consideration that a population contains a distribution of individuals of different susceptibilities. Specific models of tolerance distribution include Weibull (Weibull, 1951), Logit (Mantel & Bryan, 1961) and Log Probit (Hanes and Wedel, 1985) models. However, these models are used to a lesser extent than the mechanistic models.

38. Time-to-tumour models use data from long-term rodent bioassays, where the age of the animals at death can be used as an approximation of the time to the occurrence of a fatal tumour. From this, the probability of a tumour being observed at a specified age at a given dose can be calculated. However, the cause of death in animal bioassays cannot always be completely ascertained, which may lead to some uncertainty within the model. Examples of time-to-tumour models include an adapted multistage model (Hartley & Sielken, 1977) and a Weibull distribution that contains a time-to-tumour function (Portier *et al* 1984). These models have not been widely used and comparisons with other models are rare.

Linear Extrapolation

39. The simplest approach to extrapolate below the experimental range is linear extrapolation from a dose (or upper confidence limit) within the experimental range to a much lower dose range (Krewski, 1991). This approach, often termed ‘model free’, has a number of variations depending on selection of the dose (or upper confidence limit) to be the starting value for linear low dose extrapolation (eg US EPA, 1996). This value, known as the ‘point of departure’ should be found on the dose-response curve either within or close to the experimental dose range.
40. There are various methods of selecting a point of departure for extrapolation to low doses depending on the dataset. For example, using animal bioassays, a fixed value such as the dose needed to produce a 10% or 25% response (ED10 or ED25, respectively), or the upper 95th percentile confidence interval for the dose giving such a response (LED10 or LED25) may be used. These starting points may be derived by fitting a mathematical model to the data in the experimental range. Another approach would be to estimate the dose corresponding to excess risk of 1% above background (ED01) calculated using similar modelling methods. It has been suggested that this ED01 method may provide a more stable estimate of low dose carcinogenic potency compared to the 95% upper confidence limit (Gaylor *et al* 1994). Alternatively, data on precursor effects might be used to extend the observed range below what can be observed in carcinogenicity bioassays. Such data, together with tumour data, may be used for deriving a point of departure as such information can associate the precursor response level with that of a particular tumour response.

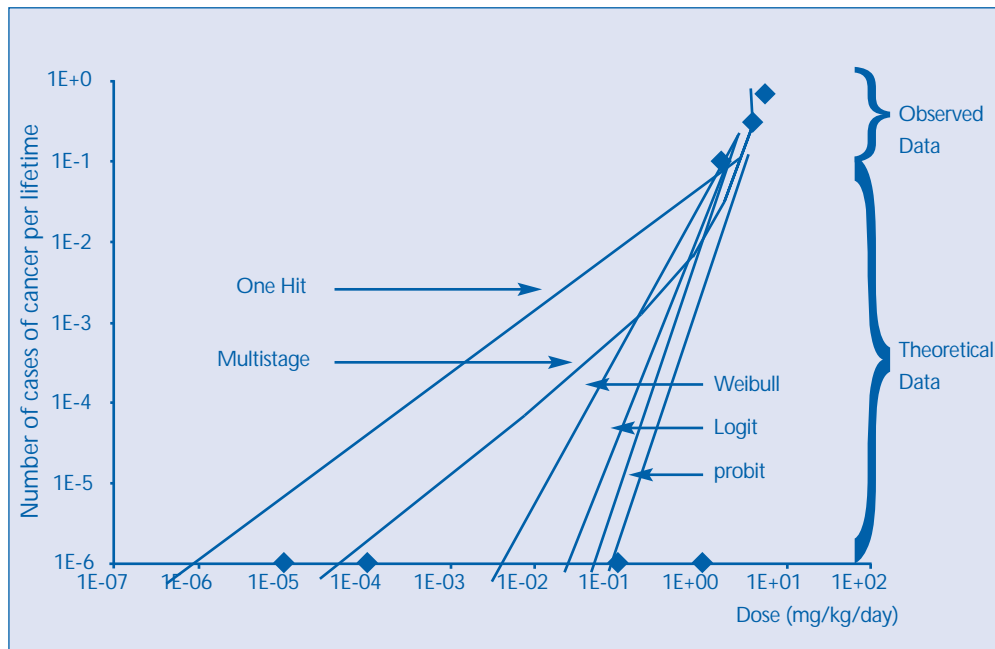
Summary of Dose-Response Modelling

41. The Committee has concerns over the use of mathematical models for carcinogen risk assessment when extrapolating from the relatively high dose levels used in animal bioassays to well below the observed region of a dose-response relationship.

42. The Committee reaffirms its view presented in the 1991 guideline that the approach of mathematical modelling does not take into consideration the complexity of events that occur between exposure to a chemical carcinogen and the induction of a neoplasm. (For example absorption, distribution and metabolism; the occurrence of the ultimate carcinogen in the susceptible organ or tissue; and the processes, including damage to DNA and differences of the repair process in different organs and species, that finally result in cancer formation) (UK Department of Health, 1991). In addition, many of the models make a number of assumptions that may be incorrect for particular carcinogenic chemicals or responses. These assumptions include i) that the time taken to induce cancer is proportional to the expected lifetime of the species (70 years in human, 2 years in rodents); ii) that the duration of the administration of the chemical should be extrapolated linearly between species although it is known that cancer incidence increases in proportion to the third or fourth power of the duration of exposure; iii) that the dose-response is linear and without threshold at low doses in spite of different repair capacities; iv) that the tissue dose of the ultimate carcinogen is proportional to the external dose administered to the species; v) and that organ/tissue specificity, which often differs between species, is quantitatively unimportant in risk assessment.
43. The Committee believe that the data requirements needed for extrapolation are too specific and are usually directly related to the sophistication of the model to be used. For example, sufficient test groups are needed to define the dose-response within the experimental range. However, the usual study design of one control plus three dose groups does not provide enough data points to be able to differentiate between different mathematical models. Furthermore, the outcome of the model should be less dependent on the absolute value of the top dose than on the slope of the dose response curve. In practice, the outcome often shows a direct proportionality to the high dose selected in the bioassay (Lovell & Thomas, 1996; ECETOC, 1996).
44. The Committee have concerns relating to extrapolating over many orders of magnitude, ie from the tumour incidence data of standard carcinogenicity bioassays to a dose that is predicted to produce tumour incidence levels of the order of 1×10^{-6} . Modelled extrapolations over even a single order of magnitude can be highly inaccurate (ECETOC, 1996). In addition, as it is a feature of mathematical models that the assumption is linearity at low doses. If the true response below the experimental range is sublinear, this assumption will result in overestimation in the extrapolation. Therefore, the Committee believe that firm evidence is needed on which to base the shape of the dose response curve below the observable range in animal studies.

45. The Committee agrees that the dose-response extrapolation procedures used in these mathematical models rarely take human variability into account. One approach that has been claimed to do this is the use of the upper 95th confidence limit of the dose-response relationship, because the variability in response will be reflected in the variability in the experimental data. However, much of this will relate to variability arising from the small size of the experimental groups, and will relate to variability within the test species and not within humans. Therefore, the use of the upper 95th confidence limit may not be appropriate to represent human variability. Indeed, different age groups of the human population would also have different dose-response relationships. Species differences can be taken into account by correcting the dose in animal studies to a human equivalent dose by interspecies scaling, or by the incorporation of a PBTK model giving the target organ or tissue dose of the active chemical species. However, despite such refinements, the final risk estimate is still determined largely by the mathematical model selected for extrapolation. Species differences in the target organ or tissue response in relation to the tissue concentration of the compound or active metabolite have also been investigated, and can also be included to modify the dose-response relationship. A biologically based dose-response model, which includes toxicokinetic and toxicodynamic aspects, can be derived. The development of biologically based dose-response models could allow the mode of action to determine the shape of the curve used for low dose extrapolation, although assumptions about the shape of the dose-response of the different toxicokinetic and toxicodynamic processes at low doses would still be necessary.
46. The Committee notes that, in practice, the slope of the extrapolated linear portion of the dose response curve differs for each of the models used (UK Department of Health, 1991; ECETOC, 1996). As a result, the extrapolated low dose can vary by several orders of magnitude for the same experimental data (see Figure 2). This variation would also be expected for newer models such as the multiplicative model, which involve limitations similar to those in other mathematical models.

Figure 2: Variance of models when modelling the same data set (redrawn from 1991 Guidelines and Cothorn 1985)



47. As a result of considering all aspects of mathematical modelling, the Committee agrees that these mathematical models of dose-response do not simulate the carcinogenic processes adequately and therefore accuracy at extrapolated low doses cannot be determined. Therefore the Committee do not recommend their use for routine risk assessment.
48. Information on the potential use of biomarker studies to aid in carcinogen hazard characterisation is given in paragraphs 52-57 overleaf.

Stage 3: Exposure Assessment

49. The objective of exposure assessment is to estimate probable human exposure by determining source, magnitude and duration of contact with the chemical of concern, as well as routes of entry (See Figure 1). Although exposure assessment in humans is a crucial stage in the assessment of risk, the Committee is aware that it is frequently identified as the main area of uncertainty in the overall risk assessment process.
50. Many epidemiological studies have major limitations related to measurement of exposure to carcinogens over long periods, as well as recall bias in certain study designs. In addition, exposure data will not be available for novel substances with no history of human exposure resulting from use (van den Brandt *et al* 2002). Although the alternative of personal monitoring (eg dermal patch studies) is able to measure exposure directly, assumptions are required concerning the relationship between results from short-term sampling and predicted long-term exposure. Scenario modelling studies may become useful when predicting exposure for potential use patterns. Approaches used in exposure assessment and the characterisation of uncertainties and variability in the resulting estimates have been extensively reviewed elsewhere (Environmental Health Criteria No. 210, 1999; Ferrier *et al* 2002)
51. Information on the potential use of biomarker studies to aid in the evaluation of exposure to carcinogens is given in paragraphs 53-56 below.

Use of Biomarkers of Exposure and Effect

52. As noted in the introduction section (see paragraph 12 above), Biomarker studies can provide valuable information for use in the risk assessment process. There are a number of criteria that should be considered when selecting and validating a suitable biomarker (Environmental Health Criteria 153; Environmental Health Criteria 222; Albertini *et al* 2000). On selection of a potential biomarker, it is important to obtain adequate evidence to support the relationship between exposure, biomarker and end-point of interest (ie cancer). Furthermore, it is essential that the sensitivity and specificity of individual biomarkers are evaluated. This should involve assessment of the consequence of health outcome or pathological change, evaluation of limitations (ie with respect to detection limit, precision and accuracy), and an investigation of intra- and inter-individual variation for a non-exposed population. It is also essential that a relationship between dose and biomarker response be established. Finally, consideration is needed with respect to the nature of the specimen required for analysis, such as integrity over time and with storage, as well as a preference for non-invasive

techniques. The Committee agree that it is essential that a biomarker is appropriately characterised and validated before any conclusions are drawn from its use.

Markers of exposure

53. Markers of exposure indicate the presence of a carcinogenic compound or its interactions by assaying levels of the chemical, a metabolite or a reaction product in blood, urine, saliva, cerebrospinal fluid, and other biological samples. In this way, exposure biomarkers can provide the most direct evidence of human exposure to a carcinogen as well as the internal dose (exposure assessment: stage 3). However, unless a relationship can be established between biomarker levels and internal dose, data from exposure biomarkers cannot be used to back-calculate the initial dose. It is also important that interfering reactions are accounted for or ruled out, for example, the presence of a chemical cannot be attributed to the metabolism of unrelated chemicals, and background levels must not mask the measurement.
54. Direct measurement of a putative carcinogen or its metabolites has been used within the risk assessment process. For example, a number of such biomarkers of exposure have been proposed for benzene, vinyl chloride and butadiene (Albertini *et al* 2003). In particular, for benzene these have included measuring the concentration of the parent compound in blood (Weisel *et al* 1996) or measuring its metabolites, such as S-phenylmercapturic acid, excreted in urine, (Weisel *et al* 1996, Boogaard and Van Sittert, 1995).
55. An alternative approach is to measure specific reaction products with macromolecules (eg DNA or protein adducts) (Schut & Shiverick 1992, Farmer 1999, Farmer 2003), which can provide evidence of exposure, uptake and distribution. For example, haemoglobin adducts have been used as a biomarker of exposure to 1,3 butadiene (Osterman-Golkar *et al* 1996) and to styrene (Yeowell-O'Connell *et al* 1996). Numerous methods of varying sensitivity exist to measure DNA adducts. These include ³²P-postlabeling (Beach & Gupta, 1992; Phillips, 1997), mass spectrometry (Farmer & Sweetman, 1995) and immunoassays using adduct-specific polyclonal or monoclonal antibodies (Shuker, 1999). For example, DNA adducts of 2-amino-1-methyl-6-phenylimidazo[4,5- b]pyridine (PhIP), a food-derived heterocyclic aromatic amine, have been shown to be quantifiable in tissues (Friesen *et al* 1994). Other examples of DNA adducts include carcinogen modified DNA bases such as alkylated purines (Shuker *et al* 1993), aflatoxin-guanine adducts (Groopman *et al* 1993), and PAH-derived adducts with DNA (Schoket *et al* 1993). Urinary DNA adducts in particular can provide a powerful tool to evaluate human exposure to carcinogenic agents (Shuker & Farmer, 1992; Groopman *et al* 1993)

56. A number of studies of the role of aflatoxin B1 in human liver cancer have clearly illustrated the advantages of using biomarkers to determine exposure status (Groopman and Kensler, 1999). In addition, these biomarkers have sufficient predictive value for cancer outcome to be used as short term indicators for intervention trials (Kensler *et al* 2003)

Biomarkers of effect

57. Biological markers of effect may be used to signify the presence of disease, early precursors of disease or events that occur concurrently with a disease process that may predict the development of impaired health. These types of biomarkers may be used in hazard identification to facilitate screening and/or identification of a carcinogenic chemical (hazard identification: stage 1). Biomarkers of effect implicated in a carcinogenic mechanism may further be used to characterise the hazard (stage 2). It is important to consider that in some instances biomarkers of effect may not represent injury, impairment of health, or disease. In addition, biomarkers of effect are frequently not specific to a given exposure or a specific agent. The Committee believe that a relationship between exposure (acute, subacute, or chronic), the biomarker of effect, and carcinogenic event must be established in order to determine validity. The need to fulfil this requirement, particularly with regard to the association with tumour outcome, presents difficulties. To date no biomarker of carcinogenic effect has been fully evaluated.

Stage 4: Risk Characterisation

58. The final stage of the evaluation strategy involves determining the probability that an adverse health effect (ie cancer) will be produced by exposure to a hazardous chemical. This procedure could be used to draw the conclusion that any exposure is associated with some increase in carcinogenic risk (although this may be very small), or conversely a level of exposure could be identified that is considered acceptable or tolerable (see Figure 3). In order to arrive at these estimates, consideration needs to be given to the toxicity profile of the chemical, particularly the nature of the carcinogenic effect, its characteristics (in terms of species variation and mechanism or mode of toxicity) and the dose-response relationship.

Threshold Carcinogenicity [Non-genotoxic carcinogens]

59. Risk assessment of chemical carcinogens is dependant on the mechanisms of carcinogenicity and the relationship between dose and tumour response. For most non-genotoxic carcinogens it is accepted that there is a threshold dose, below which no effect is observed. Many non-genotoxic carcinogens induce tumours as a secondary adverse effect arising from an initial toxicological effect, which has a threshold (Ashby *et al* 1996). It follows that for these substances there is no carcinogenic risk at dose levels that do not produce the primary toxicological event, ie at doses below the threshold (Williams, 2001). Therefore, where there is adequate evidence to support a threshold for carcinogenicity (ie the compound and metabolites do not have in-vivo mutagenic activity and there is an adequate evaluation of the mode of action for tumours observed in animal studies), the Committee believe that an approach based on the use of uncertainty factors should be adopted. The risk characterisation of non-genotoxic carcinogens can also be improved by adopting proposals such as those published by the IPCS on mode of action in animals and the ILSI human reference framework. These approaches can serve to enhance the clarity and transparency of the risk assessment process (Sonich- Mullin C *et al* 2001; Cohen SM *et al* 2003; 2004; Meek *et al* 2003)

Uncertainty Factor Approach

60. The risk characterisation for non-genotoxic carcinogens based on animal studies relies on the elucidation of a no observable adverse effect level (NOAEL) for carcinogenicity or on a precursor event linked to tumour induction. The robustness of this evaluation is dependent on the quality of the animal bioassays and dose setting procedure and on the available information to support the mode of action. In some instances it may only be

possible to derive a lowest observable adverse effect level (LOAEL) from the bioassay data. The NOAEL or LOAEL is divided by an appropriate uncertainty factor to give an estimated dose without appreciable risk in humans (or reference dose) eg derivation of Acceptable Daily Intake (ADI), such as is used for food additives or pesticide residues in food, or Tolerable Daily Intake (TDI), such as is used for environmental contaminants. Clearly, when setting the overall ADI or TDI for such a compound consideration has to be given to the overall toxicological profile, as there may be more sensitive end-points than carcinogenicity.

61. The uncertainty factor reflects the difficulties concerning extrapolating findings in animals to humans as well as interindividual variation, and also takes into account the quality of the toxicity data as well as the nature of the toxic effect. Its numerical value needs to be considered on a case-by-case basis, but as a general default a value of 100 (based on a factor of 10 for interspecies variation and a factor of 10 for interindividual variation) is frequently used when based on adequate animal data. Higher uncertainty factors might be used for non-genotoxic carcinogenicity depending on the quality of the animal data and uncertainties in evaluation of the toxicological data. If available data provide adequate information on interspecies or human variability, the default values may be replaced in part or entirely by chemical-specific adjustment factors (Meek *et al* 2002). The approaches to deriving uncertainty factors have been reviewed in detail by the Interdepartmental Group on Health Risks of Chemicals document (IGHRC, 2003) and are under re-evaluation by the Variability and Uncertainty in Toxicity working group for the Committee on Toxicity (<http://www.food.gov.uk/science/ouradvisors/toxicity/COTwg/wgvut/>).
62. The application of uncertainty factors generates a single estimate of a dose (or exposure) for a human that is considered to be without appreciable risk, the so-called deterministic approach. Normally, no numerical estimate is provided of the confidence limits for this value. Any exposure below the derived reference dose is believed to produce no significant risk. Qualitative estimations of risk above this level need to be considered on a case-by-case basis, taking into account the frequency, duration and extent by which it is exceeded, and the nature and dose-response relationship for carcinogenicity of the substance in question. The Committee believe that this approach may be used for non-genotoxic carcinogens provided that the underlying mode of action is adequately understood. In such situations, it is clearly necessary to consider all possible forms of toxicity, as a level set to protect against carcinogenicity may not be adequate to protect against other toxicological effects, such as developmental effects.
63. A Margin of Safety (MOS), calculated by dividing the NOAEL for carcinogenicity derived from long-term bioassays by the estimated exposure, can be informative to risk managers in deriving risk management policies.

Non-threshold Carcinogenicity [Genotoxic carcinogens]

64. From what is known about the mechanism of action of genotoxic carcinogens, it is currently assumed that, in the absence of mechanistic data to suggest a threshold for genotoxicity, no threshold for carcinogenicity exists. Risk estimates must therefore rely on the extrapolation of the dose response obtained from epidemiology or experimental animal studies to give estimates of risk for human exposure. The consideration of dose response modelling in stage 2 (Hazard Characterisation) above has indicated that it is not possible to give an acceptable estimate of risk at environmental levels of exposure. Therefore, a review of alternative approaches considered by the Committee for characterising risk of genotoxic carcinogens is presented below.

Minimal Risk Levels for Genotoxic Carcinogen Contaminants and Impurities

65. The Committee considers that under certain specific circumstances, for example very low exposures to genotoxic carcinogen contaminants or impurities, a pragmatic minimal risk level for these compounds may be identified. A minimal risk level for a genotoxic carcinogen contaminant or impurity is defined within this document as an estimate of daily human exposure to a chemical identified by expert judgement that is likely to be associated with a negligible risk of carcinogenic effect over a specified duration of exposure (usually a lifetime). However, it should always be recognised that for any genotoxic carcinogen there is still a carcinogenic risk (although this may be very small) at any exposure level, and thus the policy adopted by risk managers of controlling levels to 'as low as reasonably practicable' (ALARP) should always apply. Indeed this advice applies whether or not a minimal risk level for a genotoxic carcinogen contaminant or impurity can be estimated. This approach would apply solely to contaminants for which exposure was unavoidable and to impurities in materials, products and formulations which are subject to regulatory assessment schemes. The derivation of the minimal risk level for a genotoxic carcinogen contaminant or impurity involves assessment of all available carcinogenicity dose-response data to identify an appropriate dose without discernable carcinogenic effect, or the lowest dose tested, if effects are apparent at all doses, and the use of expert judgement to derive an appropriate margin of exposure. One proposal is that the maximum upper limit for the margin of exposure for carcinogenicity might be 10,000 (Gaylor, 1999; Gold *et al* 2003). A comparison of the minimal risk level for a genotoxic carcinogen contaminant or impurity with estimated exposure can be informative to risk managers.

Threshold of Regulation

66. A 'threshold of regulation' has been proposed as a method for setting a regulatory exposure level, which will be associated with a minimal risk for carcinogenic substances. The FDA originally introduced this approach in order to reduce toxicological data requirements for indirect food additives (US FDA, 1995). More recently, JECFA have used a similar method to evaluate the safety of flavouring substances, excluding genotoxic carcinogens (Munro *et al* 1999). Recent workshops have attempted to develop the concept that a *de minimus* risk value (Threshold of Toxicological Concern (TTC)) could be identified for any chemical, including those of unknown toxicity, taking chemical structure into consideration (Barlow *et al* 2001; Kroes R *et al* 2004).
67. It has been proposed to further extend the TTC, by defining a common exposure level for any unstudied chemical (regardless of its chemical class) that will not pose a risk of significant carcinogenicity (Barlow *et al* 2001, Kroes *et al* 2000, 2004, Cheeseman *et al* 1999). One proposal was derived from an evaluation (involving linear extrapolation from the TD50 down to 10^{-6} excess cancer risk) of substances in the Gold Carcinogen Potency Database (Gold *et al* 1984; 1999).
68. The threshold of regulation concept for regulatory purposes is a relatively new approach and the Committee felt that, careful consideration was needed of the biological, analytical and mathematical issues as well as a much wider database for validation. The Committee consider that it should not currently be used as a generic approach, as the proposed exclusions covered some important classes of genotoxic carcinogens (such as aflatoxin-like compounds, azoxy compounds and *N*-nitroso compounds) and a number of classes of other carcinogens, such as heavy metals and TCDD (Kroes *et al* 2004). However, as it is based on ranking by theoretical risk and exposure the Committee agree that it could be used, along with hazard identification and characterisation data, for prioritisation of chemicals, particularly for chemicals that are not subject to regulatory approval schemes.

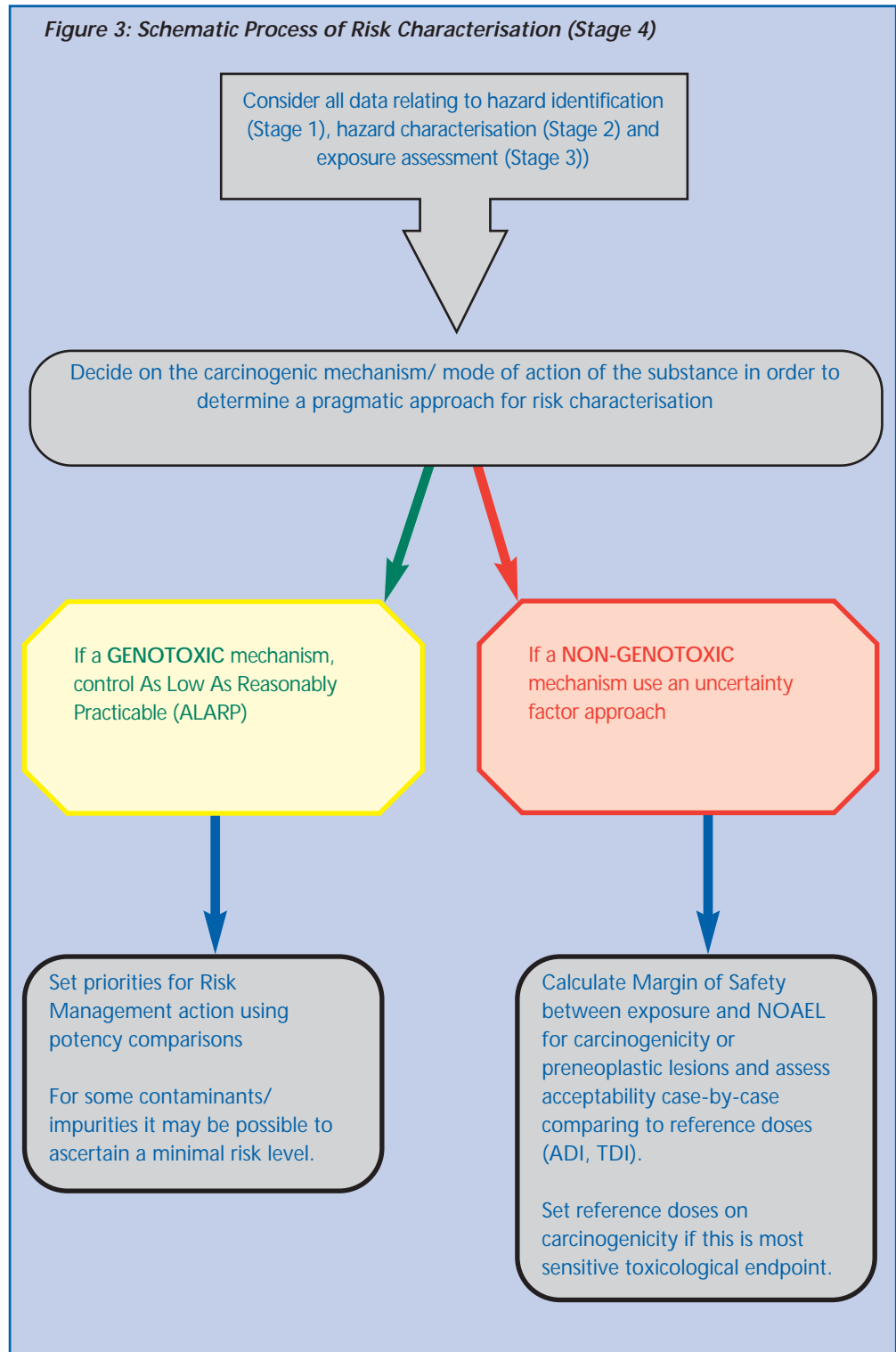
T25 Approach

69. The T25 has also been proposed as a basis for calculating risk for human exposure to carcinogens (Dybing *et al* 1997). Essentially, an appropriate animal T25 is selected (usually based on the most sensitive tumour) and converted to an equivalent Human T25 (HT25) by the use of scaling factors and linear extrapolation to low dose levels. The European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC) have recently evaluated the use of T25 estimates for regulatory risk assessment of non-

threshold carcinogens (ECETOC Technical report 83, 2002). They identified limitations of the methodology and concluded that the data and approach advocated were not sufficient to support quantitative risk assessment. The COC concurred with this view.

Summary of non threshold carcinogenicity

70. For genotoxic carcinogens, a non-threshold approach is advocated. The Committee concludes that it is inappropriate to model dose-response data for the estimation of risks at human exposure doses due to considerable uncertainties underlying the approaches used and with regard to the carcinogenic process. Therefore the Committee recommends that risk managers adopt measures such that levels should be controlled as low as reasonably practicable (the ALARP approach). However, in some cases such as contaminants or impurities, the ALARP approach may be supplemented by deriving a minimal risk level, ie a dose considered to represent a negligible carcinogenic risk, in order to aid in risk management decisions.
71. The Committee believe that the use of potency estimates such as the T25 approach has a role in the prioritisation of chemicals thought to be genotoxic carcinogens but not in the risk assessment process. The Threshold of Regulation approach can help to identify priorities for carcinogenicity evaluation particularly for chemicals not subject to regulatory approval schemes.



Gaps and Research Needs

72. The Committee consider the following to be key areas for research
- i) Clarification of the shape of the dose-response curve at very low doses and low estimated risks. Further development of PBTK/TD modelling for use in conjunction with chronic carcinogenicity studies to inform on risk assessment at low doses.
 - ii) Identification and significance for risk assessment of proposed biological markers of tumour precursors and related processes (eg pre-neoplastic foci, biomarkers, DNA adducts and repair). Further investigation of biological responses at environmentally relevant doses.
 - iii) Further development and validation of genetically modified animal models including studies to define changes to dose-response due to genetic modification, as well as to investigate their biological basis.

Overall Summary

73. The Committee on Carcinogenicity evaluates carcinogenicity data on chemicals on a case-by-case basis, taking into account the weight of all the available evidence (IGHRC, 2002). The range of data considered may differ with circumstances, for instance, it will not always be possible to obtain epidemiological data and each assessment will be considered on its own merits. It is not possible to provide a universally applicable list of data that will be needed for a carcinogenic assessment. However, it is hoped that this document will provide some guidance on a suitable strategy that could be adopted.
74. The Committee recommends a four-stage evaluation strategy for the risk assessment process of carcinogenic hazard. Initial identification of a carcinogenic hazard at stage 1 should be based upon a review of the toxicity data, the results of toxicity testing, and any knowledge of effects on human health. The Committee considers it essential to determine whether carcinogens act via a genotoxic or non-genotoxic mechanism, therefore the Committee fully endorse the strategy published by the Committee on Mutagenicity (<http://www.advisorybodies.doh.gov.uk/com/guidance.pdf>). Hazard characterisation (stage 2) should determine the dose-response relationship from epidemiological or animal data. During this stage it is important that factors such as interspecies variation in susceptibility, and information on mode of action are considered. Exposure assessment (stage 3) should estimate probable human exposure, routes of entry and levels of potential exposure taking into account the limitations of exposure models. The final stage (stage 4) should characterise the carcinogenic risk by summarising the previous stages and developing appropriate approaches to genotoxic and non-genotoxic carcinogens.
75. If a putative carcinogen is found to be non-genotoxic, the Committee recommends the adoption of a threshold approach. Thus a method based on the identification of a NOAEL and the use of uncertainty factors is appropriate, as is used in other areas of chemical risk assessment.
76. If a putative carcinogen is found to be potentially genotoxic, the Committee recommends a non-threshold approach for risk assessment. The assumption of no threshold, together with the practical difficulties of using low doses of human relevance in animal carcinogenicity studies, has led to the development of mathematical models that attempt to provide a 'best estimate' of the likely extrapolation of the dose-response curve below the lowest experimental data points. These models may give an impression of precision, which cannot be justified from the approximations and assumptions upon

which they are based. Therefore, the Committee on Carcinogenicity recommends that the ALARP (as low as reasonable practicable) approach should be adopted by risk managers. This can be supplemented in specific situations eg low exposures to contaminants or impurities by the setting of a minimal risk level for genotoxic carcinogen contaminants and impurities. This represents a novel additional approach to the risk assessment of genotoxic carcinogens compared to the previous guidelines but should be based on expert judgement of available data. The use of potency estimates can be used to rank priorities for genotoxic carcinogens within a particular class of compounds (eg polycyclic aromatic hydrocarbons which are also genotoxic carcinogens). The Committee agrees that it should always remain important to keep any exposure to genotoxic carcinogens as low as reasonably practicable (ALARP).

77. The Committee emphasises the importance of further research in order to refine the process of risk assessment. This includes the development of toxicological methods to refine extrapolation between animals and humans. In addition, biomarkers of effect need to be further investigated to aid in the extrapolation of low doses and exposure. Continued research on carcinogenic mechanisms with the ultimate aim of developing appropriate models for low dose extrapolation is also required.

References

Albertini RJ, Anderson D, Douglas GR, Hagmar L, Hemminki K, Merlo F, Natarajan AT, Norppa H, Shuker DE, Tice R, Waters MD

Aitio A. (2000) IPCS guidelines for the monitoring of genotoxic effects of carcinogens in humans. International Programme on Chemical Safety. Mutation Research, 463 (2),11-72

Albertini R, Clewell H, Himmelstein MW, Morinello E, Olin S, Preston J, Scarano L, Smith MT, Swenberg J, Tice R & Travis C. (2003) The use of non-tumor data in cancer risk assessment: reflections on butadiene, vinyl chloride, and benzene. Regulatory Toxicology Pharmacology, 37:105-32

Alexander J, Reistad R, Hegstad S, Frandsen H, Ingebrigtsen K, Paulsen JE & Becher G. (2002) Biomarkers of exposure to heterocyclic amines: approaches to improve the exposure assessment Food Chemical Toxicology, 40:1131-7

Arif JM & Gupta RC (1997) Detection of DNA-reactive metabolites in serum and their tissue distribution in mice exposed to multiple doses of carcinogen mixtures: role in human biomonitoring. Carcinogenesis, 17, 2213-2219

Ashby J & Tennant RW. (1994) Prediction of rodent carcinogenicity for 44 chemicals: results. Mutagenesis, 9:7-15

Ashby J, Kier L, Wilson AG, Green T, Lefevre PA, Tinwell H, Willis GA, Heydens WF & Clapp MJ (1996) Evaluation of the potential carcinogenicity and genetic toxicity to humans of the herbicide acetochlor. Human and Experimental Toxicology, 15:702-35

Aston JP, Ball RL, Pople JE, Jones K & Cocker J. (2002) Development and validation of a competitive immunoassay for urinary S-phenylmercapturic acid and its application in benzene biological monitoring. Biomarkers, 7(2): 103-12

Australian Department of Health and Ageing and Environmental Health (enHealth) Council (2002) Environmental health risk assessment: Guidelines for assessing human health risks from environmental hazards. Department of Health and Ageing, Canberra, Australian Capital Territory.
(www.health.gov.au/pubhlth/publicat/document/metadata/env_hra.htm)

Bailer, A.J. & Portier, C.J. (1993) An index of tumorigenic potency. Biometrics, 49: 357-365

- Barlow SM, Kozianowski G, Wurtzen G, Schlatter J. (2001) "Threshold of toxicological concern for chemical substances present in the diet. Report of a workshop, 5-6 October 1999, Paris, France. *Food Chemical Toxicology*, 39:893-905
- Beach AC, Gupta RC(1992). Human biomonitoring and the ³²P-postlabeling assay. *Carcinogenesis*, 13, 1053-1074
- Bechtold WE & Henderson RF (1993) Biomarkers of human exposure to benzene. *Journal of Toxicology and Environmental Health*, 40:377-86
- Boogaard PJ & van Sittert NJ (1995) Biological monitoring of exposure to benzene: a comparison between S-phenylmercapturic acid, trans, trans-muconic acid, and phenol. *Occupational and Environmental Medicine*, 52, 611-620
- Cheeseman MA, Machuga EJ, Bailey AB. (1999) A tiered approach to threshold of regulation. *Food Chemical Toxicology*, 37:387-412
- Clewell H.J., Gentry P.R., Gearhart J.M., Allen B.C. & Anderson M.E. (2001) Comparison of cancer risk estimates for vinyl chloride using animal and human data with a PBPK model. *Science of the Total Environment*, 274(1-3), 37-66
- Cohen SM, Meek ME, Klaunig JE, Patton DE, Fenner-Crisp PA (2003). The human relevance of information on carcinogenic modes of action: overview. *Critical Reviews in Toxicology*, 33 (6): 581-9
- Cohen SM, Klaunig J, Meek E, Hill RN, Pastoor T, Lehman-McKeeman L, Bucher J, Longfellow DG, Seed J, Dellarco V, Fener-Crisp P and Patton D. (2004). Evaluating the human relevance of chemically induced animal tumours. *Toxicological Sciences*, 78, 181-186
- Collins JF, Brown JP, Alexeeff GV & Salmon AG. (1998) Potency equivalency factors for some polycyclic aromatic hydrocarbons and polycyclic aromatic hydrocarbon derivatives. *Regulatory Toxicology Pharmacology*, 28:45-54
- Counts JL & Goodman JI (1995) Principles Underlying Dose Selection for, and Extrapolation from, the Carcinogen Bioassay: Dose Influences Mechanism. *Regulatory Toxicology and Pharmacology*, 21:418-421
- Crump KS (1994) Use of mechanistic models to estimate low-dose cancer risks. *Risk Analysis*, 14, 1033-1038
- Crump KS (1996) The linearized multistage model and the future of quantitative risk assessment. *Human & Experimental Toxicology*, 15, 787-798

DEFRA and Environment Agency (2002) Contaminants in Soils: Collation of Toxicological Data and Intake Values for Humans. Consolidated Main Report, CLR Report No 9. Available from the R&D Dissemination Centre, WRC plc, Swindon.

Duggan MJ & Lambert BE (1998) Standards for Environmental, Non-threshold, Carcinogens: A Comparison of the approaches used for radiation and chemicals. *Annals of Occupational Hygiene*, 42:315-323

Dybing E, Sanner T, Roelfzema H, Kroese D & Tennant RW (1997) T25: a simplified carcinogenic potency index: description of the system and study of correlations between carcinogenic potency and species/site specificity and mutagenicity. *Pharmacological Toxicology*, 80, 272-279

Dybing E (2002). Development and implementation of the IPCS conceptual framework for evaluating mode of action of chemical carcinogens. *Toxicology*, 181-182, 121-5

Eastin, WC (1998) The National Toxicology Program Evaluation of Transgenic Mice as Predictive Models for Identifying Carcinogens. *Environmental Health Perspectives*, 106, 81-84

ECETOC Technical Report No. 83 (2002) the use of T25 estimates and alternative methods in the regulatory risk assessment of non-threshold carcinogens in the European Union

ECETOC Monograph No. 24 (1996) Risk Assessment for carcinogens

Edler L & Kopp-Schneider A (1998) Statistical models for low dose extrapolation. *Mutation Research*, 405, 227-236

Edler L, Poirier K, Dourson M, Kleiner J, Mileson B, Nordmann H, Renwick A, Slob W, Walton K & Wurtzen G (2002) Mathematical modelling and quantitative methods. *Food and Chemical Toxicology*, 40,283-326

Environmental Health Criteria No 155 (1993) : Biomarkers and Risk Assessment: Concepts and Principles. International Programme on Chemical Safety, World Health Organisation, Geneva

Environmental Health Criteria, No. 202 (1998) Selected Non-heterocyclic Polycyclic Aromatic Hydrocarbons. International Programme on Chemical Safety, World Health Organisation, Geneva

Environmental Health Criteria, No. 210 (1999) Principles for the assessment of risks to human health from exposure to chemicals. International Programme on Chemical Safety, World Health Organisation, Geneva

Environmental Health Criteria No 222 (2001) Biomarkers in Risk Assessment: Validity and Validation. International Programme on Chemical Safety, World Health Organisation, Geneva

Fang JL, Vaca CE, Valsta LM & Mutanen M. (1996) Determination of DNA adducts of malonaldehyde in humans: effects of dietary fatty acid composition. *Carcinogenesis*, 17, 1035-40

Farmer PB & Sweetman GMA (1995) Mass spectrometric detection of carcinogenic adducts. *J. Mass Spectrometry*, 30, 1369-1379

Farmer PB. (1999) Studies using specific biomarkers for human exposure assessment to exogenous and endogenous chemical agents. *Mutation Research*, 428:69-81

Farmer PB (2004). Exposure biomarkers for the study of toxicological impact on carcinogenic processes. International Agency for Research on Cancer. Scientific Publications, 157, 71-90

Ferrier H, Nieuwenhuijsen M, Boobis A and Elliott P (2002). Current knowledge and recent developments in consumer exposure assessment of pesticides: A UK perspective. *Food Additives and Contaminants*, 19, 837-852

Friesen MD, Kaderlik K, Lin D, Garren L, Bartsch H, Lang NP, Kadlubar FF. (1994) Analysis of DNA adducts of 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine in rat and human tissues by alkaline hydrolysis and gas chromatography/electron capture mass spectrometry: validation by comparison with ³²P-postlabeling. *Chemical Research in Toxicology*, 7, 733-9

Gaylor DW, Kodell RL, Chen JJ, Springer JA, Lorentzen RJ & Scheuplein RJ (1994) Point estimates of cancer risk at low doses. *Risk Analysis*, 14, 843-849

Gold LS, Sawyer CB, Magaw R, Backman GM, de Veciana M, Levinson R, Hooper NK, Havender WR, Bernstein L, *et al.* (1984) A Carcinogenic Potency Database of the standardized results of animal bioassays. *Environmental Health Perspectives*, 58, 9-319

Gold LS, Slone TH, & Bernstein, L. (1989) Summary of carcinogenic potency (TD50) and positivity for 492 rodent carcinogens in the Carcinogenic Potency Database. *Environmental Health Perspectives*, 79, 259-272

Gold LS, Manley NS, Slone TH & Rohrbach L (1999). Supplement to the Carcinogenic Potency Database (CPDB): Results of Animal Bioassays Published in the General Literature in 1993 to 1994 and by the National Toxicology Program in 1995 to 1996. *Environmental Health Perspectives*, 107, 527-600

Gold LS, Gaylor DW, Slone TH. (2003) Comparison of cancer risk estimates based on a variety of risk assessment methodologies. *Regulatory Toxicology and Pharmacology*, 37, 45-53

Groopman JD, Wild CP, Hasler J, Junshi C, Wogan GN, Kensler TW (1993) Molecular epidemiology of aflatoxin exposures: validation of aflatoxin-N7-guanine levels in urine as a biomarker in experimental rat models and humans. *Environmental Health Perspectives*, 99, 107-113

Groopman JD, Wild CP, Hasler J, Junshi C, Wogan GN, Kensler TW. Molecular epidemiology of aflatoxin exposures: validation of aflatoxin-N7-guanine levels in urine as a biomarker in experimental rat models and humans. *Environ Health Perspect.* 1993; 99:107-113

Groopman JD, Kensler TW (1999) The light at the end of the tunnel for chemical-specific biomarker: daylight or headlight? *Carcinogenesis*, 20: 1-11

Hanes B & Wedel T (1985) A selected review of risk models: one hit, multi hit, multi-stage, probit, Weibull and pharmacokinetic. *Journal of the American College of Toxicology*, 4, 271-278

Harley HO & Sielken RL(1977) Estimation of safe doses in carcinogenic experiments. *Biometrics*, 33, 1-30

Hecht SS (2002) Human urinary carcinogen metabolites: biomarkers for investigating tobacco and cancer. *Carcinogenesis*, 23, 907-22

IGHRC – The Interdepartmental Group on Health Risk from Chemicals (2002) Assessment of chemical carcinogens: Background to general principles of a weight of evidence approach. MRC Institute for Environment and Health, Leicester

IGHRC – The Interdepartmental Group on Health Risk from Chemicals (2003) Uncertainty factors: Their use in human health risk assessment by UK Government. MRC Institute for Environment and Health, Leicester

International Expert Panel on Carcinogen Risk Assessment (1996) The use of mechanistic data in the risk assessment of ten chemicals: an introduction to the chemical-specific reviews. *Pharmacology and Therapeutics*, 71:1-5 (Special Issue)

Kensler TW, Qian GS, Chen JG & Groopman JD (2003) Translational strategies for cancer prevention in liver. *Nature Reviews Cancer*, 3, 321-329

Krewski D, Cardis E, Zeise L & Feron VJ (1999) Empirical approaches to risk estimation and prediction. In Moolgavkar S, Krewski D, Zeise L, Cardis E, & Moller H (Eds) *Quantitative Estimation and Prediction of Human Cancer Risks*. IARC Scientific Publications No. 131. International Agency for Research on Cancer, Lyon. 131-178

Krewski D, Gaylor D & Szyszkowicz M (1991) A model-free approach to low-dose extrapolation. *Environmental Health Perspectives* 90: 279-285

Kroes R, Galli C, Munro I, Schilter B, Tran L, Walker R, Wurtzen G (2000) Threshold of toxicological concern for chemical substances present in the diet: a practical tool for assessing the need for toxicity testing. *Food Chemical Toxicology*, 38, 255-312

Kroes R, Renwick AG, Cheeseman M, Kleiner J, Mangelsdorf I, Piersma A, Schilter B, Schlatter J, van Schothorst F Vos JG and Wurtzen G (2004). Structure-based thresholds of toxicological concern (TTC): guidance for application to substances present at low levels in the diet. *Food Chemical Toxicology*, 42, 65-83

Lovell DP & Thomas G (1996) Quantitative risk assessment and the limitations of the linearised multistage model. *Human & Experimental Toxicology*, 15, 87-104

Mantel N & Bryan WR (1961) Safety testing if carcinogenic agents. *J National Cancer Institute*, 27, 455-470

Marczynski B, Rozynek P, Elliehausen HJ, Korn M, Baur X. (1997) Detection of 8-hydroxydeoxyguanosine, a marker of oxidative DNA damage, in white blood cells of workers occupationally exposed to styrene. *Archives of Toxicology*, 71, 496-500

Meek ME, Renwick A, Ohanian E, Dourson M, Lake B, Naumann BD & Vu V (2002) Guidelines for application of chemical-specific adjustment factors in dose/concentration-response assessment. *Toxicology*, 181-182: 115-120

Meek ME, Butcher JR, Cohen SM, Dellarco V, Hill RN, Lehman-McKeeman LD, Longfellow DG, Pastoor T, Seed J, Patton DE (2003). A framework for human relevance analysis of information on carcinogenic modes of action. *Critical Reviews in Toxicology*, 33 (6): 591-653

Mitchelmore CL & Chipman JK (1998) DNA strand breakage in aquatic organisms and the potential value of the comet assay in environmental monitoring *Mutation Research*, 399(2):135-47

- Moolgavkar S, Krewski D, Schwarz M. (1999) Mechanisms of carcinogenesis and biologically based models for estimation and prediction of risk. IARC Scientific Publications, 131,179-237
- Munro IC, Kennepohl E & Kroes R (1999) A procedure for the safety evaluation of flavouring substances. *Food Chemical Toxicology*, 37, 207-232
- Mure K, Hayatsu H, Takeuchi T, Takeshita T & Morimoto K. (1997) Heavy cigarette smokers show higher mutagenicity in urine. *Mutation Research*, 373, 107-11
- Nakijima M, Takeuchi T, Takeshita T & Morimoto K (1996) 8-Hydroxydeoxyguanine in human leukocyte DNA and daily health practice factors: Effects of individual alcohol sensitivity. *Environmental Health Perspectives*, 104, 1336-1338
- Nielsen F, Mikkelsen BB, Nielsen JB, Andersen HR, Grandjean P. (1997) Plasma malondialdehyde as biomarker for oxidative stress: reference interval and effects of life-style factors. *Clinical Chemistry*, 43, 1209-14
- Nordic Council of Ministers. Potency Ranking of Carcinogenic Substances. Report from a Nordic Working Party. Miljørapport 1985:4E. The State Pollution Control Authority, Oslo, 1986
- OECD (1998) OECD principles on good laboratory practice (as revised in 1997). Paris, Organisation for Economic Co-operation and Development, Environmental Directorate, Chemicals Group and Management Committee (OECD Series on Principles of Good Laboratory Practice and Compliance Monitoring No. 1 –ENV/MC/CHEM (98)17)
- Osterman-Golkar S, Peltonen K, Anttinen-Klemetti T, Landin HH, Zorcec V, Sorsa M. (1996) Haemoglobin adducts as biomarkers of occupational exposure to 1,3-butadiene. *Mutagenesis*, 11, 145-9
- Paulsson B, Granath F, Grawé J, Ehrenberg L, Törnqvist M (2001) The multiplicative model for cancer risk assessment: applicability to acrylamide. *Carcinogenesis*, 22, 817-819
- Peto R, Pike MC, Bernstein L, Gold LS & Ames BN (1984) The TD₅₀: a proposed general convention for the numerical description of the carcinogenic potency of chemicals in chronic-exposure animal experiments. *Environmental Health Perspectives*, 58, 1-8
- Phillips DH. (1997) Detection of DNA modifications by the 32P-postlabelling assay. *Mutation Research*, 378, 1-12

Portier CJ, Hedges JC & Hoel DG (1984) Age-specific models and tumour onset for historical control animals in the National Toxicology Programs carcinogenicity experiments. *Cancer Research*, 46,4372-4378

Repace JL, Jinot J, Bayard S, Emmons K & Hammond SK. (1998) Air nicotine and saliva cotinine as indicators of workplace passive smoking exposure and risk. *Risk Analysis*, 18, 71-83

Richard AM (1998) Structure-based methods for predicting mutagenicity and carcinogenicity: are we there yet? *Mutation Research*, 400, 493-507

Schut HAJ & Shiverick KT (1992) DNA adducts in humans as dosimeters if exposure to environmental, occupational or dietary genotoxins. *FASEB Journal*, 6, 2942-2951

Schoket B, Phillips DH, Poitier MC, Vincze I (1993). DNA adducts in peripheral blood lymphocytes from aluminium plant production workers determined by ³²P-postlabelling and enzyme linked immunosorbent assay. *Environmental Health Perspectives*, 99, 307-309

Shuker DE & Farmer PB (1992) Relevance of urinary DNA adducts as markers of carcinogen exposure. *Chemical Research in Toxicology*, 5, 450-60

Shuker DE, Prevost V, Friesen MD, Bartsch H. (1993) Noninvasive methods for measuring DNA alkylation in experimental animals and humans. *Environmental Health Perspectives*, 101 Supplement 3, 151-3

Shuker DE (1999) DNA adducts in mammalian cells as indicators of exposure to carcinogens IARC Scientific Publications 1999; 146, 287-308

Shuker D (2002) The enemy at the gates? DNA adducts as biomarkers of exposure to exogenous and endogenous genotoxic agents. *Toxicology Letters*, 134, 51-56

Sonich-Mullin C, Fielder R, Wiltse J, Baetcke K, Dempsey J, Fenner-Crisp P, Grant D, Hartley M, Knaap A, Kroese D, Mangelsdorf I, Meek E, Rice JM & Younes M (2001) IPCS conceptual framework for evaluating a mode of action for chemical carcinogenesis. *Regulatory Toxicology Pharmacology*, 34, 146-52

UK Department of Health (1991) Committee on Carcinogenicity of Chemicals in Food, Consumer Products and the Environment: Guidelines for the evaluation of chemicals for carcinogenicity. London, Her Majesty's Stationary Office (HMSO)

UK Department of Health (2000) Committee on Mutagenicity of Chemicals in Food, Consumer Products and the Environment: Guidance on a strategy for testing of chemicals for mutagenicity.

(<http://www.advisorybodies.doh.gov.uk/com/comivm.htm>)

US EPA (1996) Proposed guidelines for carcinogen risk assessment. Federal Register, 6(79): 17960-18011

US FDA (1995) Food additives: Threshold of regulation for substances used in food-contact articles (final rule). Federal Register 60(136), 36582-36596

US NAS (National Academy of Science) (1983) National Research Council, Committee on the Institutional Means for Assessment of Risks to Public Health: Risk assessment in the Federal Government: Managing the process. Washington, DC, National Academy Press, pp1-50

Van den Berg M, Birnbaum L, Bosveld A, Brunström B, Cook P, Feeley M, Giesy J, Hanberg A, Hasegawa R, Kennedy S, Kubiak T, Larsen J, van Leeuwen F, Liem A, Nolt C, Peterson R, Poellinger L, Safe S, Schrenk D, Tillitt D, Tysklind M, Younes M, Wærn F & Zacharewski T (1998) Toxic Equivalency Factors (TEFs) for PCBs, PCDDs, PCDFs for Humans and Wildlife. Environmental Health Perspectives, 106, 12, 775-792

Van den Brandt P., Voorrips L., Hertz-Picciotto I., Boeing H., Speijers G., Guittard C., Kleiner J., Knowles M., Wolk A. & Goldbohm A. (2002) The contribution of epidemiology. Food and Chemical Toxicology, 40:387-424

Verna L, Whysner J & Williams GM (1996) 2-Acetylaminofluorene mechanistic data and risk assessment: DNA reactivity, enhanced cell proliferation and tumour initiation. Pharmacology and Therapeutics, 71:83-105

Weibull (1951) Statistical distribution function of wide applicability. J Applied Mechanics, 18, 293-302

Weisel C, Yu R, Roy A and Georgopoulos P (1996). Biomarkers of environmental benzene exposure. Environmental Health Perspectives, 104, 1141-1146

WHO (1991) World Health Organisation. Evaluation of certain food additives and contaminants. Thirty-seventh report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series No. 806. WHO, Geneva

Whysner J, Clifford-Conaway C, Verna L & Williams GM (1996) Vinyl Chloride mechanistic data and risk assessment: DNA reactivity and cross-species quantitative risk extrapolation. Pharmacology and Therapeutics, 71:7-28

Williams GM (2001) Mechanism of chemical carcinogenesis and application to human cancer risk assessment. *Toxicology*, 166:3-10

Williams GM, Iatropoulos MJ & Weisburger JH (1996) Chemical carcinogenic mechanisms of action: implications for testing methodology. *Experimental and Toxicological Pathology*, 48, 101-111

Wu Y, Chen J, Ohshima H, Pignatelli B, Boreham J, Li J, Campbell TC, Peto R & Bartsch H (1993) Geographical association between urinary excretion of N-nitroso compounds and oesophageal cancer mortality in China. *International Journal of Cancer*, 54:713-719

Yeowell-O'Connell K, Jin Z & Rappaport SM (1996) Determination of albumin and hemoglobin adducts in workers exposed to styrene and styrene oxide. *Cancer Epidemiology Biomarkers Prevention*, 5 (3),205-15

Zeise L, Cardis E, Hemminki K & Schwarz M (1999) Quantitative Estimation and Prediction of Cancer Risk: Review of Existing Activities. In Moolgavkar S, Krewski D, Zeise L, Cardis E, & Moller H (Eds) *Quantitative Estimation and Prediction of Human Cancer Risks*. IARC Scientific Publications No. 131. International Agency for Research on Cancer, Lyon. 11-59

Glossary of Terms commonly used in carcinogenicity

Acceptable Daily Intake (ADI):	Estimate of the amount of a substance in food or drink, expressed on a body weight basis (eg mg/kg bodyweight), that can be ingested daily over a lifetime by humans without appreciable health risk based on all known facts at the time of derivation.
Adduct:	A chemical grouping that is covalently bound (see covalent binding) to a large molecule such as DNA (qv) or protein.
Adenoma:	A benign neoplasm arising from a gland forming epithelial tissue such as colon, stomach or respiratory tract.
ADME:	Absorption, Distribution, Metabolism and Excretion data for a chemical.
Adverse effect:	Change in morphology, physiology, biochemistry, growth, development or lifespan of an organism which results in impairment of functional capacity or impairment of capacity to compensate for additional stress or increase in susceptibility to the harmful effects of other environmental influences.
Alkylating agents:	Chemicals which leave an alkyl group covalently bound to biologically important molecules such as proteins and nucleic acids (see adduct). Many alkylating agents are mutagenic, carcinogenic and immunosuppressive.
Ames test:	<i>In vitro</i> (qv) assay for bacterial gene mutations (qv) using strains of <i>Salmonella typhimurium</i> developed by Ames and his colleagues.
Aneugen:	A chemical which induces loss or gain of chromosomes during cell division usually as a result of non-disjunction or anaphase lag. The resulting number of chromosomes following cell division is not an exact multiple of the normal haploid number.

Bias:	In the context of epidemiological studies, an interference which at any stage of an investigation tends to produce results that depart systematically from the true values (to be distinguished from random error). The term does not necessarily carry an imputation of prejudice or any other subjective factor such as the experimenter's desire for a particular outcome.
Biomarker:	Observable change (not necessarily pathological) in an organism, related to a specific exposure or effect.
Cancer:	Synonym for a malignant neoplasm – that is, a tumour (qv) that grows progressively, invades local tissues and spreads to distant sites (see also tumour and metastasis).
Carcinogenesis:	The origin, causation and development of tumours (qv). The term applies to benign as well as malignant neoplasms and not just to carcinomas (qv).
Carcinogenicity bioassay:	Tests carried out in laboratory animals, usually rats and mice, to determine whether a substance is carcinogenic. The test material is given throughout life to groups of animals at different dose levels.
Carcinogens:	The causal agents, which induce tumours. They include external factors (chemicals, physical agents, viruses) and internal factors such as hormones. Chemical carcinogens are structurally diverse and include naturally occurring substances as well as synthetic compounds. An important distinction can be drawn between <i>genotoxic</i> (qv) carcinogens, which have been shown to react with and mutate DNA, and <i>non-genotoxic</i> carcinogens, which act through other mechanisms. The activity of genotoxic carcinogens can often be predicted from their chemical structure - either of the parent compound or of active metabolites (qv). Most chemical carcinogens exert their effects after prolonged exposure, show a dose-response relationship and tend to act on a limited range of susceptible target tissues. Carcinogens are sometimes species- or sex-specific and the term should be qualified by the appropriate descriptive adjectives to aid clarity. Several different chemical and other carcinogens may interact, and constitutional factors (genetic susceptibility, hormonal status) may also contribute, emphasising the multifactorial nature of the carcinogenic process.

Carcinogenic potency: Carcinogenic potency is the potential of a substance to cause cancer. Potencies may be obtained from the gradient of the dose-response curve and are usually expressed in units of inverse dose as a potency slope (ie (mg/kg/day)⁻¹). The derivation of the carcinogenic potency values take into account the available information on pharmacokinetics and on the mechanism of carcinogenic action. Carcinogenic Potency is more often simply defined as the dose giving rise to a fixed incidence of tumours above the background incidence. Thus T25 represents the dose level giving rise to a 25% increase in the incidence of a particular tumour above the background incidence. The various methods of reporting potency are explained in the text of the guidance document.

Carcinoma: Malignant tumour arising from epithelial cells lining, for example, the alimentary, respiratory and urogenital tracts and from epidermis, also from solid viscera such as the liver, pancreas, kidneys and some endocrine glands. (See also 'tumour').

Case-control study: (Synonyms - case comparison study, case referent study, retrospective study) A comparison is made of the proportion of cases who have been exposed to a particular hazard (eg a carcinogen) with the proportion of controls who have been exposed to the hazard.

Chromosomal aberrations: Collective term of particular types of chromosome damage induced after exposure to exogenous chemical or physical agents which damage the DNA. (see clastogen).

Chromosome: In simple prokaryotic organisms, such as bacteria and most viruses, the chromosome consists of a single circular molecule of DNA containing the entire genetic material of the cell. In eukaryotic cells, the chromosomes are thread-like structures, composed mainly of DNA and protein, which are present within the nuclei of every cell. They occur in pairs, the numbers varying from one to more than 100 per nucleus in different species. Normal somatic cells in humans have 23 pairs of chromosomes, each consisting of linear sequences of DNA, which are known as genes (qv).

Chronic effect: Consequence which develops slowly and has a long-lasting course (often but not always irreversible).

Chronic exposure:	Continued exposures occurring over an extended period of time, or a significant fraction of the lifetime of a human or test animal.
Clastogen:	An agent that produces chromosome breaks and other structural aberrations such as translocations. Clastogens may be viruses or physical agents as well as chemicals. Clastogenic events play an important part in the development of some tumours.
Cohort:	A defined population that continues to exist through time.
Cohort study:	(Synonyms - follow-up, longitudinal study) The study of a group of people defined at a particular point in time (the cohort), who have particular characteristics in common, such as a particular exposure. They are then observed over a period of time for the occurrence of disease. The rate at which the disease develops in the cohort is compared with the rate in a comparison population, in which the characteristics (eg exposure) are absent.
Cytochrome P450:	A class of haemoprotein enzymes associated with the endoplasmic reticulum of cells. The carbon monoxide-reduced cytochrome P450 complex absorbs light maximally at 450nm. The principle role of cytochrome P450 enzymes is to metabolise endogenous (intermediary metabolites) or exogenous chemicals generally from lipophilic compounds to more water soluble compounds which may be eliminated from the body. A number of cytochrome P450 mediated enzyme reactions result in the activation of compounds in some cases to form proximate carcinogens (qv).
Differentiation:	A term that denotes the degree of morphological and functional organisation within cells and organs. Differentiation is implicit in normal histogenesis and organogenesis; it is to a varying extent aberrant in neoplastic cells.
DNA (Deoxyribonucleic Acid):	The carrier of genetic information for all living organisms except the group of RNA viruses. Each of the 46 chromosomes in normal human cells consists of 2 strands of DNA containing up to 100,000 nucleotides, specific sequences of which make up genes (qv). DNA itself is composed of two interwound chains of linked nucleotides (qv).

DNA repair genes:	Genes which code for proteins that correct damage in DNA sequences. When these genes are altered, mutations may be able to accumulate in the genome, ultimately resulting in disease.
Dose:	Total amount of a substance administered to, taken or absorbed by an organism
Electrophile:	A compound containing an electron-deficient centre which tends to acquire electrons during chemical reactions- for example, an ultimate carcinogen forming adducts with DNA.
Epidemiology:	Study of the distribution and the aetiology of disease in humans.
Gene:	The functional unit of inheritance: a specific sequence of nucleotides along the DNA molecule, forming part of a chromosome (qv).
Genetic polymorphism:	a difference in DNA sequence, present in all cells, among individuals, groups, or populations (eg a genetic polymorphism might give rise to blue eyes versus brown eyes, or straight hair versus curly hair). Genetic polymorphisms may be the result of chance processes, or may have been induced by external agents (such as viruses or radiation). Changes in DNA sequence which have been confirmed to be caused by external agents are generally called “mutations” rather than “polymorphisms”.
Hazard:	Set of inherent properties of a substance, mixture of substances or a process involving substances that make it capable of causing adverse effects to organisms or the environment.
Hepatic:	Pertaining to the liver
Human relevancy Framework (HRF):	The human relevance framework (HRF) proposal developed by the Risk Sciences Institute of the International Life Sciences Institute provides a systematic approach to evaluating whether the key events in the mode of action of carcinogenic responses in experimental animals would be plausible in humans.

<i>In vitro:</i>	A Latin term used to describe effects in biological material outside the living animal (literally “in glass”)
<i>In vivo:</i>	A Latin term used to describe effects in living animals (literally “in life”).
Knockout animals:	Genetically engineered animals in which one or more genes, usually present and active in the normal animal, are absent or inactive.
Meta-analysis:	In the context of epidemiology, a statistical analysis of the results from independent studies, which aims to produce a single estimate of an effect.
Metabolic activation:	Metabolism of a compound leading to an increase in its activity, whether beneficial (eg activation of a pro-drug) or deleterious (eg activation to a toxic or carcinogenic metabolite).
Metabolism:	Chemical modification of a compound by enzymes within the body, for example by reactions such as hydroxylation (see cytochrome P450), epoxidation or conjugation. Metabolism may result in activation, inactivation, accumulation or excretion of the compound.
Metabolite:	Product formed by metabolism of a compound.
Micronuclei:	Isolated or broken chromosome fragments which are not expelled when the nucleus is lost during cell division, but remain in the body of the cell forming micronuclei. Centromere positive micronuclei contain DNA and/or protein material derived from the centromere. The presence of centromere positive micronuclei following exposure to chemicals can be used to evaluate the aneugenic (qv) potential of chemicals.
Micronucleus test:	See Micronuclei.
Mode of Action:	The mode of action of a chemical carcinogen refers to the underlying events involved in the process whereby the chemical induces cancer. In order for a specific mode of action to be supported there needs to be evidence from robust mechanistic data to establish a biologically plausible explanation. Mode of action should be distinguished from the term mechanism of action. The

	<p>latter relates to having sufficient understanding of the molecular basis of the chemical carcinogenesis process to establish causality. Thus mechanism of action is at the other end of a continuum from little or no evidence of mode of action to scientific proof of mechanism of action.</p>
Multistage carcinogenesis.:	<p>The development of tumours in humans and animals is regarded as a multistage process in which both genotoxic and non genotoxic changes occur. Three separate phases- initiation, promotion and progression (qv) have been described.</p>
Mutation:	<p>A permanent change in the amount or structure of the genetic material in an organism or cell, which can result in a change in phenotypic characteristics. The alteration may involve a single gene, a block of genes, or a whole chromosome. Mutations involving single genes may be a consequence of effects on single DNA bases (point mutations) or of large changes, including deletions, within the gene. Changes involving whole chromosomes may be numerical or structural. A mutation in the germ cells of sexually reproducing organisms may be transmitted to the offspring, whereas a mutation that occurs in somatic cells may be transferred only to descendent daughter cells.</p>
Neoplasm:	<p>See 'tumour'.</p>
Neoplastic:	<p>Abnormal cells, the growth of which is more rapid than that of other cells.</p>
No observed adverse effect level (NOAEL):	<p>The highest administered dose at which no adverse (qv) effect has been observed.</p>
Non-genotoxic:	<p>See 'carcinogens'.</p>
Nucleic acid:	<p>One of the family of molecules which includes the DNA and RNA molecules. Nucleic acids were so named because they were originally discovered within the nucleus of cells, but they have since been found to exist outside the nucleus as well.</p>

- Nucleotide:** the “building block” of nucleic acids, such as the DNA molecule. A nucleotide consists of one of four bases - adenine, guanine, cytosine, or thymine - attached to a phosphate-sugar group. In DNA the sugar group is deoxyribose, while in RNA (a DNA related molecule which helps to translate genetic information into proteins), the sugar group is ribose, and the base uracil substitutes for thymine. Each group of three nucleotides in a gene is known as a codon. A nucleic acid is a long chain of nucleotides joined together, and therefore is sometimes referred to as a “polynucleotide.”
- Oncogene:** The name given to activated forms of proto-oncogenes (qv). They are cellular in origin.
- Pharmacokinetics:** Description of the fate of drugs in the body, including a mathematical account of their absorption, distribution, metabolism and excretion (see toxicokinetics).
- Pharmacogenomics:** The science of understanding the correlation between an individual patient’s genetic make-up (genotype) and their response to drug treatment. Some drugs work well in some patient populations and not as well in others. Studying the genetic basis of patient response to therapeutics allows drug developers to design therapeutic treatments more effectively.
- Potency Equivalency Factors:** A measure of relative carcinogenic potency of a chemical compared to a well-characterised reference compound. PEFs may be used to sum the carcinogenic potency of a mixture of chemicals which are all members of the same chemical class, having common structural, toxicological and biochemical properties. PEFs have been suggested for polycyclic aromatic hydrocarbons. There has been relatively little use of Potency Equivalence Factors for carcinogenicity to date.
- Procarcinogen:** An inactive carcinogen which is metabolically converted, via proximate carcinogens, to the electrophilic ultimate carcinogen that reacts with DNA.

Proto-oncogene:	A group of normal cellular genes, highly conserved, which are concerned with control of cellular proliferation and differentiation. They can be activated in various ways to forms which are closely associated with one or more steps in carcinogenesis. Mechanisms of activation include point mutations which alter the structure of the proto-oncogene, or changes in the regulatory region which alter expression. Activating agents include chemicals and viruses. The process of proto-oncogene activation is thought to play an important part at several stages in tumour development.
Proximate carcinogens:	Metabolites generated from procarcinogens which in turn give rise to ultimate carcinogens.
Relative risk:	A measure of the association between exposure and outcome. The rate of disease in the exposed population divided by the rate of disease among the unexposed population in a cohort study or a population-based case control study. A relative risk of 2 means that the exposed group has twice the disease risk compared to the unexposed group.
Risk:	Possibility that a harmful event (death, injury or loss) arising from exposure to a chemical or physical agent may occur under specific conditions.
RNA (ribonucleic acid):	a molecule similar to DNA (qv), which helps in the process of decoding the genetic information carried by DNA.
SAR:	Structure Activity Relationship; eg for carcinogenesis and mutagenesis
Safety:	Practical certainty that injury will not result from a hazard under defined conditions.
Sister chromatid exchange (SCE):	Exchange of genetic material between two sub-units of a replicated chromosome.
Systematic review:	A review that has been prepared using a documented systematic approach to minimising biases and random errors.
TDI:	See 'Tolerable Daily Intake'.

Threshold:	Dose or exposure concentration below which an effect is not expected.
Tolerable Daily Intake (TDI):	An estimate of the amount of contaminant, expressed on a body weight basis (eg mg/kg bodyweight), that can be ingested daily over a lifetime without appreciable health risk.
Toxic Equivalency Factor (TEF):	A measure of relative toxicological potency of a chemical compared to a well characterised reference compound. TEFs can be used to sum the toxicological potency of a mixture of chemicals which are all members of the same chemical class, having common structural, toxicological and biochemical properties. TEF systems have been published for the chlorinated dibenzodioxins, dibenzofurans and dioxin like polychlorinated biphenyls, and for polycyclic aromatic hydrocarbons.
Toxicodynamics:	The process of interaction of chemical substances with target sites and the subsequent reactions leading to adverse effects.
Toxicokinetics:	The description of the fate of chemicals in the body, including a mathematical account of their absorption, distribution, metabolism and excretion. (see pharmacokinetics)
Transgenic:	Genetically modified to contain genetic material from another species (see also genetically modified organism).
Transgenic animal models:	Animals which have extra (exogenous) fragments of DNA incorporated into their genomes. This may include reporter genes to assess <i>in-vivo</i> effects such as mutagenicity in transgenic mice containing a recoverable bacterial gene (<i>lacZ</i> or <i>lac I</i>). Other transgenic animals may have alterations of specific genes believed to be involved in disease processes (eg cancer). For example strains of mice have been bred which carry an inactivated copy of the p53 tumour suppressor gene (<i>qv</i> -), or an activated form of the <i>ras</i> oncogene which may enhance their susceptibility of the mice to certain types of carcinogenic chemicals.

Tumour:

(Synonym - neoplasm): A mass of abnormal, disorganised cells, arising from pre-existing tissue, which are characterised by excessive and uncoordinated proliferation and by abnormal differentiation. **Benign** tumours show a close morphological resemblance to their tissue of origin; grow in a slow expansile fashion; and form circumscribed and (usually) encapsulated masses. They may stop growing and they may regress. Benign tumours do not infiltrate through local tissues and they do not metastasise (qv). They are rarely fatal. **Malignant** tumours (synonym - cancer) resemble their parent tissues less closely and are composed of increasingly abnormal cells in terms of their form and function. Well differentiated examples still retain recognisable features of their tissue of origin but these characteristics are progressively lost in moderately and poorly differentiated malignancies: undifferentiated or anaplastic tumours are composed of cells which resemble no known normal tissue. Most malignant tumours grow rapidly, spread progressively through adjacent tissues and metastasise to distant sites. Tumours are conventionally classified according to the anatomical site of the primary tumour and its microscopical appearance, rather than by cause. Some common examples of nomenclature are as follows:

- Tumours arising from epithelia (qv): *benign* - adenomas, papillomas; *malignant* - adenocarcinomas, papillary carcinomas.
- Tumours arising from connective tissues such as fat, cartilage or bone: *benign* - lipomas, chondromas, osteomas; *malignant* - fibrosarcomas, liposarcomas, chondrosarcomas, osteosarcomas.
- Tumours arising from lymphoid tissues are malignant and are called lymphomas (qv); they are often multifocal. Malignant proliferations of bone marrow cells are called leukaemias.

Benign tumours may evolve to the corresponding malignant tumours; examples involve the adenoma → carcinoma sequence in the large bowel in humans, and the papilloma → carcinoma sequence in mouse skin.

Tumour initiation: A term originally used to describe and explain observations made in laboratory models of multistage carcinogenesis, principally involving repeated applications of chemicals to the skin of mice. Initiation, in such contexts, was the first step whereby small numbers of cells were irreversibly changed, or initiated. Subsequent, separate events (see tumour promotion) resulted in the development of tumours. It is now recognised that these early, irreversible heritable changes in initiated cells were due to genotoxic damage, usually Annual Report 2001 in the form of somatic mutations and the initiators used in these experimental models can be regarded as genotoxic carcinogens (qv).

Tumour progression: The phase in the carcinogenic process when tumours acquire one or both of the pathognomic features of malignant growth- the capacity to invade local tissues and to disseminate to distant sites (metastasis). Progression is difficult to appraise in humans when tumours arising at many sites appear to be malignant without an identifiable preceding benign phase. An important example of progression is, however, provided by the adenoma → carcinoma sequence observed in the large intestine. The mechanisms of tumour progression are obscure, but they appear to include both genotoxic and non-genotoxic events.

Tumour promotion: An increasingly confusing term, originally used, like 'tumour initiation' to describe events in multistage carcinogenesis in experimental animals. In that context, promotion is regarded as the protracted process whereby initiated cells undergo clonal expansion to form overt tumours. The mechanisms of clonal expansion are diverse, but include direct stimulation of cell proliferation, repeated cycles of cell damage and cell regeneration and release of cells from normal growth-controlling mechanisms. Initiating and promoting agents were originally regarded as separate categories, but the distinction between them is becoming increasingly hard to sustain. The various modes of promotion are nongenotoxic, but it is incorrect to conclude that 'non-genotoxic carcinogen' (qv) and 'promoter' are synonymous.

Tumour suppressor gene:	(Synonym anti-oncogene, recessive oncogene). A gene whose continued expression is thought to be essential for normal growth and differentiation of cells. Many tumour suppressor genes probably exist, deletion or suppression of which appears to be a critical event in tumour development.
Ultimate carcinogen:	The reactive (electrophilic) form of a carcinogen which forms adducts with DNA.
Uncertainty factor:	Value used in extrapolation from experimental animals to man (assuming that man may be more sensitive) or from selected individuals to the general population: for example, a value applied to the NOAEL to derive an ADI or TDI. The value depends on the size and type of population to be protected and the quality of the toxicological information available.
Unscheduled DNA Synthesis (UDS):	DNA synthesis that occurs at some stage in the cell cycle other than the S period (the normal or 'scheduled' DNA synthesis period), in response to DNA damage. It is usually associated with DNA repair.

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Mr Khandu N Mistry (Administrative)

Dr Robin J Fielder BSc PhD Dip RCPATH (Scientific) [Until August 2004]

* Lay Members

Contact address:

All correspondence for COC should be through the secretariat initially.

Khandu Mistry
Administrative Secretary
Room 692D
Skipton House
80 London Road
LONDON SE1 6LH

Tel: +44 (0)20 7972 5020

Fax: +44 (0)20 7972 5156

Email: Khandu.Mistry@dh.gsi.gov.uk

Website: <http://www.advisorybodies.doh.gov.uk/coc/index.htm>

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