1997 Annual Report of the Committees on Toxicity Mutagenicity Carcinogenicity of Chemicals in Food, Consumer Products and the Environment

1997 Annual Report of the Committees on

Toxicity
Mutagenicity
Carcinogenicity

of Chemicals in Food, Consumer Products and the Environment

Department of Health

If you require any information please contact the committee's administrative secretary at the following address:

The Department of Health
Room 652C
Skipton House
80 London Road
Elephant and Castle
London SE1 6LH

Visit: *http://www.doh.gov.uk/cot.htm*
http://www.doh.gov.uk/com.htm
http://www.doh.gov.uk/coc.htm

CONTENTS

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

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

ABOUT THE COMMITTEES

This is the seventh joint annual report of the Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (COT), the Committee on Mutagenicity of Chemicals in Food, Consumer Products and the Environment (COM) and the Committee on Carcinogenicity of Chemicals in Food, Consumer Products and the Environment (COC). The aim of these reports is to provide the toxicological background to the Committees' decisions for the concerned professional. Those seeking further information on a particular subject can obtain relevant references from the Committee's administrative secretary.

Members of the COT, COM and COC are appointed by the Chief Medical Officer (CMO). The Committees advise the CMO and, through the CMO, the Government.

Committee members are appointed as independent scientific and medical experts on the basis of their special skills and knowledge. They are appointed for fixed time periods, generally three years, and are eligible for reappointment at the end of their terms. The terms of reference are at Annex 1.

The report also contains the commercial interests of committee members. Members are required to declare any commercial interests on appointment and, again, during meetings if a topic arises in which they have an interest. If a member declares a specific interest in a topic under discussion, he or she may, at the Chairman's discretion, be allowed to take part in the discussion, but they are excluded from decision making. Guidance on this is at Annex 2.

The report contains, at Annex 4, an alphabetical index to subjects and substances considered in previous reports. A second index, at Annex 5, contains details of the subjects on which the COT has given advice since 1987 as part of its consideration of the results of surveillance for chemicals in the UK diet. These considerations are published in the Food Surveillance Papers which report this surveillance work, rather than in the Committee's annual reports.

The usual way in which committee reviews are conducted is that the relevant secretariat critically assesses all the relevant data and prepares papers for the Committee. These normally consist of appendices giving detailed summaries of the studies reviewed -methodology and results - and a covering paper in which the available data are briefly summarised, the most important points highlighted and recommendations presented for discussion by the Committee. Although original study reports are not routinely circulated to members, they are made available on request, and are circulated if the study is particularly complex. Definitive summaries are necessary because documentation on any one chemical can amount to many

hundreds of pages. The Committees cannot undertake to review information provided by industry that has not been forwarded through, or discussed with, the appropriate Secretariat.

Many of the reviews conducted by the Committees are done so at the request of other Government Departments and the Committee secretariats liaise closely with colleagues in these Departments. The Committees offer advice independent of each other in their area of expertise but will, if need be, work closely together. This is helped by the close working relationship of the secretariats. If, for example, during a review of a particular chemical by the COT, it becomes clear that there is need for expert advice on mutagenicity or carcinogenicity aspects, it will be referred to COM or COC as appropriate. These three Committees also provide expert advice to other advisory committees, such as the Advisory Committee on Novel Foods and Processes and the Food Advisory Committee. There are also links with the Veterinary Products Committee and the Advisory Committee on Pesticides.

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

Professor H F Woods (Chairman)
BSc BM BCh DPhil FFPM
FIRST HonFFOM FRCP(Lond & Edin)

PREFACE

The list of topics considered by the Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (COT) shows that the amount of work completed by the Committee has increased and our wide range of expertise has been well employed.

A general feature of our work was the number of topics that were of public interest such as our consideration of Dental Amalgam, Food Intolerance, Peanut Allergy, Polychlorinated Biphenyls and Vitamin B6. These topics raised fundamental problems relating to both the toxicology data base and its application to the population as a whole. In this regard the reader is directed particularly to the section on the COT assessment of Polychlorinated Biphenyls (at Paragraph 1.28) which illustrates the complexities of decision making that are involved in judgements concerning the risks of chemicals in food.

The COT has continued to employ its immunotoxicology expertise through the work of the Food Intolerance Working Group and the Working Group on Peanut Allergy. In the former case the work is underway and in the latter, the work of the Group was completed during 1997 and is due to be published in early 1998. I would like to acknowledge the work of experts from outside the COT membership who have made an invaluable contribution to these deliberations.

In last year's report I mentioned the COT's relationship with other Committees, particularly the Food Advisory Committee (FAC) and the Advisory Committee on Novel Foods and Processes (ACNFP). These working relationships have continued to prosper. One area of work that has expanded is that of the consideration of the toxicology of novel foods and three examples are given in this report. This work has presented special challenges and has led to a careful consideration of the need for special approaches to the assessment of such materials.

In conclusion, the Committee wishes to document its appreciation of the work of its Secretariat and of all other officials from the Department of Health, the Ministry of Agriculture, Fisheries and Food and other Government Departments who have supported and helped the work of the Committee during the year.

FRANK WOODS

Ad Hoc Expert Group on Vitamins and Minerals

1.1 In 1997, the European Commission circulated a discussion document on the possible harmonisation of regulations on the maximum permitted levels of vitamins and minerals in dietary supplements and fortified foods [1].

1.2 In order to inform the United Kingdom consideration of any proposals for harmonisation, officials from the Department of Health and the Ministry of Agriculture, Fisheries and Food have proposed that an ad hoc Expert Working Group should be established to consider the general principles for setting maximum safe levels for vitamins and minerals and, where possible, recommending maximum levels for individual vitamins and minerals. It was proposed that the ad hoc group should consist of Members of the Committee, of the Food Advisory Committee, of the Committee on the Safety of Medicines and of the Committee on Medical Aspects of Food Policy in order to provide a broad range of scientific expertise, with additional specialised expertise being co-opted as necessary.

1.3 The Committee agreed that the proposed Ad Hoc Expert Group should be established.

Bisphenol A

1.4 Bisphenol A is a chemical substance used in the manufacture of polycarbonate plastics used in products such as baby bottles and in the internal coatings of some food cans. The Committee considered the health implications of a study carried out at the University of Missouri to investigate the potential oestrogenic activity of bisphenol A; the Committee issued the following statement:

(i) We have been asked to comment on the health implications of a study which investigated the potential oestrogenic activity of bisphenol A, in its context as a monomer used in polymeric food packaging material. Concern has been expressed about the potential endocrine activity of both naturally occurring and man-made chemicals. We are aware that many research groups worldwide are studying the possible effects of endocrine disruptors and that Government departments and regulatory bodies are considering this issue.

[1] Addition of vitamins and minerals to Foods and Food Supplements. A discussion paper. Prepared by DGIII of the European Commission. III/5934/97

(ii) Bisphenol A is a component of epoxy resins which are used in the manufacture of internal coatings for some food cans and in plastics used in consumer products. We are aware that the Ministry of Agriculture, Fisheries and Food has commissioned research projects to develop robust methods of testing for migration of bisphenol A from can coatings and polycarbonate bottles. The study on polycarbonate feeding bottles for infants has indicated that there was no detectable migration of bisphenol A into the feed or bottle washings at a limit of detection of 0.03 milligram per kilogram (mg/kg). We *welcome* this result, and wish to support the continued research and surveillance in the area of food contact materials.

(iii) We *note* that the European Commission's Scientific Committee for food recommended (in 1986) a Tolerable Daily Intake (TDI) of 0.05 milligram per kilogram body weight per day (mg/kg bw/day) for bisphenol A in the context of its uses in food contact plastics. However, we *note* that the TDI for bisphenol A was set before recent evidence on the potential oestrogenic activity of this substance had been reported in the literature. Therefore, we gave specific consideration to the study by Nagel *et al.* in the context of other recent work on potential oestrogenicity and examined its relevance to other information on the reproductive toxicity of this substance.

(iv) The 1997 study by Nagel *et al.* confirmed that bisphenol A is active at inducing the *in vitro* proliferation of the breast cancer cell line MCF-7 under special conditions. However, it has been shown that the activity of bisphenol A is at least 3 to 4 orders of magnitude lower than that of 17ß-oestradiol. The oestrogen-responsive MCF-7 cell line provides a preliminary screen for identifying potential xenoestrogens but its value for predicting *in vivo* activity is largely not validated.

(v) The *in vivo* activity of bisphenol A was also addressed in the Nagel *et al.* study. The exposure of pregnant mice to very low oral doses of 2 and 20 micrograms (µg)/kg bw/day during gestation days 11 to 17, apparently resulted in a 30 to 35% increase in prostate weight of male offspring at 6 months of age. This study showed some shortcomings which makes the results of limited value for risk assessment. As the authors did not support their findings with histopathological examination of the prostate, the nature of the change is not known and its toxicological relevance is uncertain. A no-effect level for the changes was not established. The mechanism of the effect of bisphenol A on the observed increase in prostate weight is unclear, as is the relative contribution of exposure *in utero* and during suckling. Furthermore, the measurement of prostate weight is not an accepted *in vivo* endpoint for

oestrogenic activity. Because of these limitations of the model we *consider* that it would have been more helpful if the authors had reported on other indications of male reproductive function.

(vi) We also gave consideration to a compilation of toxicity studies, some unpublished, put together by the American Society of Plastics Industry which included reproductive toxicity (fertility), and developmental studies of bisphenol A. We *note* that effects on reproductive parameters in rats and mice were observed, but only at very high doses: these conventional toxicity studies demonstrated no-effect levels of 50 mg/kg bw/day, a value 25000 times higher than the low dose reported to be active in the study of Nagel *et al*..

(vii) In conclusion, with respect to the studies by Nagel *et al.*, we *note* that a mechanism for the potential effect of bisphenol A on the mouse prostate has not been established and its relevance to humans is unknown. At the present, we do not *consider* that it would be justified to draw any conclusions about the health implications of human exposure to bisphenol A based on these results.

Bisphenol A diglycidyl ether

1.5 Bisphenol A diglycidyl ether (BADGE) is used in the production of some plastic food contact materials and in can linings. It may also be used in some food contact plastics as one of the basic building blocks of the plastic. It is also used as an intermediate during the manufacture of the epoxy resins used to line some food cans. Neither of these uses was considered likely to lead to significant migration of BADGE into food unless there was incomplete incorporation of BADGE into the respective polymers. BADGE is also used as a heat stabilising additive in flexible ("Organosol") coatings for "easy open" cans such as may be used for fish. Recent analytical data from laboratories, particularly in Switzerland, suggested that migration could occur from Organosol coatings in cans into food. There is potential for greater migration of BADGE from Organosol coatings because it is not incorporated into the polymer backbone of these coatings.

1.6 The Committee on Mutagenicity had previously advised on the mutagenicity of BADGE in 1986 and updated its advice in 1996, in the context of the use of BADGE for relining water mains as well the use of epoxy resins in food contact materials. During 1997 the Committee considered the results of a recent MAFF survey on the migration of BADGE into canned foods in the United Kingdom and issued the following statement:

(i) We were provided with details of the recent MAFF surveillance work on bisphenol A diglycidyl ether (BADGE) in a range of canned foods. The information was presented in the form of a Food Surveillance Information Sheet.

The Survey

(ii) The survey was conducted to investigate whether BADGE migrated from can coatings into food. BADGE is used in the manufacture of lacquers which coat the inside of some food cans. We understand that the first published survey on BADGE was carried out in Switzerland; this found BADGE in the oil from canned fish products. The UK survey was therefore carried out to confirm the Swiss one, by examining both fish and oil, and to extend the work further by surveying samples of other canned foods that might contain BADGE. However, we understand that, due to the need to avoid hydrolysis of BADGE, the analytical procedure was complex and therefore the UK survey was lengthy.

(iii) The UK survey of retail samples of canned foods found that BADGE was not detected in canned tuna, corned beef, milk and infant formula at a detection limit of 0.02 milligrams per kilogram (mg/kg) of food. The levels of BADGE in 4 of 12 samples of sardines (purchased in 1995) and in 7 of 15 samples of anchovy (purchased in 1996) exceeded the limit of 1 mg/kg set by the European Commission's Scientific Committee for Food (SCF) in June 1996. However, the level of BADGE in all 11 samples of anchovy purchased in 1997 was well below the SCF limit. A similar downward trend in BADGE levels was also noted for sardine samples but further analyses are being done to ensure that the decline is maintained. BADGE was also detected in some samples of canned mackerel, pork luncheon meat, chopped ham and pork, pate and meat spread but the levels did not exceed the SCF's temporary limit.

(iv) Food consumption data indicated that the intake of BADGE from canned food is very low; even for extreme consumers, the estimated intake would not exceed 3 micrograms per kilogram body weight per day (µg/kg bw/day). The results obtained with the sardine samples showed that the greatest proportion of BADGE was likely to be present in the oil phase. Thus, exposure to BADGE is reduced considerably by the usual practice of draining the oil prior to consumption of the can contents. The survey results pointed to a link between can coating types and BADGE levels. There was some evidence that the use of Organosol lacquers led to the highest levels of BADGE. Although we *accept* that the use of BADGE in Organosol coatings confers technological advantages, we wish to *encourage* further work by manufacturers and fillers of

cans to ensure that the levels of BADGE migration in canned fish remain below 1 mg/kg food; if other substances are used in place of BADGE these should be of demonstrable safety at the levels at which they are likely to be found in canned foodstuffs.

SCF Opinion on BADGE

(v) We are aware that the SCF is currently reviewing the safety of BADGE in the context of its uses in food contact materials. In recommending the temporary limit of 1 mg/kg in June 1996, the SCF took into account the available toxicity data on BADGE. Multigeneration reproductive toxicity and teratogenicity studies of BADGE in the rat did not show any adverse effects on reproduction at a dose level of 20 mg/kg bw/day. There was no evidence that BADGE was carcinogenic when applied directly to the skin. However, the compound did have mutagenic properties in *in vitro* studies and, in 1996, the SCF requested further *in vivo* studies. Since then reports of negative *in vivo* assays in bone marrow and liver have been received and the SCF is currently giving further consideration to the toxicology of this substance.

(vi) The SCF has also addressed the issue of possible oestrogenicity of BADGE, which has been tested *in vitro* in human breast cancer cell line MCF-7. We *note* that BADGE did not bind to the oestrogen receptor and *agree* that the small effect on cell proliferation relative to 17ß-oestradiol is questionable. The significance of these *in vitro* data is unclear but we have *noted* that BADGE has been investigated in a comprehensive range of reproductive toxicity studies including a single- and a very recent multi-generation study together with two developmental toxicity studies. The results indicated that BADGE did not have any significant adverse effect on reproduction.

Mutagenicity

(vii) The Committee on Mutagenicity of Chemicals in Food, Consumer Products and the Environment (COM) also advised specifically on the mutagenic potential of BADGE later in 1996. The *in vitro* data indicated that the compound itself was a direct acting mutagen, but when tested in experimental animals in a range of tissues (bone marrow, liver and germ cells) it produced negative results. It is known that BADGE is rapidly detoxified by enzymes in the liver. However, in the absence of an adequately conducted carcinogenicity bioassay using the oral route, the COM recommended that further data were needed to provide reassurance that genotoxic effects did not occur in the

gastro-intestinal tract following ingestion. We *endorse* this recommendation and understand that it has been passed to the SCF to facilitate its further consideration of this compound.

Nutritional Aspects

(viii) The UK Committee on Medical Aspects of Food Policy (COMA) has recommended an increase in the consumption of oily fish to an average of one portion per person per week. We *note* that there is evidence that increasing intake of long-chain n-3 polyunsaturated fatty acids, principally from oily fish, reduces mortality after heart attacks.

Conclusions

(ix) In conclusion, we *consider* that, on the basis of the available information, the levels of BADGE identified in the recent MAFF survey of canned foods are unlikely to pose a risk to health. However, as a matter of prudence, we *recommend* that continued efforts be made by industry to ensure that the level of BADGE in canned fish products remain below 1 mg/kg food. In addition, we *recommend* that further surveillance for BADGE be carried out to ensure that the SCF temporary limit is not exceeded.

Carrageenan

1.7 Carrageenan is the generic term for a group of similar chemicals obtained by aqueous extraction of certain types of red seaweeds. It is used in food as a gelling agent and stabiliser and is particularly useful in milk products.

1.8 Carrageenan had been considered by the Committee as part of the review of a class of food additives termed emulsifiers and stabilisers. In its report of this review, the Committee recommended that consideration should be given to tightening the specification for carrageenan to restrict the proportion of lower molecular weight material (degraded carrageenan) in food-grade material, so as to minimise the absorption of this material from the gut. Carrageenan is well known to have effects on the immune system of laboratory animals if administered parenterally and some studies have shown effects following oral administration. The Committee was concerned that even limited uptake of carrageenan by humans could result in immunological effects and, in particular, that it might enhance the likelihood of allergic-type reactions.

Therefore, it also asked that studies be carried out to investigate further the extent of absorption and bioavailability of orally administered food-grade carrageenan, particularly by the immature gut, and of possible effects on the immune system due to uptake by the gut-associated lymphoid tissues. In the meantime, the Committee altered its classification of carrageenan from fully acceptable to provisionally acceptable, pending the provision of the data requested.

1.9 Following the publication of this advice in 1992, submissions were received from the International Food Additives Council and from the Centre de Liaison des Industries de Traitement des Algues de la CEE (CLITAM) containing data not previously seen by the Committee. The Committee concluded that the submissions did not fully consider the potential for immunological effects of carrageenan acting via the gut-associated lymphoid tissue and still considered it possible that orally administered carrageenan could influence the development of allergic food intolerance. Further work was still, therefore, required. The Committee also recommended that the specification for carrageenan should be altered to introduce a maximum level of 10% for the molecular weight fraction of less than 100,000 daltons.

1.10 In 1997, further submissions were received from CLITAM. These included studies on a potential model for the induction of allergic food intolerance using known allergens but not carrageenan itself. Since it had not been possible to demonstrate the induction of allergy in the experimental system used, CLITAM concluded that it would not be possible, given the present state of scientific knowledge, to provide the data sought by the Committee on carrageenan.

1.11 The Committee concluded that the data provided were insufficient to demonstrate that the proposed model for allergic food intolerance would not be successful and that CLITAM's view that it was not possible to evaluate the potential induction of food intolerance by carrageenan was not supported, particularly since carrageenan itself had not been tested as part of the experimental work conducted. The Committee therefore concluded that their advice given in 1992 and 1993 was still effective.

Chlorobenzenes

1.12 The Committee were asked to consider the consequences for public health of the levels of various chlorobenzenes that had been detected in samples of foodstuffs collected during the 1995 Total Diet Study and also in various retail foods collected during 1995-1996 in the UK.

1.13 The chlorobenzenes are environmental contaminants which can accumulate in fatty foodstuffs. One or more of the following chlorobenzene congeners had been detected at concentrations above the reporting limit in one or more samples of foodstuffs: 1,4-dichlorobenzene, 1,2-dichlorobenzene, 1,2,4-trichlorobenzene, 1,2,3,4-tetrachlorobenzene and 1,2,4,5-tetrachlorobenzene.

1.14 The Committee has not previously recommended any Tolerable Daily Intakes (TDIs) for these chlorobenzenes. TDIs have been recommended by a group convened jointly by the United Nations Environment Programme, the International Labour Organisation, and the World Health Organization. The Committee considered possible intakes of these compounds by members of the UK population and agreed the following statement which was published with the analytical results in Food Surveillance Information Sheet No. 141:

> The COT have considered the results of this survey for chlorobenzenes and have agreed that it is reasonable to use the TDIs published by the World Health Organization in 1991. In addition, the Committee advised that, since estimates of intakes of the chlorobenzenes from the various food products are below the TDIs, the results of the survey do not give any cause for concern regarding human health.

Dental Amalgam

1.15 In 1986, following public concerns about the safety of mercury amalgam used in dental fillings, the Committee was asked to advise the Committee on Dental and Surgical Materials on the toxicity of mercury in dental amalgam. The Committee advised that the use of dental amalgam is free from risk of systemic toxicity and that only a very few cases of hypersensitivity occur.

1.16 Subsequently, the European Commission established an Ad Hoc group of experts to consider dental amalgam in the context of the General Medical Devices Directive (93/42/EEC). This group has reported and the draft report has been circulated by the Commission to Member States for comment. The Committee on Toxicity was asked by the Medical Devices Agency to consider the draft report in order to assist in the formulation of the United Kingdom's response.

1.17 The Committee reviewed the toxicological hazards and risks of the use of dental amalgam and agreed the following statement:

Introduction

(i) The Committee has been asked to advise the Medical Devices Agency on the toxicity of mercury in dental amalgam. This request was made in order to help formulate the United Kingdom's response to the report of an ad hoc group of experts established by the European Commission to consider dental amalgam within the context of the Medical Devices Directive. Particular topics on which the Committee's views were sought included: the risk assessment, the risks of neurotoxicity or nephrotoxicity, the risks of amalgam use during pregnancy or in patients with renal disease, and the adequacy of the toxicological database.

Background

(ii) The Committee last considered the safety of dental amalgam in 1986. At that time we recognised that some mercury may be released from completed restorations but were of the opinion that the use of dental amalgam is free from risk of systemic toxicity and that only a very few cases of hypersensitivity occur.

(iii) Recently there has been a resurgence of interest in the toxicity of mercury in dental amalgam. We have been informed that dental restorative materials, including dental amalgam, are considered to be medical devices under the terms of the Medical Devices Directive 93/42/EEC and have seen the report of the ad hoc group established to review dental amalgam in relation to this directive.

Exposure to dental amalgam and intake of mercury

(iv) We have been informed that the use of dental amalgam in the UK has declined. Whereas the annual placement rate in 1986 was 30 million amalgam restorations per year, the estimate for 1996 in National Health Service patients in England and Wales of 12 to 13 million restorations is considerably lower. The decline is possibly due to a decrease in the incidence of dental decay as well as a reduced usage of amalgam by dentists.

(v) The Committee noted that there were problems in accurately measuring the intake of elemental mercury from amalgam fillings and that other sources of exposure, such as the diet, might result in the absorption of mercury in other chemical forms (e.g. as cations or as organo-mercury compounds). It was

understood that the contribution of dietary intake to mercury exposure was of a similar order as that from amalgam fillings. It was agreed that the placement or removal of such fillings were occasions during which the greatest exposure of individuals to mercury from the amalgam could occur.

(vi) We also noted that when studies of metabolism and excretion of mercury have been carried out these were undertaken most frequently in individuals who were exposed occupationally to mercury. Such exposures might not be relevant to the individual exposed to the trace quantities (estimated as 1 to 5 micrograms (µg) per day, or in a more recent paper as 1 to 2 µg per day) released from dental restorations *in situ*.

Toxicity of mercury to humans

(vii) The Committee considered the toxicity of mercury to the kidney and noted that epidemiological studies of the effects of dental amalgam on renal function have been conducted only in healthy subjects. In these individuals mercury exposure from dental amalgam was not associated with the urinary excretion of *N*-acetyl-ß-D-glucosaminidase, which is an enzyme that is a sensitive indicator of kidney damage. The Committee concluded that, in healthy subjects, exposure to mercury from dental amalgam was not associated with nephrotoxicity. On the basis of the available data it was not possible to draw any conclusions about the effects of mercury from amalgam on persons with pre-existing renal disease.

(viii) The Committee agreed that immunologically-mediated mercury-induced glomerulonephritis (a form of kidney damage) was poorly understood and that studies in occupationally-exposed individuals indicated the existence of a possible dose-response relationship for this effect. This could be an appropriate subject for further research. In our last consideration of dental amalgam we noted the occurrence of a few cases of hypersensitivity but considered that this area did not warrant further study.

(ix) The Committee recognised that neurotoxicity was of potential concern. Both elemental mercury and organo-mercury compounds can contribute to this. The major source of organo-mercury compounds is the diet but the Committee noted that methylation and demethylation of mercury compounds by micro-organisms in the large bowel might occur. Evidence on the balance of these reactions is limited. The Committee accepted that exposure to mercury vapour is of greater concern for dentists and their staff than for patients.

(x) The Committee noted that there was some evidence that mercury could be taken up by the fetus and placenta during pregnancy, however there was a lack of data that would determine whether the mercury was present in an unreactive, metallothionein-bound form. Apart from one study which had been severely criticised, and which was discounted, there was no evidence that occupational exposure to mercury during pregnancy in modern dental practice was harmful. There is no evidence that the placement or removal of amalgam fillings during pregnancy is harmful.

Conclusions and Recommendations

(xi) The Committee welcomes the report and appreciates the opportunity to comment on it. Although the report includes information published since the Committee last reviewed dental amalgam we *consider* that our former conclusions regarding hypersensitivity and the lack of risk of systemic toxicity remain unchanged.

(xii) The Committee *concluded* that nephrotoxicity was not associated with exposure of healthy subjects to mercury amalgam from dental restorations. Also, we *consider* that neurotoxicity caused by exposure to mercury vapour is a matter of more concern in the occupational setting than in dental patients.

(xiii) We *conclude* that there is no available evidence to indicate that the placement or removal of dental amalgam fillings during pregnancy is harmful. We *are of the opinion*, however, that the toxicological and epidemiological data are inadequate to assess fully the likelihood of harm occurring in such circumstances. Until appropriate data are available we *concur* with the view that it may be prudent to avoid, where clinically reasonable, the placement or removal of amalgam fillings during pregnancy.

(xiv) Accordingly, we *consider* that pregnant women and patients with kidney disease are groups who should be included in future studies. We *recommend* that such studies should incorporate measurements of dietary intake of the various chemical forms of mercury. Additionally, we *consider* that studies to elucidate the mechanism of immunologically-mediated mercury-induced glomerulonephritis should also be included in further research. Studies should be done to ascertain the kinetics of mercury in the body at low doses and verify whether the kinetics determined from occupational studies are applicable to patients with dental amalgam restorations.

Food Intolerance

1.18 The Food Intolerance Working Group has met twice during the year to define the details of topics which will be covered in its report. It has advertised in the medical press seeking submissions from individuals or groups who might contribute relevant information. It is expected to complete its work in 1999.

Iodine in Cows' Milk

1.19 The Committee were asked to review iodine concentrations in cows' milk in 1989 and, in conjunction with iodine concentrations in total diet samples, also in 1992. In the latter year the Committee was reassured by surveillance data provided by MAFF which indicated that iodine concentrations in milk appeared not to be increasing. It recommended that monitoring of milk should continue.

1.20 The results of further surveys undertaken for MAFF in 1995/1996 were made available for review by COT during 1997 and the Committee agreed the following statement:

Introduction

(i) Iodine is an important nutrient element and is required for the synthesis of thyroid hormones. An inadequate intake of iodine can lead to a broad spectrum of iodine deficiency diseases. However, the intake of high levels of iodine, as the element, as anionic salts or as organo-iodine compounds, can result in functional impairment of, and tissue damage to, the thyroid gland and fatalities may result from extreme exposure. In infants the potential consequences of an excessively low intake of iodine include impaired brain function and hypothyroidism with an increased susceptibility to the adverse effects of high intakes of iodine in later life. The consequences of a high intake in infancy are those mentioned above, although generally the hypothyroidism is transient.

(ii) For protection against iodine deficiency the Department of Health's Committee on Medical Aspects of Food Policy has recommended a Reference Nutrient Intake (RNI) for adults of 140 micrograms per day (µg/day). The World Health Organization recommends that a mean iodine intake of 200 µg/day meets the requirements of pregnant and lactating women, the population group with the greatest requirement. In order to protect individuals against the toxic effects of iodine the Joint Expert Committee on Food Additives (JECFA) of the Food and

Agriculture Organization and the World Health Organization has recommended a Provisional Maximum Tolerable Daily Intake of 17 micrograms per kilogram body weight per day (µg/kg bw/day); this is applicable to adults and is equivalent to 1000 µg/day in a 60 kg adult.

Dietary iodine intake

(iii) In 1992 we reviewed iodine concentrations in the total diet and noted that the estimated daily mean intake of iodine by adults in the United Kingdom was 190 µg/day. Although this was in excess of the recommended RNI of 140 µg/day we considered that it gave little cause for concern because higher levels of iodine have been ingested with no toxic effects.

(iv) We have previously reviewed iodine concentrations in cows' milk in 1989 and 1992. We *note* the concentrations, representing the country as a whole, measured in the course of a survey undertaken in 1995/1996 for the Ministry of Agriculture, Fisheries and Food. We *note* also that there is no evidence of any difference in iodine levels between whole milk and skimmed or semi-skimmed milk and that the levels are higher in the winter months (a mean concentration of 40 µg/100 g compared with 20 µg/100 g in the summer). The winter and summer levels are higher than those measured in 1990/1991 and 1982 (winter: 40 µg/100 g in 1995/1996 compared with 21 µg/100 g in 1990/1991 and 37 µg/100 g in 1982; summer: 20 µg/100 g in 1995/1996 compared with 9 µg/100 g in 1990/1991 and 7 µg/100 g in 1982). The higher levels in winter may reflect the greater use of supplemented compound feeds at this time as pasture is less available.

(v) There are few data on which to base estimates of the potential intake of iodine by different age groups. Calculations for adults suggest that the average consumer of milk would ingest about 220 µg/day from the total diet. Similarly, the average consumption by children aged 1½-4½ would give an intake of 150 µg/day (11 µg/kg bw/day). High level (97.5th percentile) consumers in this age group might obtain 300 µg/day (23 µg/kg bw/day) from milk in winter and 150 µg/day (11 µg/kg bw/day) from milk in summer. Therefore children in this age group might exceed the JECFA limit by consumption of milk alone during the winter months.

Conclusions and recommendations

(vi) We *consider* that there is a paucity of data which might allow the accurate determination of the daily intake of iodine that can be safely ingested by infants and children over long periods. We *encourage* research to address this point. Nevertheless, we take reassurance from a study in which 1-11 year old children were given doses of iodide up to 1000 µg/day over a period of four months without signs of toxicity. Since this dose corresponds to 59-94 µg/kg bw/day in children aged 1½-4½, over three times the JECFA Provisional Maximum Tolerable Daily Intake, we *consider* that the intake of iodine (23 µg/kg bw/day in winter, 11 µg/kg bw/day in summer) at the concentrations that have been found in cows' milk is unlikely to pose a risk to health.

(vii) We *recommend* that the monitoring of iodine concentrations in cows' milk should continue. In addition, we *encourage* investigation of the bioavailability of iodine in iodophors and of the forms of iodine in infant formulas.

Long Chain Polyunsaturated Fatty Acid for use in Infant Formula

1.21 The COT was asked by the Advisory Committee on Novel Foods and Processes (ACNFP) to advise on the toxicology data submitted on a novel oil for use in infant formula. The submission had been made under the voluntary scheme for assessment of novel foods that existed until May 15, 1997. The oil contains high levels of the long chain polyunsaturated fatty acid arachidonic acid. The toxicology data consisted of a 13-week study and a neo- and post-natal study in rats. The oil had also been tested in an Ames test and in an *in vitro* cytogenetics assay and was found to be non-mutagenic.

1.22 In the 13-week study a number of changes were observed in the liver. These included an increase in liver weight and an increased incidence of centrilobular hypertrophy and periportal vacuolation. Serum levels of the enzymes alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase were also increased. Such increases may be indicative of liver cell damage. The effects in the liver were more apparent in male rats than in females and were present at the mid (1.7 g/kg bw) and high (5 g/kg bw) dose levels. The liver changes were still evident in male rats at the end of a 5-week recovery period. Histopathological examination of livers from pups in the neo- and post-natal study revealed no adverse effects. However, liver weight was not measured in this study. The Committee recommended that the cause of the liver effects be investigated further in order to determine whether they were due to cytochrome P450 enzyme induction or to peroxisome proliferation.

1.23 A further 2-week study was conducted in rats and parameters indicative of enzyme induction (levels of the enzymes CYP 1A1, 4A1 & 3A2) and peroxisome proliferation (acyl CoA and enoyl CoA hydratase activity) were assessed. However, there was no evidence of an increase in liver weights nor of centrilobular hypertrophy in the new study. Similarly, cytochrome P450 enzyme levels were not increased. A small (1.7-fold) but statistically significant increase in the activity of the enzyme acyl CoA oxidase was observed at the top dose level of 5 g/kg bw. However, examination of liver sections by electron microscopy did not reveal any evidence of peroxisome proliferation.

1.24 The COT advised that the new study had not provided any additional information on the cause of the liver hypertrophy observed in the 13-week rat study. The cause of these effects still needed to be explained. This advice was passed to the ACNFP to be taken into account in any final evaluation of this material.

Ochratoxin A

1.25 Ochratoxin A is a naturally occurring toxicant produced by various species of moulds. It has been associated with kidney lesions identified in pigs at slaughter and subsequently has been found in foodstuffs other than meats. When it was last considered by the COT the Committee were advised by the COC and COM that it is possibly a carcinogen in humans and, accordingly, advised that it would be prudent to reduce the level of ochratoxin A contamination of foodstuffs to the lowest level that is technologically achievable. At that time the Committee expressed a wish that it should be kept informed of future surveillance work for ochractoxin A.

1.26 A survey of ochratoxin A concentrations in foodstuffs purchased in the UK in 1997 was carried out by MAFF and the COT were asked to provide advice on the results. The Committee produced the following report:

Introduction

(i) The Committee has been asked to advise the Joint Food Safety and Standards Group of the Department of Health and the Ministry of Agriculture, Fisheries and Food on the significance for public health of concentrations of the mycotoxin, ochratoxin A, found in samples of various foodstuffs purchased in the UK during 1997. The Committee last considered ochratoxin A in 1992 in the context of its presence in samples of cereals and of porcine products and noted that it could be found in both UK-produced and imported foodstuffs.

Background

(ii) Ochratoxin A is a naturally-occurring mycotoxin made by some fungal species of the *Aspergillus* or *Penicillium* genera. The formation of ochratoxin A is affected by moisture and temperature; storage in damp conditions favours production of ochratoxin A. It has been found in a wide variety of agricultural crops including cereals and coffee beans and, because of its presence in animal feeds, as residues in eggs or the tissues of non-ruminant animals, e.g. pigs.

(iii) When the Committee considered ochratoxin A in 1992 we noted that, in several animal species, it was toxic to the kidneys and that it could adversely affect the developing foetus. It can also affect the immune system. At that time we were advised by the Committee on Carcinogenicity (COC) and the Committee on Mutagenicity (COM) of Chemicals in Food, Consumer Products and the Environment that ochratoxin A is a genotoxic carcinogen. Ochratoxin A has induced renal adenocarcinomas in both mice and rats at doses which are associated with nephrotoxicity; also, it has induced hepatocellular adenocarcinomas in both male and female mice. The COM has concluded that ochratoxin A should be regarded as an *in vivo* mutagen.

(iv) Ochratoxin A has been detected in the blood and foodstuffs of individuals living in areas in which a human kidney disease (Balkan Endemic Nephropathy) and associated tumours of the upper urinary tract are endemic. The numbers of samples of blood or food which contained ochratoxin A were generally greater in the endemic areas than in non-endemic areas. In 1991 the COC concluded that no definite causal link had been established between ochratoxin A exposure and Balkan endemic nephropathy and urothelial cancer. The International Agency for Research on Cancer has classified ochratoxin A as possibly carcinogenic to humans (Group 2B).

(v) The Committee is aware that the Joint Expert Committee on Food Additives (JECFA) of the World Health Organization and the Food and Agriculture Organization has recommended that a Provisional Tolerable Weekly Intake (PTWI) of ochratoxin A is 100 nanograms per kilogram body weight (ng/kg bw), approximating to 14 ng/kg bw per day or 1 microgram (µg) daily for a person of 70 kg body weight. This value derives from a short term study in a small number of pigs in which enzyme activities in the kidney and other measures of kidney function were affected.

(vi) We have been informed that ochratoxin A will shortly be considered by the European Commission's Scientific Committee on Food (SCF). However, in the absence of a recommendation from the SCF for an acceptable daily intake, we propose to use a daily equivalent of the JECFA PTWI for ochratoxin A in our consideration of the concentrations found in the recent survey of foodstuffs in the UK. This provides a very large safety margin (greater than one million) below the doses causing kidney damage and tumours in rodents.

The 1997 Survey

(vii) We have seen the analytical data from the 1997 survey and note that, although most of the foodstuffs have ochratoxin A concentrations of less than 0.2 µg/kg, there are some samples with concentrations more than 50-fold higher (≥10 µg/kg). These include one of seventeen samples of red kidney beans, one of twelve samples of butter beans, one of twenty samples of raisins, four of twenty samples of sultanas and five of twenty samples of currants. These high concentrations are a matter of concern, particularly since they appear to occur more commonly in samples of dried vine fruits. These foodstuffs had not been included in previous surveys.

(viii) Estimates of the consumption of different foodstuffs are available from dietary surveys of various age groups that have been carried out in the UK. We have been informed of calculations which used these estimates together with the maximum concentrations of ochratoxin A measured in currants, raisins and sultanas to determine the possible intakes of ochratoxin A by consumers of dried vine fruits. We note that the average consumer of dried vine fruits is unlikely to exceed a daily intake of ochratoxin A of 14 ng/kg bw. However, it is a matter of some concern that in infants or, particularly, pre-school children (age 1½-4½) high level or 97.5th percentile consumers of dried vine fruits contaminated at the higher concentrations measured in the survey might exceed a daily intake of ochratoxin A of 14 ng/kg bw.

Conclusion and Recommendations

(ix) We *conclude* that, in view of the very large safety margin reported above, it is most unlikely that consumers of the dried vine fruits that were found to be contaminated with ochratoxin A in the recent survey will have suffered any adverse health effects as a result of this contamination. However, in view of our concerns as to the toxicity of ochratoxin A in humans, we reiterate our

previous opinion and *recommend* that it would be prudent to reduce the level of ochratoxin A contamination of UK foodstuffs to the lowest level that is technologically achievable. We also *recommend* that further surveillance should be undertaken as a matter of priority in order to confirm that reductions in the levels of ochratoxin A in dried vine fruits are achieved.

Peanut Allergy

1.27 The Working Group of the Committee considering whether there is an association between early exposure to peanuts and peanut products and the incidence of peanut allergy later in life met four times in 1997 and prepared a report which was reviewed by the main Committee at its December meeting.

Polychlorinated Biphenyls

1.28 During 1997 the Committee completed its assessment of the health hazards of the polychlorinated biphenyls (PCBs). This has been a lengthy and complicated process because of the number of isomers of these compounds that exist and also because different isomers may cause toxicity by different mechanisms and with different endpoints.

1.29 As a part of the review the Committee considered whether it would be possible to set Tolerable Daily Intakes for the mixtures of isomers that have, in the past, had commercial applications. Differences between the mix of isomers that exist in the commercial preparations and in foodstuffs led the COT to conclude that this would not be a valuable procedure.

1.30 Since many of the isomers exert their toxicity by a mechanism that is similar to that by which the polychlorinated dibenzo-*p*-dioxins (PCDDs) act, the COT also considered whether it would be feasible to use the concept of toxic equivalents that has been used with the PCDDs to assess the hazards of mixtures of isomers. Although it had some reservations, the COT concluded that, as a pragmatic approach, this can be done.

1.31 The Committee were asked to comment on the results of surveillance exercises which had measured the concentrations of PCBs and PCDDs in foods, fish oils and human breast milk. It used the procedures which it had endorsed to comment on the risks to human health of exposure to the concentrations found in the survey. An abbreviated version of the full statement produced by the Committee is given below:

Introduction

Background

(i) Polychlorinated biphenyls (PCBs) are a group of 209 chlorinated hydrocarbons which were widely manufactured from the 1930s to the 1970s for a range of industrial applications. They are environmentally stable, lipophilic chemicals which accumulate in biological systems. The recognition that PCBs have a substantial environmental impact has led to severe restrictions on their use and to international commitments to phase out the use of PCBs and of electrical equipment containing them. Nevertheless, PCBS may still be released to the environment, mainly during the disposal of transformers and capacitors, and, because of the properties of these chemicals, they are likely to persist as contaminants of the environment and of food for many years to come.

(ii) We last considered PCBs in 1983, when we recommended that the PCB content of the average national diet was unlikely to constitute a health hazard. Since then, a considerable amount of new toxicity data has become available and developments in the analysis of PCBs have produced more specific information on the levels of individual PCB congeners in food. Therefore, we have updated our review of the human health hazards of PCBs and our conclusions are given in this statement. We have also been asked to advise on the health significance of current intakes of PCBs from the diet and from fish oil dietary supplements by the UK population and on intakes of PCBs by breast-fed babies in the UK. This advice is given below.

General Observations

(iii) There is a large scientific literature on the toxicity of PCBs which reports the results of conventional animal toxicology studies and of mechanistic studies to investigate further the action of PCBs on specific organ systems or toxicological endpoints. There are also many reports documenting effects in humans following high level exposure to PCBs, either in an occupational setting or following accidental exposures of the general population, such as in the Yusho and Yu-Cheng[2] contaminated rice oil incidents in Japan and Taiwan, respectively. A few studies have been published on populations with background environmental or food exposure to PCBs. This information was

[2] The Yusho and Yu-cheng incidents involved the contamination of edible oils with polychlorinated biphenyls and resulted in human toxicity, including chloracne a skin condition assoicated with exposure to PCDD-like compunds.

presented to us largely in the form of detailed review papers prepared by our Secretariat, summarising the data from approximately 700 references. The quantity of information is such that it is not appropriate to make a detailed summary of it in this statement but we *encourage* the Secretariat to publish the detailed reviews prepared for our consideration in an appropriate scientific journal. In view of the large number of references used, in this statement we have only referenced those papers which are specifically referred to or which are key to our risk assessment of PCBs.

(iv) There are 209 individual PCB congeners. The chemical structures of PCBs are given in Figure 1 [not included here], together with those of the structurally similar environmental contaminants, polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs). From a toxicological viewpoint, it is useful to subdivide PCBs into two categories: di-*ortho*-substituted PCBs, which are mostly non-coplanar, and non-*ortho*-substituted and other PCBs, which exist in a coplanar configuration. PCBs were sold and used commercially as mixtures of congeners which varied in their composition according to the manufacturer and the intended use. The mixtures were marketed under trade names such as Aroclor, Clophen or Kanechlor. The numbering system used to describe these commercial products usually includes a description of the percentage of chlorine present in the products eg Aroclor 1260 and Clophen A60 contain approximately 60% chlorine which is present in a range of different congeners. Until recently, most toxicological studies were carried out on these commercial mixtures. The individual congeners and the relative proportion of these congeners in the commercial mixtures are different from those found in food, because of differential uptake and metabolism of individual congeners along the food chain. Also, many of the commercial mixtures of PCBs used in these studies were contaminated with low levels of other pollutants, such as PCDFs, which may have confounded the results. A large number of studies are available investigating aspects of the toxicity of individual congeners but few of these are standard toxicology studies which can be used in risk assessment. All these factors have made the assessment of the human health hazards of PCBs a lengthy and difficult task.

Toxic Equivalency Factors

(v) It is believed that PCDDs and PCDFs exert most of their toxicological effects by a common mechanism involving binding to an intracellular protein called the Ah (aryl hydrocarbon) receptor. This has enabled the development of "Toxic Equivalency Factors (TEFs)" for individual PCDD and PCDF

congeners which have been developed and accepted on an international basis. This approach uses the available biological data and knowledge of the structural similarities among the PCDDs and PCDFs to generate a set of weighting factors for each congener, which expresses the potency of the congener as a proportion of that of the best studied PCDD congener, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). Multiplication of the concentration of PCDD or PCDF congener by its TEF gives a TCDD Equivalent or Toxic Equivalent (TEQ). The toxicity of any mixture of PCDDs and PCDFs, relative to TCDD, is taken to be the sum of the individual TEQs. Where humans are exposed to that mixture, the risk to health can be assessed by reference to the Tolerable Daily Intake (TDI) of 10 pg/kg bw/day for TCDD. It should be noted that the TEQ approach is a simplistic approach, in that it assumes that there are no toxicological interactions between the individual PCDD and PCDF congeners; synergistic interactions would increase the toxicity whereas antagonistic interactions would reduce the toxicity of the mixture compared with that calculated using TEQs.

(vi) The toxicological data for some individual PCB congeners (notably certain non-*ortho* coplanar, mono-*ortho* coplanar and di-*ortho* coplanar congeners) indicate that the structure-activity relationships for a number of effects are consistent with an Ah-receptor mediated mechanism (ie "dioxin-like" activity). Therefore, as part of our review, we considered whether it would be appropriate to assign TEFs to these congeners and to assess the safety of a mixture of PCBs, PCDDs and PCDFs by reference to the TDI for TCDD.

Toxicity Assessment

Toxicokinetics

(vii) PCBs are well absorbed from the gastrointestinal tract. Some congeners may undergo metabolism in the liver to hydroxychlorobiphenyls and subsequent elimination via the faeces. There are wide differences in the rates and extent of metabolism and elimination among individual congeners. Thus there is a large range of elimination half-lives, for example, in rats, a range from 0.15 days for PCB 4 to 460 days for PCB 153 (2,2',4,4',5,5'-hexachlorinated biphenyl). PCB congeners which are not readily metabolised accumulate in lipid-rich tissues, predominantly adipose tissue. PCBs can cross the placenta. They can also be eliminated in milk, thus reducing the adipose tissue stores. Because PCBs are both stable and highly fat-soluble, they accumulate in the food chain.

Toxic Effects of PCBs

(viii) A number of toxic effects of PCB mixtures and individual PCB congeners have been documented in laboratory animals and the major effects are described briefly here.

General Toxicity

(ix) At high doses, PCBs cause overt, non-specific toxic effects in laboratory animals such as retardation of weight gain and poor clinical condition, with more selective effects at low doses. More specific effects include skin lesions (chloracne and/or lesions of the fingernails and gums); liver toxicity; and impairment of the function of both the humoral and cell-mediated immune systems. Effects have also been reported on the neurochemistry of the central nervous system in that treatment of animals with PCBs can cause a reduction in the concentrations of dopamine and its metabolites in a number of brain regions.

(x) PCBs also have effects on the endocrine system such as reducing serum concentrations of the thyroid hormones thyroxine and triiodothyronine. The effect on thyroid hormones has been associated with induction by PCBs of the hepatic enzyme thyroxine-uridine diphosphate-glucuronyltransferase (UDPGT). We note the results of a recent human study on 105 mother-infant pairs which reports a correlation between PCDD, PCDF and PCB levels in human milk, expressed as TEQs, and lower thyroid hormone levels in maternal serum, and with higher plasma levels of thyroid stimulating hormone (TSH) in the infants. Although we have reservations about the methods used to estimate exposure in this study, these findings indicate a need for further work in a larger population to define better whether current human body burdens of PCDDs, PCDFs and PCBs are affecting thyroid hormone levels.

Reproductive Toxicity

(xi) Studies in a number of different animal species indicate that PCBs can affect reproduction and development. Among the effects seen are prolonged oestrus cycles in rats and mice and menstrual cycles in monkeys, impaired fetal or postnatal survival, and reduced birth weight in the offspring of PCB-treated animals. Some individual congeners are teratogenic in mice, producing a similar pattern of malformations to that documented in mice exposed to

TCDD. Increased testis weight and daily sperm production have been reported in male rats receiving commercial PCB mixtures by injection in the early postnatal period although studies in older rats using dietary administration have not reported an effect on the testis. Effects on postnatal neurobehavioural development have been seen in rodents and in monkeys, although the interpretation of the findings in monkeys is complicated by the fact that the offspring and/or their mothers were exhibiting signs of severe PCB intoxication prior to testing for neurobehavioural performance.

(xii) Because of the importance of effects on reproduction in the animal toxicity profile of PCBs, we convened a sub-group with additional, co-opted expertise in epidemiology and psychology to examine the available data on PCB exposure and reproduction and postnatal effects in humans. The sub-group concluded that accidental exposure to high levels of PCBs, as occurred during the Yu-Cheng and Yusho rice oil contamination incidents, were probably associated with adverse effects on reproduction and postnatal development in humans. The available epidemiological studies of individuals exposed to high background dietary levels of PCBs have been inadequately conducted. There was no consistency in the effects on birth weight and gestational age reported in these latter studies which would suggest a link with exposure to PCBs. It is not possible to reach any conclusions regarding reported effects on reflexes and muscle tonicity in neonates as the magnitude of the reported effects was small and there are reservations about the methods used to assess these endpoints. Some of the epidemiological studies have documented effects on cognitive performance in infants and children but it is not possible, in view of the inadequacy of the published studies, to associate any of the findings with background dietary exposure to PCBs. In summary, the sub-group's conclusion was that the available exposure and epidemiological data are not of sufficient quality to enable a quantitative estimate of risk of reproductive dysfunction following dietary exposure to be made. We *endorse* the conclusions of this subgroup. We have reviewed the report of a recent follow-up study on a group of children born to mothers with high background dietary exposures to PCBs, which concludes that *in utero* exposure to PCBs in concentrations slightly higher than those in the general population can have a long-term impact on intellectual function. We have concerns about the methodology used in this study and the lack of data on confounding factors and, because of these uncertainties, consider that the report is not sufficient to alter the sub-group's conclusions.

Mutagenicity and Carcinogenicity

(xiii) We sought advice from our sister committees, the Committee on Mutagenicity (COM) and the Committee on Carcinogenicity (COC), on these aspects of the toxicity of PCBs. The COM has advised that most of the available data on the mutagenicity of PCBs relates to commercial preparations, principally Aroclor 1254, but also Aroclor 1242 and Kanechlor 500. These products consist of mixtures of a large number of compounds, and trace amounts of other chemical contaminants, such as PCDFs. Very limited data are available on specific PCBs. It concluded that commercial PCBs can be regarded as essentially 'non-genotoxic' and that any carcinogenesis in animal studies is likely to be produced by 'non-genotoxic' mechanisms. On specific PCB congeners, the COM advised that there is limited evidence that PCB 77 (3,3',4,4'-tetrachlorinated biphenyl) and mixtures of PCB 77 and PCB 52 (2,2'5,5'-tetrachlorinated biphenyl), but not the pure form of PCB 52, have clastogenic potential in an *in vitro* assay using human lymphocytes. There are, however, no adequate *in vivo* data on specific congeners and the absence of activity with the commercial PCBs in this regard would suggest that these congeners do not have significant mutagenic potential.

(xiv) The COC noted that there was some evidence that PCBs acted as tumour promoters by a variety of mechanisms, possibly involving the Ah receptor or by stimulating cell proliferation and enzyme induction. A number of commercial PCBs have been shown to be carcinogenic in rats. Aroclors 1254 and 1260 and Clophen A60 induce hepatocellular carcinomas. Limited studies in mice indicate that Aroclor 1254 and Kanechlors 400 and 500 induce liver tumours in this species. The lowest effect level reported in these studies is 50 ppm (parts per million) in the diet (approximately 2.5 milligrams per kilogram bodyweight per day (mg/kg bw/day) given for up to 2 years. Although the data suggest that the less extensively chlorinated PCBs may be of lower carcinogenic potency, the COC has advised that it cannot be concluded that it is only the highly chlorinated PCBs that give rise to concern. Individual commercial PCB mixtures need to be considered on a case by case basis when estimating carcinogenic potency.

(xv) The COC also advised that there are few data available on the carcinogenicity of PCBs in humans. In all the available studies the numbers involved were small and the results very difficult to interpret. Investigations on patients who consumed PCB-contaminated rice oil suggest that there was an increased incidence of liver cancer. Mortality studies in workers exposed to PCBs at capacitor manufacturing plants in the USA also suggest an increase in mortality

from liver cancer. A report from a manufacturing site in Italy was uninterpretable because of the use of unrepresentative controls and lack of exposure data. The COC concluded that because of the inadequacies in these data it is not possible to reach a definite conclusion from the epidemiological data.

(xvi) Several groups have investigated whether commercial PCBs (Aroclor 1254 and Kanechlor 500) have the potential to produce promoting effects in 2 stage *in vivo* models of chemically induced hepatocarcinogenesis in rats or mice. In all cases evidence of promoter activity was obtained.

(xvii) Overall, the COC concluded that it would be prudent to assume that all PCB congeners are potential human carcinogens.

Discussion

(xviii) A number of toxic effects of PCB mixtures and individual PCB congeners have been documented in laboratory animals. With the exception of effects on brain neurochemistry and certain effects on reproduction, the spectrum of toxicological effects seen with commercial PCB mixtures is similar to those previously reported for 2,3,7,8-TCDD and the structure-activity relationships for a number of effects are consistent with an Ah-receptor mediated mechanism (ie "dioxin-like" activity). There is evidence that effects on postnatal neurobehavioural development may be mediated through another, hormonal, mechanism but there are insufficient data available to specify the critical hormonal pathways which are affected.

(xix) After reviewing the animal toxicity data on commercial PCB mixtures, we *conclude* that the most critical effects, ie those seen at the lowest level of exposure, are those on the skin, the immune system, reproduction and postnatal behavioural development. It was not possible to derive No Adverse Effect Levels (NOAELs) for these effects from the available data but it is possible to derive Lowest Observed Adverse Effect Levels (LOAELs). A LOAEL of 5 micrograms (µg)/kg bw/day Aroclor 1254 has been identified for effects on the skin and on the immune system. The LOAEL for effects of commercial PCBs on reproduction is considered to lie in the range from 5 to 30 µg/kg bw/day, derived from studies in the monkey. There are problems in comparing the effects of PCBs on postnatal neurobehavioural development observed in different species of laboratory animals. However, the data indicate that the monkey is the most sensitive species and the LOAEL for this effect is

8 µg/kg bw/day based on a single generation study in the rhesus monkey using Aroclor 1016. These LOAELs have been derived after consideration of all the available toxicological data on commercial PCB mixtures.

(xx) We have considered whether to set a Tolerable Daily Intake (TDI) for total PCBs by using the LOAELs for commercial PCB mixtures and application of appropriate safety factors. However, the pattern of PCB congeners present in foodstuffs does not accord with that present in commercial mixtures. We *conclude* that a TDI derived from studies on a commercial mixture of PCBs might be of value in evaluating the significance of occupational exposure to PCB mixtures, but not in evaluating the significance of dietary exposures to PCBs. Therefore, we have not set a TDI for total PCBs. We discuss below, in the context of advising on the health risk of current dietary exposures to PCBs, how the toxicological data on the commercial mixtures can be used to assess the significance of PCB contamination of food to human health for those congeners which are not "dioxin-like".

(xxi) We also considered whether to adopt the TEF approach to estimate the risk of exposure to PCBs. We have reservations about this approach for a number of reasons. As stated above, not all toxicological endpoints of PCBs are mediated by an Ah-receptor mechanism. Many of the TEFs which have been derived for individual PCB congeners are based on limited data. Also, it should be borne in mind that most of the congeners found in food are not "dioxin-like". Interactions may exist between the "dioxin-like" and non-"dioxin-like" PCBs in a mixture which would affect a risk assessment based on strict additivity of TEFs. Nevertheless, the use of TEFs offers a pragmatic approach to the evaluation of "dioxin-like" PCBs and we *conclude* that they can be tentatively accepted and used in a limited manner for this purpose. The TEFs recommended by the WHO-ECEH/IPCS task force in 1994 represent a consensus of current expert opinion and we *recommend* that these are used at present (see Table 1 [not included here]). The TDI of 10 pg TEQ/kg bw/day can therefore be used to assess the health risks of the intake of combinations of PCDDs, PCDFs and dioxin-like PCB congeners.

Health risk of current dietary exposures to PCBs

Adults

(xxii) The recent data provided for our consideration are derived from a survey by the Ministry of Agriculture, Fisheries and Food (MAFF) of PCBs in UK Total Diet Study (TDS) samples collected in 1982 and 1992. A total of 53 individual

congeners were selected for analysis, after consultation with us, on the basis of their biological activity or distribution in food and other media.

(xxiii) We *welcome* the large decline in the estimated average UK dietary intake of PCBs from 1.0 µg/person/day in 1982 to 0.34 µg/person/day in 1992. We note that estimated high level (97.5 percentile) intakes have also decreased considerably, from 1.9 µg/person/day in 1982 to 0.6 µg/person/day in 1992.

(xxiv) The 1992 figures are equivalent to an average intake of PCBs of 0.006 µg/kg bw/day and a high level intake of 0.01 µg/kg bw/day for a 60 kg individual. These are approximately 900 times and 500 times, respectively, below the LOAELs for effects of commercial PCB mixtures on the skin, on the immune system and on reproduction in animal studies; and over 100,000 times lower than the lowest effect level for liver carcinogenicity in rodents. Although, as stated above, the mixtures of congeners in food and those tested in toxicity studies are not the same, these large margins are reassuring.

(xxv) The upper bound average dietary intakes of the individual congeners which contribute most to the overall intakes of PCBs in 1992 are given in Table 2 [not included here]. For those for which toxicity data are available, a comparison is made with the LOAEL identified from toxicity studies. For the two congeners for which data exist, the margins between intake and LOAEL are substantial.

(xxvi) Another comparison which can be made is between the lowest LOAEL identified in our review for an individual non-coplanar congener and the 1992 intake figures for PCBs. A LOAEL of 2.6 µg/kg bw/day was estimated for depletion of dopamine levels in rat brain in a 13-week study on PCB 128 (2,2',3,3',4,4'-hexachlorobiphenyl). This is over 400 times and 200 times, respectively, above the average and high level intakes of total PCBs in 1992. Again, these are reassuringly high margins.

(xxvii) We have been given an estimate of the contribution of the 13 "dioxin-like" PCB congeners to the total intake of TEQs from the 1992 UK TDS samples. The WHO-ECEH/IPCS TEFs were used to calculate the PCB TEQs. Average intake was 143 picograms (pg) TEQ/person/day, of which 50 pg TEQ/person/day came from PCBs. High level intake was 252 TEQ pg/person/day, of which 90 pg TEQ/person/day came from PCBs. These are equivalent to 2.4 and 4.2 pg TEQ/kg bw/day, respectively, for a 60 kg adult. Both figures are below the TDI for PCDDs and PCDFs of 10 pg/kg bw/day, which we set in 1992 and confirmed in 1995.

Schoolchildren (10-15 years)

(xxviii) We have also been provided by MAFF with estimates of the intakes of PCBs, and of the "dioxin-like" PCBs, PCDDs and PCDFs, expressed as TEQs, by schoolchildren. The estimates are based on the 1992 TDS data, combined with food consumption data from a national survey of 10-15 year olds. The upper bound average PCB intake was estimated to be 0.007 µg/kg bw/day and the high level intake was 0.012 µg/kg bw/day (Table 3 [not included here]). In terms of TEQs, the average intake was estimated to be 2.8 pg TEQ/kg bw/day, of which 0.9 pg TEQ/kg bw/day came from PCBs; and the high level intake was estimated to be 4.6 pg TEQ/kg bw/day, of which 1.5 pg TEQ/kg bw/day came from PCBs (Table 4 [not included here]). These figures are only slightly higher than adult intakes.

Toddlers (1½-4½ years)

(xxix) MAFF has also provided us with provisional estimates of the intakes of PCBs, and of the "dioxin-like" PCBs, PCDDs and PCDFs, expressed as TEQs, by toddlers (ie children aged between 1½ and 4½). This was derived by calculating the average PCB content of the UK diet, in terms of the sum of the 53 congeners measured, from the mean energy and PCB intakes of adult consumers (1992 TDS data). Multiplying this PCB content by the average daily energy intakes on a bodyweight basis recorded in a recent dietary and nutritional study of toddlers gave the estimated PCB intakes by toddlers given in Table 3 [not included here].

(xxx) Taking the highest intakes by toddlers (ie those estimated for 1½ to 2½ year olds), the intakes on a body weight basis are approximately twice those of adults. Average intakes are approximately 360 times below the LOAELs for PCBs in animal studies and 97.5 percentile intakes are approximately 230 times below these LOAELs. Although these margins are not as high as for adults, they do not indicate any major cause for concern.

(xxxi) In terms of total TEQs, average intakes ranged from 5.9 pg TEQ/kg bw/day for 1½ to 2½ year olds to 5.0 pg TEQ/kg bw/day for 3½ to 4½ year old girls (see Table 4 [not included here]). 97.5th percentile intakes ranged from 9.3 pg TEQ/kg bw/day to 7.6 pg TEQ/kg bw/day. These intakes are below the TDI of 10 pg/kg bw/day.

Human Milk

(xxxii) As stated above, PCBs accumulate in lipid-rich secretions such as milk and thus human milk is a major source of exposure to these chemicals. Recent UK data indicate that the mean estimated intake of PCBs by breast-fed babies is 930 nanograms (ng)/kg bw/day at 2 months, falling to 460 ng/kg bw/day at 6 months. It should be noted that these are worst-case estimates for two reasons. Firstly, the milk samples analysed in this study were collected from mothers no more than two months after delivery. A mother's body burden of PCBs and other fat-soluble contaminants will decrease during the several months of breast-feeding and, consequently, the levels in her milk will decrease. Secondly, all samples were obtained from mothers nursing their first child. Since breast-feeding reduces the maternal body burden, levels of contaminants in milk fed to subsequent children are expected to be lower.

(xxxiii) Comparison of these worst-case estimated intakes with the LOAELs for non-carcinogenic effects of commercial PCB mixtures shows that the safety margins are low - approximately 5 at 2 months and 25 at 10 months. In comparison, the margin between these intakes and the LOAEL for carcinogenic effects is 2700 at 2 months and over 10,000 at 10 months. As with other dietary exposures, the mixture of PCB congeners present in human milk is different from that found in commercial mixtures.

(xxxiv) Comparison of the estimated mean intake of certain individual PCB congeners by infants aged 2 months with the relevant NOAEL or LOAEL identified from toxicity studies are given in Table 5 [not included here]. These margins range from 1,700 to 800,000, which is reassuring.

(xxxv) We have also been provided with mean estimated intakes by breast-fed infants of the 13 "dioxin-like" PCB congeners, expressed as TEQs. The TEQs were also calculated using the WHO-ECEH/IPCS TEFs. Estimated intakes range from 58 pg TEQ/kg bw/day at 2 months of age to 13 pg TEQ/kg bw/day at 10 months of age. The mean total intake of PCDDs, PCDFs and dioxin-like PCBs is estimated to be 170 pg TEQ/kg bw/day at 2 months, falling to 39 pg TEQ/kg bw/day at 10 months. These intakes exceed the TDI of 10 pg TEQ/kg bw/day.

(xxxvi) It is clearly a matter of some concern that the margin of safety between the LOAELs for commercial PCB mixtures in animals and intakes of PCBs by breast-fed babies is so small; and that intakes of TEQs exceed the TDI, in some cases substantially. We have commented previously on the health significance of PCDDs and PCDFs in human milk and have advised that, despite the presence of these chemicals in milk, breast-feeding should continue to be

encouraged. In formulating this advice we took into account the fact that infants may be more susceptible to these compounds, and that a high level of exposure during a critical period of early postnatal development may have adverse effects whereas exposure to the same level in later life would not. However, we also took into account the fact that the period of breast-feeding is short compared with the time needed to accumulate these compounds in the body. We took into account the known benefits of breast-feeding: that it confers immunological protection on the infant, that it is less likely to cause gastrointestinal disturbances, and that breast-feeding protects against the development of allergies in families where there is a history of allergies. Studies have shown that there is a cognitive advantage for breast-fed babies over formula-fed babies, even after adjustment for socio-biological confounding. After consideration of all these factors, we *reaffirm* our previous advice that, despite the presence of PCDDs and related compounds in human milk, breast-feeding should continue to be encouraged on the basis of convincing evidence of the benefits of human milk to the overall health and development of the infant. Nevertheless, we *recommend* that the PCB, PCDD and PCDF levels in human milk are surveyed at regular intervals and, if levels do not continue to fall, that a review is instigated to investigate whether inputs of these pollutants to the environment can be reduced further, so as to reduce human exposure. It would be desirable to investigate the potential for accumulation of those PCB, PCDD and PCDF congeners which occur in breast milk, that is their disposition in and elimination from the body, especially in early life.

Survey of fish and fish liver oil dietary supplements and licensed medicines, 1994 and 1996

(xxxvii) As previously discussed, PCBs, PCDDs and PCDFs accumulate in lipid-rich tissue, and this is particularly apparent in the livers of certain marine fish. Fish body and fish liver oils are popular dietary supplements and medicinal products in the form of either liquid oils or capsules which are consumed for their vitamin and long chain fatty acid content. Fish oil products are available as unlicensed dietary supplements and as licensed medicinal products on general sale. We have been provided by MAFF with data from a survey of the levels of PCBs, PCDDs and PCDFs in fish and fish liver oils purchased in 1994. These data confirmed findings that fish body and fish liver oils contain relatively high levels of PCBs, PCDDs and PCDFs. The survey was repeated in December 1996 to establish whether the levels of the contaminants had declined as a result of reported changes in the manufacturing process. The levels of PCBs, PCDDs and PCDFs in certain recently manufactured samples of salmon oil and halibut liver oil supplements appear to have declined, though it

is difficult to draw firm conclusions due to the limited number of samples analysed. However, the levels of the contaminants present in other contemporary samples, particularly of cod liver oil, have not changed significantly since 1994. Data from the survey are summarised in Table 7 [not included here].

Estimated intakes of PCBs, PCDDs and PCDFs from fish oil dietary supplements and licensed medicines.

(xxxviii) The intake of PCBs, PCDDs and PCDFs from consumption of bottled or encapsulated fish oil dietary supplements and medicinal products has been estimated from the data obtained by MAFF using the daily dosage recommended on the product packaging ("the recommended dose"). This ranged from 2.5 to 5 ml (2.3 to 4.6 g) for infants, 5 to 10 ml (4.6 to 9.2 g) for toddlers and one capsule (0.1 g) to 10 ml (9.2 g) for adults and schoolchildren. When calculated on a TEQ basis, the data show that:

Breast-fed infants

a) Consumption of the recommended dose of fish oil dietary supplements and medicinal products would increase the margin by which breast fed infants exceed the TDI of 10 pg/kg bw/day for PCBs, PCDDs and PCDFs. The maximum estimated intake from fish oil supplements is 11 pg TEQ/kg bw/day as compared to an intake of 39-170 pg TEQ/kg bw/day from breast milk.

Toddlers (1½ - 4½)

b Consumption of the recommended dose of certain bottled fish oil dietary supplements or medicinal products, either alone or in combination with the diet, could lead to the TDI of 10 pg/kg bw/day for PCBs, PCDDs and PCDFs being exceeded by toddlers. The maximum estimated intake from fish oil supplements is 12 to 16 pg TEQ/kg bw/day as compared to a dietary intake of 5.2 to 5.9 (mean consumer) or 7.6 to 9.3 (97.5 percentile consumer) pg TEQ/kg bw/day. The maximum estimated total intake of PCBs, PCDDs and PCDFs is 19.6 to 25.3 pg TEQ/kg bw/day for toddlers aged 1½- 2½ and 3½ - 4½ respectively.

Schoolchildren

c) Consumption of the recommended dose of certain bottled fish oil dietary supplements or medicinal products, in combination with the diet, could lead to the TDI of 10 pg TEQ/kg bw/day for PCBs, PCDDs and PCDFs being exceeded by schoolchildren. The maximum estimated intake being 9.1 pg TEQ/kg bw/day in addition to an intake of 2.8 to 4.6 pg TEQ/kg bw/day from the diet. The maximum estimated total intake of PCBs, PCDDs and PCDFs is 13.7 pg TEQ/kg bw/day.

Adults

d) For adults, the maximum estimated intake of PCBs, PCDDs and PCDFs from fish oil dietary supplements or medicinal products is 6.6 pg TEQ/kg bw/day compared to an intake of 2.4 to 4.2 TEQ/kg bw/day from the diet. Thus, adults who were 97.5 percentile consumers would just exceed the TDI, consuming a maximum of 10.8 pg TEQ/kg bw/day.

Discussion of PCB, PCDD and PCDF intake from fish oil supplements

(xxxix) Consumption of the recommended dose of encapsulated fish oils (which are not recommended for infants or toddlers) does not lead to the TDI being exceeded by adults or schoolchildren. This is a result of the smaller quantity of oil present in the recommended dose of encapsulated oils compared to bottled oils, rather than any difference in the oils used in the two types of product.

(xl) The levels of PCBs, PCDDs and PCDFs present in the recommended doses of fish oil dietary supplements and medicinal products are unlikely to pose a risk to the health of breast-fed infants, toddlers, schoolchildren or adults. Consumption of the more highly contaminated oils by toddlers and schoolchildren could lead to the TDI being exceeded for a sustained period and would thus reduce the safety margin between intake and the toxicity observed in animal studies. This is undesirable but it is reassuring that the margin by which the TDI is exceeded decreases with age as the body weight increases.

Conclusions

a. PCBs produce a wide spectrum of adverse effects in experimental animals, including reproductive toxicity, immunotoxicity and carcinogenicity.

b. We *accept* the advice of the COM that PCBs do not have significant mutagenic activity and that any carcinogenesis in animal studies is likely to be due to a "non-genotoxic" mechanism.

c. We *accept* the advice of the COM and COC that it would be prudent to assume that all PCB congeners are potential human carcinogens.

d. We *note* the results of preliminary work which indicates that current human body burdens of PCBs may be affecting thyroid hormone levels. We *consider* that further work in a larger population is required to investigate this further.

e. We *conclude* that the available epidemiological studies of individuals exposed to high background dietary levels of PCBs are not of sufficient quality to allow a quantitative estimate of risk to be made of reproductive dysfunction following dietary exposure.

f. Most animal studies have been conducted using commercial mixtures of PCBs. The individual congeners and the relative proportion of these congeners in these mixtures are different from those found in food. There are only limited data on individual congeners. For these reasons, we have not been able to set a Tolerable Daily Intake for total PCBs.

g. We are of the opinion that, despite the limitations in the derivation of the TEFs (see paragraphs 5 and 21 of the main text), the use of TEFs to assess the health risks of certain coplanar ("dioxin-like") PCB congeners offers a pragmatic approach to the evaluation of these compounds and we *recommend* that TEFs be tentatively accepted and used in a limited manner for this purpose. We *recommend* that the TEFs proposed by the 1994 WHO-ECEH/IPCS task force are used at present and that a TDI of 10 pg TEQ/kg bw/day can be employed to assess the health risks of combinations of PCDDs, PCDFs and dioxin-like PCB congeners. Considerable further work is necessary to improve the scientific basis for validating TEFs.

h. Further work is needed to develop an approach to assessing the health risks of the non-coplanar PCB congeners.

i. We *welcome* the new data on UK dietary intakes of both total and individual congeners of PCBs provided by MAFF. We note that the data on intakes by toddlers are provisional and *suggest* that further work is carried out to give more reliable estimates

of intake by this age group. We *consider* that there is unlikely to be a health risk from current intakes of PCBs from food.

j. We *welcome* the new data on levels of PCBs in human milk. Although intakes of PCBs by breast-feeding babies are higher than is desirable, we *reaffirm* our previous advice that breast-feeding should continue to be encouraged on the basis of convincing evidence of the benefits of human milk to the overall health and development of the infant.

k. We *note* the data on levels of PCBs, PCDDs and PCDFs in fish oil dietary supplements and medicinal products. The intake of such levels is undesirable, since it potentially leads to the TDI for PCBs, PCDDs and PCDFs being exceeded by toddlers and schoolchildren for a sustained period and thus reduces the safety margin between intake and the toxicity observed in animal studies. However, we *consider* that this intake is unlikely to pose a risk to health.

l. We *recommend* that it would be desirable to carry out studies to improve the understanding of PCB, PCDD and PCDF accumulation and disposition in humans in the first year of life, eg by physiologically-based toxicokinetic modelling.

m. We consider that it would be prudent to seek action to ensure that consumer exposure to these contaminants is kept below the TDI. However, we recognise that PCBs are likely to persist as contaminants of the environment for many years to come. Therefore, we *recommend* that levels in food and in human milk should continue to be monitored at regular intervals to confirm that the downward trend continues. If it does not, we *recommend* that a further review is instigated to determine how human exposure can be reduced.

Short and long chain triacyl glycerol molecules (Salatrims)

1.32 During 1997 the Committee completed its review, undertaken at the request of the Advisory Committee on Novel Foods and Processes (ACNFP), of the toxicological and clinical data on the Salatrims, a group of chemically similar products which have been proposed as low-calorie fat substitutes and which had been submitted to the ACNFP for consideration.

1.33 The review process was extensive and included discussions of a joint working group of COT and ACNFP with representatives of the sponsoring company. A statement summarising the COT views was prepared and passed to the ACNFP. It is outlined below. References and tabular data were provided in an Annex which is available from the COT Secretariat.

Introduction

(i) We have been asked by the Advisory Committee on Novel Foods and Processes (ACNFP) to comment on specific aspects of a large submission of data received by the ACNFP in respect of Salatrims, a family of low calorie fat products. The ACNFP reviewed the available toxicological and clinical safety data on Salatrim at its 32nd meeting on 26 September 1996. The ACNFP requested advice from the Committee (COT) in respect of:

a) the adequacy of the animal toxicological database and, in particular, the arguments proposed by Cultor Food Science, who wish to market these products in the UK, regarding limited testing of Salatrims in animals.

b) an evaluation of the increases in serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) documented in human clinical studies and, for comments regarding the lack of predictivity of the animal toxicology studies in respect of these effects on the liver.

(ii) Salatrims comprise a family of structured glycerides composed predominantly of mixtures of long chain fatty acids (LCFAs; principally stearic acid) and short chain fatty acids (SCFAs; acetic, propionic and/or butyric) which are intended as low caloried fats for use in soft sweets, coatings (eg wafers and confections), dairy products, shortening and potentially in table spreads. Predicted intakes will vary according to the uses of Salatrims, level of substitution for existing fats, and the extent to which these fatty acids are consumed. We have considered several reports provided by Cultor Food Science which present calculations regarding potential intakes. The estimated intake for the whole population assuming selected uses varied between 11-29 g/day (mean), and 18-65 g/day (97.5th percentile). Estimates of intake using all potential uses varied between 18-46 g/day (mean) and 30-88 g/day (97.5th percentile). We also *note* that the Joint Food and Agriculture Organization/World Health Organization Expert Committee on Food Additives estimated intakes in children aged 3-5 years to be approximately 26 g/day (90th percentile). We *note* that there are considerable uncertainties regarding the methods and precision of the calculated estimates of potential Salatrim intake and that the various figures provided by the company show a wide variation. We are also *aware* that children have a caloric intake which on a body weight basis is higher than that in adults, on which basis their exposure to Salatrim would also be higher. Thus it would have facilitated interpretation of the intake data if the latter had been calculated and expressed in terms of grams Salatrim/kg bw/day and grams

Salatrim/energy intake/day. However, we *consider* that the available figures provided by the company can be used as a guide to the evaluation of the clinical and toxicological data provided in the submission.

(iii) An abbreviated nomenclature has been used by the manufacturers and also throughout this statement to describe the various Salatrims which have been evaluated in toxicological and clinical safety studies. An account of this nomenclature and details regarding all Salatrims tested are given in Tables 1 and 2 of the Annex to this statement. (For example, in Salatrim 43SO tributyrin and tripropionin are the SCFAs and the LCFA source is hydrogenated **S**oybean **O**il.)

(iv) The specific questions raised by the ACNFP with regard to Salatrims were considered by the COT during 1996 and at a joint ACNFP/COT Working Group in early 1997 where representations from the company were heard. Following this latter meeting, additional information regarding intakes and evaluation of the animal toxicity and human clinical studies was submitted to the COT. A short summary of the available animal and human toxicology data on Salatrims is given below for information so that our consideration of the questions raised by the ACNFP can be placed into context.

Animal Metabolism/Toxicity Data

(v) Initial *in-vitro* studies of the hydrolysis of Salatrim products using porcine pancreatic lipase demonstrated that a wide range of Salatrim triacylglycerides underwent rapid hydrolysis. *In-vivo* metabolism experiments in rats were designed to compare the metabolism of a specified Salatrim (23CA) with triolein and the results showed that Salatrim 23CA was metabolised in an analogous way to triolein (a normal dietary fat).

(vi) Five 90-day feeding studies were undertaken in rats using a range of Salatrim products selected to include different combinations of short chain fatty acids (ie acetate, propionate, butyrate) and different sources of stearate (ie canola oil, cottonseed oil and soybean oil). Details regarding the Salatrim products tested are given in Table 2 of Annex 1. The results of the toxicity studies were consistent, showing no toxicologically significant effects at up to 10% w/w in the diet (ie approximately 6-8 g fat/kg bw/day). The observed changes in clinical chemistry and histopathological findings noted in these studies, which occurred predominantly at 10% w/w in the diet and consisted of alterations in bone mineral levels (eg increased bone zinc concentrations), and of renal mineralisation in female rats, were considered by the authors to be consistent

with alterations in mineral metabolism induced by a reduced proportion of polyunsaturated fats in the diet. The authors of the animal toxicology studies noted that published evidence is available which reported diet-induced alterations in mineral metabolism, and in particular reduced bone zinc concentrations in rats following administration of diets containing high levels of polyunsaturated fatty acids. Dietary administration of 10% w/w Salatrim 23SO to rats for up to 17 days had no effect on serum activity of aspartate aminotransferase (AST), alanine aminotransferase (ALT) or γ-glutamyltransferase (GGT) activities, although increases in the levels of serum enzymes have been documented in the human clinical studies. No toxicologically significant effects were documented in a 28-day study in minipigs fed a diet containing 10% w/w Salatrim 23SO. Samples of caecum were taken during routine necropsies of the rats fed Salatrim 23CA or 32CA for 13 weeks, frozen and then subsequently analysed for gut microflora and for evidence of any Salatrim induced changes in caecal pH, and primary/secondary metabolites of bile acids and phytosterols and for cholesterol and coprostanol levels. There was no evidence of any effects on gut microflora from these experiments. We *note* that the morphological methods used in these investigations to assess effects on gut microflora were insensitive. Salatrim products do not contain any structural alerts for potential mutagenicity. There was no evidence of genotoxicity in an adequate range of *in-vitro* or *in-vivo* studies. No studies to evaluate potential carcinogenicity or effects on reproduction are available.

Human Toxicity Data

(vii) Four clinic-based studies using adult volunteers have been undertaken which utilised double blind study designs. The experimental protocols used exposures up to 60 g Salatrim/day for 1, 4 or 7 days and study designs which included a 4 day triple cross-over experiment. This type of design allowed the effects of three different fat sources on clinical chemistry and recording of symptomatology to be compared in the same individuals, but we *note* that only a very short exposure period of 4 days was used. The results of the clinic based studies with tested Salatrim products showed similar effects on gastrointestinal function (ie nausea, stomach cramps, diarrhoea, flatulence were reported) at 60 g/day. These effects subsided when the Salatrim administration was stopped. Overall, the authors concluded that 30 g Salatrim/day in the clinic-based studies did not result in any gastrointestinal effects. We *note*, however, the limited duration of these studies and that clinical investigations were only performed on healthy adults and thus potential effects in children or individuals with compromised gastrointestinal function were not considered.

(viii) A 28-day free living study was undertaken using groups of at least 12 male and 12 female volunteers consuming a range of food products containing one of three Salatrim products (23SO, 43SO, 4SO) at levels of up to 60 g Salatrim/day. The purpose of this study was to allow an extended evaluation of the Salatrim products and to confirm the effects seen in the clinic based studies and also to determine if any of these effects were reversible. The adverse effects reported in this study, predominantly at 60 g Salatrim/day, were consistent with those documented during the clinic based studies (ie gastrointestinal disturbances and increases in serum transaminase levels). The authors considered that the effects on serum transaminase levels were transient with noticeable decreases in serum activities of AST and ALT occurring towards the end of the study period. We *note*, however, that convincing evidence of a decline in the activities of serum AST and ALT to baseline levels was not provided. Although increases in serum AST and ALT activities rarely reached clinical significance in the 28 day free living study, we *note* that there were concomitant increases in the activities of some liver function enzymes (ie AST and ALT) and reduced serum cholesterol documented in the clinic-based studies at 60 g Salatrim/day. There is limited published evidence to support the view that an increased load of high caloric materials such as proteins or sucrose in the diet may induce alterations in serum transaminase enzyme levels.

(ix) The authors noted that none of the subjects in the 28-day free living study reported severe gastrointestinal effects which might impair normal function. The effects were considered to be "mild" or "annoying". They considered that 30 g Salatrim/day would have little or no effect on the health of individuals. We *note* that complaints of adverse effects on gastrointestinal function persisted for at least 10 days in a small number of individuals at all dose levels, including two who consumed 30 g Salatrim/day.

(x) There are a number of proposals concerning the mechanism(s) for the effects of Salatrim on gastrointestinal function. Increased stool weight and faecal water content as reported in the 4 day triple cross over study could have resulted in a bulking effect and hence may have contributed to the gastrointestinal symptoms. The authors have also proposed that the introduction of Salatrim, a poorly digested fat, into the diet may have caused transient gastrointestinal disturbance since similar effects have been documented following an abrupt increase in dietary fibre intake. The authors of the clinic-based studies have also speculated that the level of short chain fatty acids at high Salatrim doses (ie 60 g/day) might temporarily overwhelm the ability to utilise acetate which might be sufficient to induce some adverse gastrointestinal symptoms. Overall, we *conclude* that there is no convincing explanation regarding the mechanism of the adverse gastrointestinal effects of

Salatrims seen in the clinical studies. Additionally there is no information available regarding the likelihood of adverse gastrointestinal effects in children or individuals with compromised gastrointestinal tract function.

Consideration of the adequacy of the animal toxicological database

(xi) We *consider* that the animal toxicity studies were adequately conducted and can be used in the safety assessment of Salatrims. However, we *note*, that there are inadequate data available in respect of the potential effects of bolus doses of SCFAs on reproduction following their release from Salatrims during the metabolism of Salatrims in the gastrointestinal tract. We are *aware* that butyrate and propionate have been shown to have teratogenic potential *in-vitro*. We reviewed the additional data provided by Cultor Food Science including calculations regarding potential blood levels of butyrate following consumption of a meal containing 30 g of Salatrim 4SO and *conclude* that additional pharmacokinetic studies to evaluate blood levels of SCFAs in volunteers following consumption of individual Salatrim products are required before any conclusions regarding the teratogenic risk of Salatrim(s) can be drawn. We *agree* that there is no requirement for additional mutagenicity studies or for the provision of carcinogenicity bioassays with Salatrim(s).

Consideration of the increases in aspartate aminotransferase and alanine aminotransferase documented in human clinical studies

(xii) We have considered all of the data available from the clinical studies with regard to the potential effects of Salatrim(s) on serum enzymes which can be used to evaluate potential adverse effects on liver function and have also considered the further information supplied by Cultor Food Science regarding an evaluation of the individual clinical chemistry for all of the clinical studies. We *conclude* that increases in both AST and ALT occurred in a higher proportion of individuals consuming 30 g or 60 g Salatrim per day for 28 days compared to controls, although only a few of these reported increases can be regarded as reaching the level of clinical significance. Whereas there were differences between individuals in respect of the magnitude of the increase in AST and ALT in response to the ingested dose level of Salatrim and also between that induced by different Salatrim products, we *conclude* that the evidence is consistent with a weak treatment related effect which did not appear to decline during the 28 day treatment period. The analysis of individual clinical chemical data for all of the clinical studies is complicated by the absence of detailed background information on the individuals included in

the clinical studies and also, in respect of the 28 day study, by the identification of inconsistencies and transcription errors both in the original and published reports of this study. There was also additional evidence of concurrent increases in other serum enzymes consistent with liver dysfunction, such as alkaline phosphatase, lactate dehydrogenase and GGT, in a small number of individuals who ingested Salatrims. Overall, we *consider* that a No Observable Adverse Effect Level (NOAEL) with respect to clinical chemical markers of liver function cannot be identified from the clinical safety studies undertaken with Salatrims and that no conclusions can be drawn with regard to the mechanism or biological significance of the Salatrim-induced effects on AST and ALT.

(xiii) We *consider* that there are insufficient data available to derive any firm conclusions regarding the reasons for an absence of effects on serum liver enzymes in the animal studies. We *conclude* that it would be appropriate to proceed on the basis that the effects on serum liver enzymes documented in the clinical safety studies were treatment-related and thus require additional evaluation, particularly with respect to the identification of an appropriate NOAEL. In this regard the discrepancies in the reporting of the results from the 28 day free living study limit the value of this investigation. We *recommend* that a suitable long term clinical evaluation study of the individual Salatrims to be marketed should be undertaken with particular reference to the identification of a NOAEL.

Discussion and Recommendations

(xiv) We have, during our consideration of the questions raised by the ACNFP, evaluated all of the available toxicological and clinical safety studies on Salatrim and have considered the representations made by Cultor Food Science at the joint ACNFP/COT Working Group. The following two conclusions respond to the specific requests made by the ACNFP. An additional conclusion based on a consideration of all the toxicological and clinical data submitted to the COT is given in paragraph 15.

a) Animal toxicological data alone on Salatrims are insufficient to evaluate the proposed use of these materials as fat replacers. We *recommend* that additional pharmacokinetic studies to evaluate blood levels of SCFAs in volunteers following consumption of individual Salatrim products are required before any conclusions regarding the teratogenic risk of Salatrim(s) can be drawn.

b) The clinical safety studies demonstrate a weak treatment related effect of Salatrims on serum levels of marker enzymes for liver dysfunction. There are insufficient data available to derive any firm conclusions regarding the reasons for an absence of effects on serum liver enzymes in the animal studies. The documented discrepancies in the reporting of the results from the 28 day free living study limit the value of this investigation with regard to evaluating the potential effects of Salatrims on liver function. We *recommend* that a suitable long term clinical evaluation study of the individual Salatrims to be marketed should be undertaken with particular reference to the identification of a NOAEL for this effect.

(xv) Regarding overall conclusions, we *note* that evidence of adverse effects on gastrointestinal function and on marker enzymes for liver dysfunction in humans were reported following the consumption of 30 g Salatrim 23SO for 28 days and that there was clear evidence of adverse effects at 60 g/day regarding several Salatrim products. We are *concerned* that there would appear to be no margin of safety between these levels of consumption and the calculated potential intakes reported above in paragraph 2. Additionally children and individuals with compromised gastrointestinal function might be more susceptible to these particular effects associated with Salatrim consumption. We therefore *recommend* that the additional clinical studies requested in paragraph 14 (ii) should further investigate the potential effects of the Salatrim(s) to be marketed on gastrointestinal function with a view to also identifying a NOAEL for this effect.

Soluble Fibre derived from Guar Gum

1.34 In 1996 the COT had been asked by the ACNFP for its advice on the toxicology data submitted on a novel soluble fibre. The data were part of a submission made under the voluntary scheme for assessment of novel foods that existed until April 15, 1997. The novel fibre is derived from guar gum and is intended to be added to a range of processed liquid and solid foods to increase the fibre content. The COT had advised that the novel fibre should be considered in its own right, as a substance distinct from guar gum, and that the toxicology data cited on guar gum could not be used to support the safety-in-use of the novel fibre. In addition the Committee considered that the toxicology studies carried out on the novel fibre itself were inadequate. The COT indicated that further data, including a 90-day rat study, were required on the novel fibre.

1.35 The results of a 90-day study on the fibre were submitted to the COT in 1997. The Committee was content with the new study and satisfied that the increased caecum weights observed in the study were due to the increased fibre levels in the diet and not an effect of the fibre *per se*. However, the Committee had noted previously that the novel fibre reduced transit time through the gut and was concerned that this may have an affect on the absorption of minerals and trace elements. The Committee considered that the new study had not adequately addressed this concern and recommended that some further work be carried out. The Committee also noted that there were no adequate human studies on the novel fibre and considered that further human studies were advisable.

Tetrachloroethylene

1.36 The Committee was asked to advise the Health and Safety Executive on the implications of an epidemiological study in the dry-cleaning industry. The study had investigated the potential risks of reproductive and developmental toxicity arising from exposure to tetrachloroethylene in dry-cleaning establishments. It produced the following statement:

Introduction

(i) The Committee has been asked to advise the Health and Safety Executive (HSE) and other Government Departments on the implications of an epidemiological study in the dry-cleaning industry. This concerns the potential risks of reproductive and developmental toxicity from tetrachloroethylene to employees, the general public, and consumers arising specifically from its use in dry-cleaning establishments. We understand that our conclusions will be presented to the Health and Safety Commission's Advisory Committee on Toxic Substances.

Background

(ii) Tetrachloroethylene (perchloroethylene or ethylene tetrachloride, C_2Cl_4) is a colourless, non-flammable chlorinated hydrocarbon solvent with widespread industrial application. It has been used in the dry-cleaning industry for over thirty years and currently it accounts for about 80% of the total solvent use in dry-cleaning.

(iii) The use of tetrachloroethylene in the workplace is controlled in the UK under the Health and Safety at Work etc. Act 1974 and the Control of Substances Hazardous to Health Regulations, 1994. Under these regulations employers are required to prevent, or if this is not reasonably practicable, adequately control exposure of employees and other persons to substances hazardous to health. The 8-hour Occupational Exposure Standard is currently 50 parts per million (ppm) and the short term exposure limit is 100 ppm.

(iv) In the European Union (EU) tetrachloroethylene is classified as a category 3 carcinogen (possible risk of irreversible effects), but not as a substance toxic to reproduction.

(v) The Committee considered tetrachloroethylene in 1993 following the publication of studies which suggested that exposure to tetrachloroethylene may be linked to spontaneous abortion (miscarriage). At that time we concluded that the available epidemiological evidence, although based on studies that were methodologically weak, was consistent with the view that tetrachloroethylene may be a reproductive toxicant in humans. However, we recognised that the available animal data did not support this view. After we reached our conclusions, HSE commissioned a further epidemiological study of the possible reproductive toxicity of tetrachloroethylene in the dry-cleaning industry.

(vi) A draft risk assessment of tetrachloroethylene has been prepared by the UK (jointly by HSE and the Department of the Environment, Transport and the Regions) under the EU Existing Substances Regulation (ESR). The review had access to information which was not available when the Committee considered the issue in 1993. The section on toxicity for reproduction, including human developmental toxicity, was made available to us.

The study funded by the Health and Safety Executive

(vii) In 1994 the HSE commissioned a retrospective occupational cohort study of reproductive outcome in women currently or previously employed in dry-cleaning shops or laundry units. We have been given the results in the form of a report to HSE and also as a manuscript submitted for publication. So far, only the data on spontaneous abortions have been fully analysed.

(viii) The key findings of the research were that:

a) within the dry-cleaning group the risk of spontaneous abortion was statistically significantly higher in the operator group than in the non-operator group; adjusted odds ratio 1.63 with 95% Confidence Interval (CI) 1.01-2.66, p=0.04, equivalent to 63% excess risk;

b) consistent findings were obtained for exposed pregnancies of dry-cleaning operators compared both with unexposed pregnancies prior to the first exposed pregnancy and with unexposed pregnancies after the last exposed pregnancy; adjusted odds ratios 1.67, 95% CI 1.17-2.36 and 1.82, 95% CI 1.09-3.05, respectively. These are based on non-overlapping data sets;

c) the risk of spontaneous abortion for pregnancies conceived while women were employed as non-operators in dry-cleaning establishments was no greater than that for pregnancies occurring while women were not employed in either the dry-cleaning or laundry industries; adjusted odds ratio 1.02, 95% CI 0.65-1.60 (compared with unexposed pregnancies prior to first exposed pregnancy).

(ix) The Committee considered five questions:

a) Whether it agreed that the study's main conclusion (that dry-cleaning machine operators had a higher risk of spontaneous abortion than non-operators) was supported by the data?

b) If so, could any conclusions be drawn about the rôle of tetrachloroethylene in the observed increased risk?

c) Whether the effect, whatever the cause, was restricted to operators and if so, by implication, is there no risk to the general public and consumers (on dry-cleaning premises)?

d) Does the study provide sufficient evidence that exposure to tetrachloroethylene is not associated with low birthweight?

e) Whether the research alters the balance of the argument in the ESR review in terms of its assessment of tetrachloroethylene's toxicity for reproduction?

Conclusions

(x) Our conclusions are:

a) We *consider* that the study showed that dry-cleaning machine operators had an increased risk of spontaneous abortion compared with non-operators. This is consistent with other published work. We *agree* that there is an epidemiological association between the job category and spontaneous abortion.

b) We *are of the opinion*, however, that there is no evidence for a plausible biological mechanism by which tetrachloroethylene could cause this effect and that other factors could have contributed to the observed risk. We *conclude*, therefore, that the increased risk of spontaneous abortion could not be specifically attributed to exposure to tetrachloroethylene.

c) The evidence that non-operators were at no increased risk leads us to *conclude* that, by implication, there is no risk to the general public who visit dry-cleaning premises.

d) We are aware that the researchers are conducting further analyses on the relationship between low birthweight and job category. We cannot comment on this aspect of the study until this work has been completed.

e) The draft ESR review concludes that 'Although there is no convincing evidence that tetrachloroethylene causes developmental toxicity in humans, concern has been raised regarding the risk of spontaneous abortion in, particularly, dry-cleaning workers.'. We *conclude* that this study adds a little weight to the existing epidemiological evidence for the risk of spontaneous abortion in dry-cleaning workers, but that on balance it does not change the conclusion in the draft ESR review concerning the rôle of tetrachloroethylene.

Thiabendazole

1.37 Thiabendazole (TBZ) is a benzimidazole derivative used as a fungicide in agriculture, as a food additive to prevent the growth of mould on the skin of citrus fruit and bananas and as an anthelminthic in human and veterinary medicines. The COT reviewed a submission of new data regarding the use of thiabendazole as a food

additive in September 1995 and at that time recommended an extension of the temporary ADI of 0.05mg/kg bw until 1 January 1997 pending the receipt of further data on the mechanism underlying the occurrence of the thyroid tumours seen in rats and advice from the COM in respect of new mutagenicity data. In 1996, COM concluded that the ingestion of thiabendazole at current dietary levels does not give rise to concern with respect to mutagenic hazard.

1.38 During 1997 the COT received the mechanistic study of thyroid tumours that they had requested in 1995. They reviewed this study and concluded that this toxic end point is not relevant to man. Having been informed of the conclusions of COM as regards mutagenicity, the COT agreed to recommend a full ADI of 0.1 mg/kg bw/day on the basis of a NOAEL of 10 mg/kg bw/day and a safety factor of 100.

Vitamin B6

1.39 The Committee had been asked in 1995 to review the toxicity of vitamin B6 following concerns expressed by the Consumers' Association regarding the high level of vitamins and minerals in dietary supplements in general, and of vitamin B6 products in particular.

1.40 The Committee were then asked to advise on the toxicity of vitamin B6 for a second time in 1997 when it became apparent that interested parties were concerned that the Committee might not have taken important information into account in its earlier assessment. The Secretariat invited interested parties to submit relevant information for consideration by the Committee. After completing its assessment of the additional information the Committee prepared the following statement:

Introduction

(i) We were asked to review the safety of vitamin B6, following concerns expressed about the potential toxicity of high dose dietary supplements. One vitamin B6 supplement was highlighted since a single dose contained 100 mg - more than 50 times the amount required to maintain normal bodily function. Dietary supplements, classified as foods, are freely available to the general public and are consumed by individuals who wish to supplement their diet. We note that vitamin B6 products with Marketing Authorizations (ie licensed as medicines) are also freely available under the General Sales List Order to individuals for the self-treatment of specific medical conditions. Other licensed vitamin B6 products, which contain higher doses, are available from pharmacies or on prescription from General Practitioners.

(ii) The generic term vitamin B6 includes six vitamers: the alcohol pyridoxine, the aldehyde pyridoxal, the amine pyridoxamine and their 5'-phosphates. At one time *pyridoxine* was used as a generic descriptor but since 1973, IUPAC-IUB and IUNS nomenclature has been used. The vitamers are interconvertible.

(iii) Vitamin B6 is essential for good health and plays a major role in amino acid metabolism. Protein intake affects vitamin B6 requirements. For example, adults maintained on vitamin B6-deficient diets develop abnormalities in tryptophan and methionine metabolism faster and their blood vitamin B6 levels fall faster when they have a diet high in protein (approximately 80-160 g/day) in comparison with low protein intakes (30-50 g/day). During repletion of vitamin B6-deficient subjects, tryptophan and methionine metabolism and blood vitamin B6 levels are returned to normal faster at low levels of protein intake. The EC's Scientific Committee for Food and the UK Government's Committee on Medical Aspects of Food Policy have both reported that 15µg vitamin B6 per gram protein per day is sufficient for the needs of healthy people. This approximates to a daily amount of between 1.1 mg and 1.5 mg for those with an average protein intake. Results of the National Food Survey show that the average amount of vitamin B6 in the diet of the population is sufficient to meet the estimated requirements.

(iv) Pyridoxal phosphate, one of the vitamers, is a coenzyme in transamination, deamination, decarboxylation and trans-sulphuration reactions within the body. Individuals with deficiencies in some enzymes such as cystathionine synthetase, cystathionase, peroxisomal glyoxylate amino transferase and glutamine decarboxylase, may show improvement following vitamin B6 administration. For example, homocystinuria, an autosomal recessive aminoacidopathy resulting from a defect in cystathionine ß-synthase, is characterised by excessive homocysteine in plasma and urine. Plasma methionine levels are elevated and patients develop ectopia lentis, mental retardation, and hepatomegaly as well as deformities in the cardiovascular system and skeleton. Because of the many metabolic differences in individuals with this and other similar hereditary diseases, it is not appropriate to use the data from case studies of such patients receiving vitamin B6 medication when assessing the safety of vitamin B6 supplements sold as foods.

Human Toxicity Data

(v) Severe sensory peripheral neuropathy in individuals following ingestion of large doses of vitamin B6 was first described in the early 1980s. Symptoms of toxicity include hyperaesthesia, paraesthesia, muscle weakness, numbness and

loss of proprioception and vibration sense. Electrophysiological measurements and examination of nervous tissue by biopsy in some individuals have demonstrated nerve damage. The lowest dose reported to have been followed by symptoms consistent with sensory nerve damage is 50 mg per day. Signs of toxicity were observed after an average of 35 months. We are aware that the study by Dalton and Dalton has some methodological deficiencies but, nevertheless, the symptoms reported by the patients are consistent with the well described clinical syndrome of peripheral sensory neuropathy. The observations of Dalton and Dalton are also consistent with the reported symptoms of patients in other studies undertaken at higher doses which contained objective measures as well as subjective observations. In most instances, the clinical signs of toxicity were reversible once ingestion of high doses of vitamin B6 had ceased. However, in some instances where the dose of this vitamin was especially high, signs of damage remained. The clinical studies suggested an inverse relationship between the daily dosage and the time required before symptoms were detected. We consider that the small number of individuals involved and/or the short duration of administration may explain the absence of signs of sensory peripheral neuropathy in some studies. As stated above in paragraph iv, we consider that studies of individuals with hereditary diseases such as homocystinuria, are inappropriate in the assessment of vitamin B6 toxicity from dietary supplements.

Animal Toxicity Data

(vi) Several studies have shown that administration of high doses of vitamin B6 to different animal species, including the rat and dog, resulted in ataxia and muscle weakness. Neuropathological damage, including degeneration of the dorsal root ganglia, axonopathy and demyelination have been observed. The lowest reported adverse effect level in animals is 50 mg/kg bodyweight/day in the dog after approximately 16 weeks administration. We are aware of a report of a no observed adverse effect level in the dog of 20 mg/kg bodyweight/day for 80 days but, bearing in mind the age of the study and without further experimental detail, we have not used this figure in our consideration. With a safety factor of 300 (10 for the use of animal data, 10 for inter-individual human variation, 3 for the use of a lowest observed adverse effect level) and assuming that an individual weighs 60 kg, extrapolation from the lowest observed adverse effect level in dogs (50 mg/kg bw/day) would give a maximum daily safe dose for humans of 10 mg.

Conclusions

(vii) There is no doubt that consumption of vitamin B6 by humans in excess of the amount required to maintain bodily function can result in symptoms which are consistent with sensory peripheral neuropathy. Furthermore, the animal toxicity data are consistent with the study of Dalton and Dalton which reported adverse effects at daily intakes of 50 mg in humans. Electrophysiological measurement and examination of nerve tissue confirm neuropathological changes. With the exception of the instances where especially high doses (in the order of grams) of this vitamin were ingested by some individuals, the signs of toxicity are reversible after cessation of ingestion. The lowest dose reported to have adverse effects in humans is 50 mg per day; although there are methodological deficiencies in the study showing effects at this level of intake, we consider it would be unwise to ignore this evidence in the light of other supporting human and animal data.

Recommendation

(viii) Allowing for a margin of safety between the lowest observed adverse effect level in humans and bearing in mind the supporting animal toxicity data, we *recommend* that the maximum daily intake of vitamin B6 from dietary supplements should be 10 mg per day.

Topics Still Under Consideration

1.41 The following topics, which were discussed by the Committee at meetings held in 1997, are still under review: consideration of the safety of a high intensity artificial sweetener, a review of the toxicity likely to result from use of a cerium-containing fuel additive.

1997 Membership of the Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment

CHAIRMAN

Professor H F Woods BSc BM BCh DPhil FFPM FIRST HonFFOM FRCP(Lond & Edin)

Sir George Franklin Professor of Medicine
Division of Molecular and Genetic Medicine
University of Sheffield

MEMBERS

Professor P Aggett MB ChB FRCP MSc DCH
Vice Chairman.
Head of Lancashire Postgraduate School of Medicine and Health

P N Bennett MB ChB MD FRCP
Reader in Clinical Pharmacology, University of Bath and Dean of the School of Postgraduate Medicine

N A Brown BSc PhD
Senior Scientist, Medical Research Council Experimental Embryology and Teratology Unit and Honorary Senior Lecturer, Department of Child Health, St Georges Hospital Medical School, University of London

Professor J K Chipman BSc PhD CBiol FIBiol MRCP
Professor of Cell Toxicology, University of Birmingham

Professor A D Dayan BSc MD FRCP FRCPath FFOM FFPM CBiol FIBiol
Director of DH Toxicology Laboratory, St Bartholomew's and the Royal London School of Medicine and Dentistry

Professor G G Gibson BSc PhD FRCPath
Reader in Molecular Toxicology, University of Surrey

Professor G Hawksworth BSc PhD
Senior Lecturer in Medicine, Therapeutics and Biomedical Sciences, University of Aberdeen

M Joffe MD MScEcon FRCP FFPHM
Senior Lecturer in Public Health, Imperial College of Science, Technology and Medicine, St Mary's Hospital Medical School

I Kimber BSc MSc PhD FIBMS CBiol MIBiol
Research Manager, Zeneca Central Toxicology Laboratory

D E Prentice MA VetMB MVSc FRCPath MRCVS
Independent consultant, animal histopathology

Professor A G Renwick BSc PhD DSc
Clinical Pharmacology, University of Southampton

A G Smith BSc PhD CChem FRSC
Molecular Toxicologist, Medical Research Council Toxicology Unit in Leicester

A Thomas MB ChB PhD FRCP
Consultant Physician with special responsibility for the elderly, Plymouth District Health Authority

M Tucker BSc PhD FRCPath
Independent pathologist and animal histopathologist

SECRETARIAT

J Greig MA DPhil (Scientific)
Mrs K Ighavini (Administrative, until March 1997)
Mrs D Davey (Administrative from April until October 1997)
J Lighthill BA (Administrative, from November 1997)

D E Aldridge BSc PhD CBiol MIBiol (until September 1997)
J Shavila BSc MSc PhD
Ms C Mullholland BSc
Mrs C Steed BSc MSc (until October 1997)
A Wadge BSc PhD CBiol MIBiol

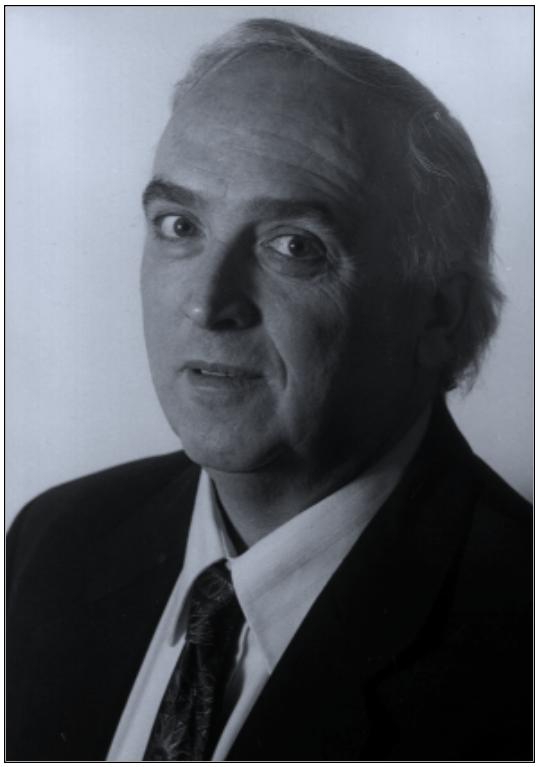
COMMITTEE ON TOXICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT

DECLARATION OF INTERESTS DURING THE PERIOD OF THIS REPORT

Member	Personal Interest		Non-Personal Interest	
	Company	**Interest**	**Company**	**Interest**
Prof H F Woods (Chairman)	Halifax Bank	Share Holder	Wide range of national & international food & chemical companies	Dean of the Faculty of Medicine, University of Sheffield, which has extensive activity in teaching and research in nutrition and toxicology and in topics related to and supported by many companies in the food and chemical industry. Harry Bottom Charitable Trust and Special Trustee for the former United Sheffield Hospitals
Prof P J Aggett	NONE	NONE	Nutrica Milupa SMA Nutrition Unilever Nestlé	Research Support Research Support Research Support Institute intellectual agreement Meeting Support
Dr P N Bennett	Glaxo Wellcome Grand Metropolitan Hanson Marks and Spencer Tomkins Shell	Share holder Share holder Share holder Share holder Share holder Share holder	Research Institute for the Care of the Elderly plc Bath Clinical Trials Ltd	Director Director
Dr N A Brown	Merck Glaxo Wellcome Shook,Hardy & Bacon Styrene Information Research Control Du Pont	Consultancy Consultancy Consultancy Consultancy Consultancy	EC (DGXI+DGXII) Glaxo Wellcome US EPA	Research Support Research Support Research Support
Dr J K Chipman	Boots Healthcare International	Consultancy	Astra Glaxo-Wellcome STD Pharmaceutical Ltd Water Research Centre Zeneca, Safety of Medicine Zeneca, Central Tox. Lab.	MRC collaborative Research Award Research Support Research Support Research Support CASE Research Award MRC Collaborative Research Award
Prof A D Dayan	British Petroleum Glaxo-Wellcome Schering Plough SmithKline Beecham TI	Share Holder Pension Consultant Consultant Share Holder	NONE	NONE

Member	Personal Interest		Non-Personal Interest	
	Company	**Interest**	**Company**	**Interest**
Prof G G Gibson	NONE	NONE	NONE	NONE
Prof G Hawksworth	NONE	NONE	Glaxo-Wellcome Pfizer Central Research Servier Research & Dev SmithKline Beecham Solvay Pharma Zeneca	Research Support Research Support Research Support Research Support Research Support Research Support
Dr M Joffe	Ilzro Woolwich Abbey National	Research Grant Share Holder Share Holder	NONE	NONE
Dr I Kimber	British Airways British Petroleum ICI ICI Zeneca Zeneca	Share Holder Share Holder Share Holder Consultant Share Holder Employee	Unilever plc	Grant for Research
Dr D E Prentice	Novartis Crop Protection Novartis Pharma Rhône-Poulenc Rorer Schering-Plough Synthélabo Wyeth-Ayerst	Consultant Consultant Consultant Consultant Occasional fee Consultant	NONE	NONE
Prof A G Renwick	International Sweeteners Association	Consultant	Hoffmann-La Roche Unilever Smith Kline Beecham Pfizer Flavor and Extract Manufacturers Association (FEMA)	Research Support Research Support Research Support Research Support Research Support
Dr A Smith	Abbey National British Telecom Halifax Bank	Share Holder Share Holder Share Holder	Rhone Poulenc	Research Support
Dr A Thomas	NONE	NONE	NONE	NONE
Dr M Tucker	Zeneca	Pension	NONE	NONE

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



Professor J M Parry (Chairman)
BSc PhD DSc

PREFACE

The Committee on Mutagenicity COM has two major functions:

a) To assess and advise on the mutagenic risk to humans of chemicals present in food, consumer products and the environment and,

b) to advise on the general principles of test methods which are in use or newly developed, which are capable of assessing the mutagenic activity of chemicals.

This advice is provided to Government Departments and regulatory agencies. The COM is made up of independent scientists and observers from Government Departments supported by a secretariat from the Department of Health. The secretariat provides the COM with comprehensive data packages upon which the Committee members use their expertise to evaluate both chemical activity and the effectiveness of test methodologies. During the year the COM provided advice on a diverse range of chemicals including the pharmaceutical thalidomide, the fire retardant antimony trioxide, the plasticiser acetyl tributyl citrate, the wood preservative 2-phenylphenol, and the pesticide phosphine.

As a complement to the detailed evaluation of animal carcinogenicity assays by our sister Committee on Carcinogenicity (COC) the COM evaluated the potential mutagenicity of a range of chemicals that had been reported as being carcinogenic in the mouse but not in the rat. Following reports of an association between childhood cancer and paternal smoking the COM considered the potential mechanisms which may account for these observations.

Test systems evaluated by the COM included a bacterial assay proposed for the assay of the potential mutagenicity of water and waste water and the mouse lymphoma assay developed to measure the mutagenic activity of chemicals in an *in vitro* mammalian cell system. Advice on the principles of test strategies and interpretation focused upon the proposals of the International Conference on the Harmonisation of the Technical Requirements for the Registration of Pharmaceuticals for Human Use (ICH).

For a number of chemicals the COM identified deficiencies in the available information on their mutagenic potential. In such cases the COM provided advice on test methods and experimental designs that could rectify these deficiencies.

JAMES PARRY

Thalidomide: Not a mutagen

2.1 The Committee was aware of considerable press publicity regarding claims that thalidomide produced heritable mutations in humans. This was based on a paper by Huang and McBride (*Teratogenesis, Carcinogenesis, and Mutagenesis*, **17**, 1-5, 1997) and earlier work by these authors which reported evidence of DNA binding (using a radiolabel method). The Committee had been asked by the MCA to consider this paper in the light of the available mutagenicity data on thalidomide and comment on any areas of concern. Members also had access to a copy of a paper[3] which reported a large number of mutagenicity studies conducted by a number of separate laboratories.

2.2 The Committee agreed that the Huang and McBride study had been poorly undertaken and the results describing a small increase in radioactivity in DNA samples prepared from whole embryos did not support any definite conclusions regarding the potential interaction of thalidomide with DNA. Members reviewed the new mutagenicity data and concurred that thalidomide was not mutagenic in *Salmonella* and was not clastogenic in culture human lymphocytes in the absence of S-9 or in CHO cells in the presence or absence of S-9. Thalidomide did not induce micronuclei in human lymphocytes (in the absence of S-9) and was not mutagenic in the mouse lymphoma L5178Y $TK^{+/-}$ assay in the presence and absence of S-9. Thalidomide was not mutagenic in *in-vivo* cytogenetics tests using two strains of mice or in rabbits.

2.3 The Committee concluded that there was no evidence to suggest that thalidomide was mutagenic and that the hypothesis proposed in the Huang and McBride paper was unfounded.

Antimony trioxide

2.4 Antimony trioxide is widely used as a fire retardant, including in consumer products such as upholstery and some PVC textiles. In the past it has been shown to be present in PVC textiles used in cot mattress covers. Although the bulk of antimony trioxide was firmly bound in these products recent evidence had suggested that small amounts may, in the presence of moisture, be leached out of PVC textiles giving rise to consumer exposure. However, exposure to antimony is relatively ubiquitous with low levels present in may foodstuffs and also in house dust. There was some uncertainty about the quality of the mutagenicity data on antimony trioxide and the Department of Health asked for advice of the COM on this issue.

[3] Ashby J et al. Thalidomide: Lack of mutagenic activity across phyla and genetic endpoints. Mutation Research, **369** 45-65, 1997.

2.5 The Committee agreed the following conclusions

(i) Antimony trioxide has been adequately investigated for its mutagenic potential *in-vitro*. Negative results were obtained on Salmonella assays for gene mutation and in a mouse lymphoma assay using the L5178Y $tk^{+/-}$system. There was limited evidence of questionable significance for genotoxicity in DNA repair assays using bacteria and in an SCE induction assay in cultured mammalian cells. Some evidence for a clastogenic effect was documented in a metaphase analysis using human peripheral lymphocytes.

(ii) *In-vivo* studies to current protocols have recently been conducted in two species. There was no evidence for clastogenicity in a bone marrow micronucleus test in mice using oral administration of either a single (5g/kg bw) or repeated dose (1g/kg bw/day) for up to 21 days. Negative results were also obtained in a rat liver UDS assay. These data suggest an absence of mutagenic activity following oral administration but members noted that no indication had been given as to whether material had reached bone marrow. Information on this was needed before definite conclusions could be drawn regarding other routes of exposure.

Acetyl tributyl citrate (ATBC)

2.6 ATBC is a plasticiser used in polyvinylidene chloride (PVdC) films used as "food contact materials". A consortium of manufacturers had submitted a number of mutagenicity studies to the COM in 1994. At that time the COM concluded that ATBC was negative in studies to investigate gene mutation in Salmonella and negative results were also obtained when the clastogenic potential of ATBC was investigated by metaphase analysis in rat peripheral lymphocytes in two separate studies. A study to investigate gene mutation in mammalian cells using the CHO/HGPRT assay was considered inadequate and the Committee therefore requested that an additional gene mutation test, preferably a mouse lymphoma study should be undertaken. The study report had now been submitted and the Committees advice was sought on the conduct of the assay and the results obtained. Although the authors of the study report claim that ATBC did not demonstrate consistent evidence of mutagenic potential in this assay it appeared that a small, statistically significant increase in mutations was seen in some of the studies in the presence of exogenous metabolic activation.

2.7 The Committee noted that ATBC contained no structural alerts and was rapidly absorbed from the gastrointestinal tract, metabolised and its metabolites eliminated, predominantly in the urine. The estimated UK intake reported to the COT in 1994 was very low at approximately 0.05 mg/person/day.

2.8 The COM reached the following conclusion regarding the new study;

> The further in-vitro mutation study submitted considered the potential mutagenicity of ATBC in mouse lymphoma L5178Y cells at the TK locus using the soft agar technique. Five separate trials were conducted. No convincing evidence of a mutagenic response was documented in tests 1 and 2 conducted in the absence of metabolic activation. Three trials were undertaken using rat liver S-9 prepared from Aroclor 1254 pre-treated animals. No evidence of an effect was seen in the first trial. A small but statistically significant increase in the mutation frequency was documented in the second trial in the presence of S-9 at the highest three dose levels but no dose related trend was evident. A small dose related statistically significant increase in mutation frequency was documented in the third trial. The results of positive control trials in the presence of S-9 yielded variable and weak responses indicating that the conduct of the assay in the presence of S-9 was suboptimal and that the test sensitivity was low.

2.9 The Committee reached the following overall conclusion with respect to the mutagenicity of ATBC;

> The Committee concluded that the newly submitted mouse lymphoma study reported a weak positive response in the presence of metabolic activation. Taking into account the lack of mutagenic structural alerts for ATBC, the rapid absorption and metabolism of ATBC to inert metabolites and the negative results reported in well conducted Salmonella and *in-vitro* cytogenetics assays, the Committee agreed that no further testing with this material was warranted at the present time. The Committee considered that the situation might need to be reconsidered should information indicating a substantial increase in intakes of ATBC become available.

2-Phenylphenol

2.10 2-Phenylphenol and its sodium salt are used as wood preservatives and surface biocides. The COM had been asked to advise on these compounds in 1988 and 1992 in respect of studies which investigated the mechanism of bladder tumours induced in F344 rats fed high dietary levels of 2-phenylphenol or its sodium salt (5,000 ppm and above). In 1992, the Committee had concluded that 2-phenylphenol (or its sodium salt) were not

mutagenic in bacterial tests in *Salmonella typhimurium* but positive results had been documented using *in-vitro* metaphase analysis studies in CHO cells and in a mouse lymphoma assay in the presence of exogenous metabolic activation. Negative results had, however, been found in well conducted bone marrow assays for clastogenicity *in-vivo* and also in a dominant lethal assay for germ cell mutations. Thus, although 2-phenylphenol had some *in-vitro* mutagenic activity, there was no evidence that it was an *in-vivo* mutagen. The COM also reviewed some studies to investigate effects on DNA in bladder epithelium which had given conflicting results and the Committee asked for further data using a sensitive *in-vivo* method to investigate DNA adduct formation in the rat bladder, although it was felt that a 'threshold' approach to risk assessment with this compound was still appropriate. The ACP had subsequently recommended a ^{32}P-post labelling study be carried out to investigate adduct formation in the urinary bladder of male rats. HSE Pesticides Registration Section had now received a report and had requested advice from the COM.

2.11 The following conclusion was agreed;

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

Phosphine and metal phosphides

2.12 Aluminium and magnesium phosphide are used in pesticides to fumigate stored food products (principally grain). In addition, aluminium and zinc phosphide are used in rodenticide products. The active compound in both cases is phosphine gas which is liberated when the phosphide comes into contact with moisture. These compounds have been used for this purpose in the UK for over 25 years. In view of their high acute toxicity their use is limited to trained operators. The Pesticides Safety Directorate (PSD) was reviewing the pesticidal use of aluminium, magnesium and zinc phosphide and had noted there were conflicting data on the mutagenicity of phosphine, including reports from one research group of clastogenic effects in humans occupationally exposed to phosphine. The Advisory Committee on Pesticides had therefore asked for advice from the COM on these data.

2.13 The Committee reached the following conclusions with respect to the mutagenicity of phosphine and metal phosphides.

(i) Aluminium and magnesium phosphide are used in pesticides to fumigate stored food products (principally grain). In addition, aluminium and zinc phosphide are used in rodenticide products. The active compound in both cases is phosphine gas which is liberated when the phosphide comes into contact with moisture. These compounds have been used for this purpose in the UK for over 25 years. The Advisory Committee on Pesticides (ACP) requested the advice of the COM on the mutagenicity of these pesticides. The Committee reviewed all the available published and unpublished data forwarded by the Pesticides Safety Directorate and placed a particular emphasis on the studies in humans. The Committee agreed the following conclusions which are applicable to phosphine and all the above mentioned metallic phosphides.

In-vitro studies

(ii) Phosphine (or zinc phosphide) has consistently given negative results in assays for gene mutation in bacteria (*Salmonella typhimurium*). There was some concern that the high toxicity of phosphine could mask potential mutagenic effects. Phosphine has however been shown to have clastogenic potential in cytogenetic studies in mammalian cells and zinc phosphide has given positive results in the mouse lymphoma assay. In both cases activity was seen in the presence and absence of an exogenous metabolic activation system. Phosphine and metallic phosphides therefore have mutagenic potential *in-vitro*

In-vivo studies in animals

(iii) Phosphine has been extensively studied *in-vivo* using bone marrow/peripheral blood assays for clastogenicity. Although some inconsistent data were obtained, overall it can be concluded that negative results were obtained. Negative results were also obtained in a liver UDS assay and in a dominant lethal assay in mice, although this latter assay was of limited quality. Overall, the Committee concluded that there was no convincing evidence that phosphine and metallic phosphides had shown mutagenic activity *in-vivo* in rodents.

Studies in humans

(iv) The Committee considered three published reports in detail.

(v) Garry VF et al (Science, **246**, 251-255, 1989) studied small groups of grain workers in Minnisota. They reported an increase in total chromosome abberations (excluding gaps) and of deletions, breaks and complex aberrations in a group of 9 individuals with reported exposure to phosphine alone compared to a control group of 24 individuals with no exposure to pesticides and a control group of 15 grain workers employed in the inspection and processing of grain. Limited personal sampling data were available to show exposures up to 4.1 ppm phosphine in enclosed space applications and up to 0.64 ppm for open air applications. The Committee noted the small number of phosphine workers examined and the limited details available regarding matching of control and exposed groups for smoking habits. The Committee observed the considerable overlap in the range of results for pesticide applicators exposed to phosphine and grain worker controls. Overall it was concluded that pesticide applicators exposed to phosphine alone had elevated levels of chromosome damage in this study.

(vi) In a subsequent study the same group of authors (Garry VF et al Cancer Epidemiology, Biomarkers and Prevention, **1**, 287-291, 1992) reported similar findings in a group of 6 pesticide applicators exposed to phosphine compared to a control group of 26 individuals. The Committee agreed that the results, ie increased levels of chromosome damage were compatible with the earlier study by these authors.

(vii) In a separate Australian study, Barbosa A and Bonin AM (Occupational and Environmental Medicine, **51**, 700-705, 1994) analysed micronuclei in peripheral blood lymphocytes taken from 31 fumigators working with phosphine and 21 control subjects. Limited exposure data were provided for 3 individuals employed as fumigators and reported exposure concentrations of 0.1-0.8 ppm for a period of 1 hour. The authors report that the groups were matched for sex age and smoking habit but few details were provided. No evidence of an increase in micronuclei in phosphine exposed fumigators was documented. No increase in urinary mutagenicity (Salmonella assay)was seen in fumigators.

Conclusions human studies

(viii) The Committee concluded that it would be prudent to assume that phosphine was a human genotoxin on the basis of the results reported in studies of Garry et al. The Committee agreed that the negative data reported by Barbosa and Bonin may be due to the fact that they studied workers exposed to lower levels of phosphine and used a methodology of lower sensitivity than Garry et al. However, there were limitations in the studies published by Gary et al, for example in subject and control selection and matching, and phosphine exposure estimations. The Committee felt that further data were needed to ascertain whether these results were reproducible (by other groups) before any definitive conclusions could be drawn. Consideration should be given to the feasibility of a study in UK pesticide applicators using modern methods for assessing genotoxic effects. The COM would be willing to advise on the design of such a study.

Childhood cancer and paternal smoking

2.14 Members of the Committee noted that a two epidemiological studies had recently been published where an association between paternal but not maternal smoking and an increase in the relative risk for total cancers. A separate study also reported an elevated risk for some specific or groups of cancers (acute lymphoblastic leukaemia, lymphoma, and brain tumours) in children aged 5 years or less whose fathers had smoked for at least 5 years prior to conception. The COM asked the COC for advice on these studies. The COC considered the data and concluded that no definite conclusions could be drawn in view the limitations of the methodology. The COC felt that further comments should be sought from the COM regarding the mechanisms which could result in the effects documented in these reports.

2.15 The Committee identified 3 potential mechanisms whereby smoking could conceivably produce a specific increase in malignancies following paternal exposure. These were:

(i) The induction of genomic instability in damaged chromosomes of sperm. Following fertilisation of oocytes this damage may be expressed at various times in the developing embryo resulting in chromosome rearrangements. Such genomic instability may be expressed differentially in cell types, with potential for lymphocyte precursors being more sensitive. A possible mechanism of this type has been indicated in studies following alpha particle exposure.

(ii) Imprinting in the male germ line resulting in the differential expression of genes derived from male and female chromosomes. Such a mechanism has recently

been suggested for differences in "social awareness" between male and female offspring (Skuse et al Nature, **387**, 705-708,1997.) Variations in imprinting could result from differences in patterns of methylation in male and female germ cells.

(iii) Differential effects of DNA damage (most probably oxidative) upon germ cells which may have a lower probability of repair in male germ cells. Such damage may play a role in instability and/or imprinting as in (i) and (ii).

2.16 The Committee agreed that the higher number of cell divisions from stem cell to mature gamete in male compared to female germ cells provided an increased number of divisions where genomic instability, imprinting and DNA damage could take place preferentially in the male germ line. It was noted that the mechanisms suggested were theoretical and that there was little direct evidence to support them, but a number of experimental approaches were discussed which might potentially provide information upon which more definitive conclusions could be based. The Committee indicated its willingness to design an experimental approach to investigate the potential role of paternal smoking on childhood cancer.

Iso Water quality standard: Determination of the genotoxicity of water and waste water using the umu test

2.17 The Committee was asked to consider this subject by the Department of Health. An ISO Working Group had recently recommended a water quality standard for determination of the genotoxicity of water and waste water using the *umu*-test (based on the SOS-chromotest). Although the ISO biological standards relate to effects on environmental organisms it was possible that some results would be extrapolated to the assessment of potential human health effects. The Department was concerned that the ISO guidelines were developed in the absence of any input from any recognised mutagenicity expertise.

2.18 The Committee noted that the *umu* test had never been validated as a mutagenicity assay. In respect of environmental monitoring, it was agreed that it would be necessary to develop mutagenicity tests in the target species such fish, amphibians, lug worms etc. The Committee agreed that *in-vitro* methods such as the *umu* test were not suited to the mutagenicity assessment of complex mixtures of chemicals which might be found in water samples. The Committee endorsed an approach to the European Environmental Mutagen Society (EEMS) in order that appropriate mutagenicity expertise could be made available to the ISO water quality standard.

Utility of carcinogenicity bioassays in the mouse

2.19 Animal carcinogenicity bioassays in two species (namely the rat and mouse) have been traditionally used for over two decades as the critical scientific data required to identify potential human carcinogenic chemicals in the absence of adequate epidemiological studies. The International Conference on the Harmonisation of Technical Requirements for the Registration of Pharmaceuticals for human use (ICH) has agreed that bioassay data from only one species, (ie the rat), supported by appropriate mutagenicity and pharmacokinetic data and also information from new (unvalidated) short term *in-vivo* screening tests for potential carcinogenicity could be used for the licensing of human medicines.

2.20 The COC reviewed the available published data on mouse carcinogenicity studies part of their consideration of the utility of the mouse carcinogenicity bioassay. This work was not restricted to any one chemical category and the COC therefore requested advice from the COM in respect of the mutagenicity data on the chemicals considered in the review. The COM's conclusions in respect of the 'genuine' mouse-specific carcinogens identified by the COC have been summarised in tabular form in the COC section (3.24) of this Annual report. Detailed conclusions have been set out below in accordance with the chemical group categories agreed by the COC and using the same order of individual chemicals;

Mouse-specific: genotoxic chemicals

2,6-dichloro-p-phenylenediamine

(i) 2,6-Dichloro-p-phenylenediamine gave positive results in the Salmonella assay in the presence of S-9 and in the mouse lymphoma L51787 $TK^{+/-}$ in the absence of S-9. These *in vitro* data clearly show that the compound has mutagenic potential.

(ii) There is some weak evidence of *in vivo* activity from conflicting results reported from bone marrow studies to investigate clastogenicity in mice. No data are available from other tissues.

(iii) 2,6-Dichloro-p-phenylenediamine should be regarded as a possible *in vivo* mutagen.

N-methylolacrylamide

(i) N-methylolacrylamide gave negative results in assays for gene mutation in Salmonella. The Committee noted that the strains used in the bacterial assays were inappropriate to detect cross linking agents. The compound was clastogenic in CHO cells indicating that the compound has mutagenic potential.

(ii) The one bone marrow assay using the micronucleus test was negative but no definite conclusions can be drawn in view the absence of any data to assess whether the top treatment dose was adequate. A positive result was obtained in a limited dominant lethal assay in mice.

(iii) These data suggest that N-methylolacrylamide should be regarded as an *in vivo* mutagen.

Mouse-specific Equivocal evidence of mutagenicity

2,4-Diaminophenol (Dihydrochloride)

(i) 2,4-diaminophenol has been shown to produce gene mutations in the Salmonella assay with TA98 in the presence of rat or mouse S-9. There is limited evidence that it produces mutations in the mouse lymphoma L5178Y $TK^{+/-}$ assay in the absence of metabolic activation. Some of the *in vitro* data indicate that the compound has mutagenic potential.

(ii) In the absence of any *in vivo* data in mammals it is not possible to draw any definite conclusions about activity of 2,4-diaminophenol in intact animals.

Dipyrone

(i) It is not possible to drawn any definite conclusions from the very limited data using the Salmonella assay but dipyrone has been shown to produce clastogenicity in a metaphase analysis study in CHL cells. The *in vitro* data indicate that dipyrone has mutagenic potential.

(ii) There are limited published data available from *in vivo* bone marrow assays for clastogenicity (2 negative, 1 positive) but in view of the absence of any details of these studies it is not possible to draw any conclusions. No data are available from tissues other than the bone marrow.

(iii) *In vitro* data indicate that dipyrone has some *in-vivo* mutagenic potential. The limited published studies in mammals are inadequate to allow definite conclusions to be drawn.

Zearalenone[4]

(i) Negative results have consistently been obtained when zearalenone has been investigated for its ability to induce gene mutation in bacteria. Negative results were also obtained when the compound was investigated in the yeast *Saccharomyces cerevisiae* D3.

(ii) Only one limited metaphase study has been carried out using zearalenone and this provided no convincing evidence for clastogenicity. There was some evidence that zearalenone could induce SCEs but only in the absence of S-9 and at very high concentrations (above 10,000 µg/ml). Overall negative results were obtained when using the mouse lymphoma assay.

(iii) One group has demonstrated DNA adduct formation using ^{32}P-post labelling techniques in the liver and to a lesser extent in the kidney and ovaries of female mice. Further data are desirable on the reproducibility of this study, and to define any sex/species specificity. At present it would appropriate to provisionally regard zearalenone as being genotoxic *in-vivo*.

2.21 The COM has also been asked by the COC to review the mutagenicity data on ozone during 1998. Ozone has been identified by the COC as a 'genuine' mouse-specific carcinogen. The COM did not specifically consider the mutagenicity of vinylidene chloride, the remaining chemical allocated to this group by the COC (see section of this report for COC conclusions on this chemical).

Mouse-specific: mutagenicity inadequate

Benzaldehyde

(i) Benzaldehyde has consistently given negative results in the Salmonella assay and also in the one clastogenicity assay that has been adequately reported. However equivocal results were obtained in the mouse lymphoma L5178Y TK$^{+/-}$ assay in the absence of exogenous metabolic activation. Negative results

[4] Provisional conclusions. The COM will undertake a further assessment of the data on zearalenone during 1998.

in assays for SCE induction in mammalian cells.

(ii) Negative results were obtained in a sex linked recessive lethal assay in drosophila using feeding or injection. No *in vivo* data are available in mammals.

(iii) Overall, the COM concluded that the dataset on benzaldehyde was inadequate to come to any definite conclusions regarding mutagenic potential. As with acetaldehyde (see 1995 COM Annual report) benzaldehyde would only be expected to have potential *in vivo* activity at sites where it was not rapidly hydrolysed.

2.22 Members considered that the carcinogenicity of benzaldehyde (forestomach, mice) might have been exerted by a similar mechanism to that of acetaldehyde (forestomach, rats and hamsters) probably by a direct effect on the squamous epithelium of the forestomach.

Piperonyl sulphoxide

(i) Piperonyl sulphoxide has given negative results in assays for gene mutation in bacteria, and in a limited metaphase assay for clastogenicity and SCE induction in CHO cells. A single experiment with S-9 gave a positive result in an assay for mutation at the TK locus in mouse lymphoma cells, but there was no evidence for mutation in a similar assay using TK6 cells.

(ii) Negative results were obtained in the sex linked recessive lethal mutation assay in *Drosophila melanogaster.*

(iii) No final conclusion can be reached at the present time.

Ripazepam

(i) The compound does not have significant structural alerts and is apparently negative when assayed for gene mutation in Salmonella. These data suggest absence of significant mutagenic potential, but in view of the very limited published information it is impossible to draw any definite conclusions.

Mouse-specific: not-genotoxic

2.23 The COM concluded there were either recent COM conclusions and/or extensive essentially negative databases as summarised by IARC supporting a 'non-genotoxic' conclusion in respect of captan, dieldrin and diethylhexyladipate (DEHA). The following conclusions were reached regarding the two remaining chemicals allocated to this group

2-aminobiphenyl.hydrochloride (2-biphenylamine.HCl)

(i) Interpretation of the available mutagenicity data on 2-aminobiphenyl is complicated by the fact that nearly all studies used technical material, and this contains up to 1-2% 4-aminobiphenyl a known mutagen and human carcinogen.

(ii) 2-aminobiphenyl has structural alerts for mutagenic potential. Some evidence of weak activity was seen in assays using Salmonella typhimurium TA100, and equivocal results were obtained with TA98 in the presence of S-9. Essentially negative results were obtained using purified compound and Salmonella typhimurium TA 100. A doubtful positive result was also reported in the mouse lymphoma tk^+/tk^-assay using L5178Y cells in the presence of exogenous metabolic activation. Negative results were obtained in an *in vitro* UDS assay in primary rat hepatocytes and in an assay for mitotic recombination in the yeast *Saccharomyces cerevisiae* (a clear positive result was obtained with 4-aminobiphenyl in all these assays). It is considered likely that the weak activity seen with technical 2-aminobiphenyl is probably due to contamination with 4-aminobiphenyl.

(iii) Negative results were obtained in a cell transformation assay using a Rauscher leukaemia virus infected rat embryo cell line; again 4-aminobiphenyl gave a positive result in this assay.

(iv) Negative results were obtained with 2-aminobiphenyl in the wing spot test in Drosophila. (Positive results were obtained with 4-aminobiphenyl). The only *in vivo* assay in mammals is a host-mediated type test measuring activity in urine, as detected by Salmonella typhimurium TA 1538, as marker. Negative results were obtained following intraperitoneal administration of technical 2-aminobiphenyl (4-aminobiphenyl again gave a clear positive result in this assay).

(v) 2-aminobiphenyl itself does not have any significant mutagenic potential, the weak activity being seen with the technical material in the Salmonella assay (but not other *in vitro* tests) being due to the 4-aminobiphenyl present as an impurity.

Probenicid

(i) Probenicid does not have any structural alerts for mutagenicity. Negative results were obtained in assays for gene mutation in Salmonella and in a limited assay for clastogenicity in CHO cells. Negative results were also obtained in assays for SCE induction in CHO cells, the slight increase in SCEs seen in the absence of S-9 being of no biological significance.

(ii) Probenicid does not have any significant mutagenic potential.

Test Strategies and evaluation

2.24 The Committee finalised its advice on test strategies and interpretation of genotoxicity tests in the context of the discussions of the Genotoxicity Working Party of the International Conference on Harmonisation of the Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH). The Committee heard a presentation from Dr Lutz Muller, ICH rapporteur on genotoxicity who gave a detailed presentation outlining the background to the ICH proposals and further on-going work in this area. The finalised COM conclusions regarding this guideline are given below. The Committee also discussed the current status of the Mouse lymphoma assay in detail and heard a presentation from Dr Jane Cole from the MRC Cell Mutation Unit, University of Sussex) regarding a poster presentation submitted at the 7th ICEM congress, Toulouse, September 1997. A paper was also drafted for UK regulatory departments, which outlined some general principles for evaluating genotoxicity assays drawn from the Committee's discussion of items during 1996.

ICH guideline: Genotoxicity: A standard battery for genotoxicity testing of pharmaceuticals (S2B) and consideration of the mouse lymphoma assay

2.25 The International Conference on Harmonisation of the Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) had been discussing the harmonisation of genotoxicity testing for pharmaceuticals for several years and during this time the COM had made available several written contributions which had been

forwarded to the Dr Lutz Muller as ICH rapporteur in respect of the guideline on the standard battery of tests for genotoxicity testing of pharmaceuticals. There were a number of issues on which the COM considered there was a need for further data and discussion before it would be possible to reach a final conclusion. Of most importance was the suggestion by the ICH Working Group that the mouse lymphoma assay was capable of detecting a wide range of chemically induced genetic effects including clastogenic and aneugenic changes. Dr Muller had agreed to update the Committee on the most recent information available to the ICH Working Group.

Presentation by Dr Muller

2.26 In his presentation Dr Muller summarised that the standard 3 test battery for genotoxicity testing was ;

(i) A test for gene mutation in bacteria.

(ii) An *in-vitro* test with cytogenetic evaluation of chromosomal damage with mammalian cells or an *in-vitro* mouse lymphoma tk assay.

(iii) An *in-vivo* test for chromosomal damage using rodent haematopoietic cells.

2.27 There were a number of situations which would require a modification to the three test battery and which might include additional testing. These included situations where there were limitations to the use of bacterial test organisms (eg antibiotics), compounds bearing a structural alerts for genotoxicity, new/unique chemical structural classes, compounds where *in-vivo* tests would not provide useful information (eg compounds that are not systemically absorbed), and compounds which are negative in the three test battery but have shown evidence of carcinogenicity in a bioassay. Dr Muller reviewed the results of the collaborative study on the mouse lymphoma tk assay and most recent investigations regarding the genetic changes in mutant colonise. Overall, the results of this trial had been reported to show that the microtitre version of the mouse lymphoma assay could detect *in-vitro* gene mutagens and clastogens and also non-DNA targeting clastogens and aneugens provided the treatment time was extended to 24 hours. The agar version of the mouse lymphoma assay might not be able to reliably detect clastogens and aneugens .

COM consideration

2.28 The following conclusions were agreed:

(i) The Committee agreed that there was still limited data to justify the routine use of the mouse lymphoma assay to identify potential clastogens particularly in view of a lack of appropriate basic research on the nature of cytogenetic changes induced by clastogens in this test system using the microtitre protocol. Such information could be provided by studies to demonstrate the loss of TK gene.

(ii) There were insufficient data to assess the ability of the mouse lymphoma assay to detect potential aneugens.

(iii) There were still considerable methodological problems particularly concerning the reliability of the soft agar method. Members noted that poor growth conditions particularly in Nobel agar had lead to inadequate detection of small colonies (and hence the potential for inter-laboratory variation in overall results). In addition there was no absolute method for distinguishing between small and large colonies. Members noted the wide range of spontaneous mutant frequencies in the Japanese studies.

(iv) The Committee agreed that colony sizing was an essential element in the quality control of mouse lymphoma assay and had provided positive results with a number of clastogens when tested using appropriate methodology.

(v) The Committee did not wish to oppose the strategy being developed by ICH for human medicines, provided that the need for additional 'mechanistic' validation was recognised and that the status of the assay could be reviewed, in due course, in the light of such data.

Presentation by Dr Jane Cole on the Mouse Lymphoma Assay

2.29 Dr Cole gave a short presentation regarding a poster which she and Dr Martha Moore (from the US EPA) had submitted to the 7th ICEM congress, Toulouse, September. She told the Committee that there were two main themes to consider. Firstly, the differences between the results obtained using microwell and agar techniques and secondly the adaption of mouse lymphoma assay test protocols to detect aneugenic activity. It was noted that the microwell and agar methods were essentially identical except for the way in which cells are cloned to determine the mutant frequency. In general the two

methods gave comparable results when mutant colonies were scored manually by trained personnel. However some agar batches have been shown to have an adverse effect on small colony growth and the use of automated counters could reduce the detection of small colonies in the agar technique, thus underscoring of small colonies might be a significant problem with many earlier tests. There was an urgent need to run the two techniques concurrently for a range of compounds in order to evaluate the extent of the differences between the two techniques. Regarding the detection of aneugens, there was, at present, no logical reason for preferentially using 24 hour exposures to detect aneugens as the standard exposure times of 3-4 hours would in an exponentially growing discontinuous culture result in exposure of all stages of the cell cycle to the compound under test.

2.30 The Committee agreed with their previous conclusion that there were insufficient data to assess the ability of the mouse lymphoma assay to detect potential aneugens. It was agreed that there was a need for mechanistic studies to examine the utility of the mouse lymphoma assay to detect aneugenic activity before any definite conclusions could be drawn regarding its use for screening of pharmaceuticals.

ECETOC Monograph on Aneuploidy

2.31 An ECETOC task group produced a comprehensive review of aneuploidy the results of which have recently been published.[1] This report made recommendations regarding screening of chemicals for aneugenicity which placed much emphasis on the induction of polyploidy in metaphase analysis *in vitro*. It also implied that any concern arising from such studies could be discounted if there was a negative *in vivo* somatic cell micronucleus assay (presumably a bone marrow assay) available. The current COM guidelines stated that the recommended *in-vitro* studies in the screening strategy, which included an *in vitro* metaphase analysis, "did not provide information on interaction with the spindle apparatus or induction of aneuploidy" and thus the Committee was asked to consider the evidence presented in this new report.

2.32 Members considered that the finding of polyploidy in mammalian cells in *in-vitro* metaphase analyses in the absence of cytotoxicity or chromosomal damage was rare and it was possible that such compounds might also have aneugenic potential. However, the Committee agreed that polyploidy in *in-vitro* metaphase tests was not a specific marker for aneugenicity. The following conclusion was agreed.

The ECETOC report entitled 'Aneuploidy: a report of an ECETOC task force[5] provides a valuable review of the published literature on aneugenic chemicals. The Committee considered that there were currently insufficient data to recommend that the finding of polyploidy in *in-vitro* metaphase analyses could be used to specify additional testing for potential aneugenicity. The Committee wished to review the subject when appropriate information from the *in-vitro* micronucleus assay currently under development was available.

Topics under consideration

2.33 The following topics, which were discussed by the Committee at meetings held in 1997, are still under review:

Childhood cancer and paternal smoking.
Aspects of the COM guidelines.
Mutagenicity of ozone.
Mutagenicity of zearalenone.

[5] European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC). Monograph No 27, Aneuploidy, August 1997, Brussels, ISSN -0773-6347-27.

1997 Membership of the Committee on Mutagenicity of Chemicals in Food, Consumer Products and the Environment

CHAIRMAN

Professor J M Parry BSc PhD DSc
Professor of Genetics. School of Biological Sciences, University of Wales, Swansea

MEMBERS

Professor J Ashby BSc PhD CChem FRCS
Senior Research Associate, Zeneca Central Toxicology Laboratory

Dr Peter E Bryant BSc MSc PhD DSc
Cancer Biology Group, School of Biomedical Sciences,
University of St Andrews

Professor C Cooper BSc PhD DSc
Head of Molecular Carcinogenesis Section, Institute of Cancer Research, Haddow Laboratories

Professor D S Davies BSc PhD CChem FRCS FRCPath Hon MRCP
Section of Bio-Chemical Pharmacology, Hammersmith Hospital

Professor M H L Green BSc PhD
MRC Cell Mutation Unit, University of Sussex

Professor R F Newbold BSc PhD CBiol FIBiol
Professor of Cancer Genetics, Dean of Faculty of Science and Head of Department of Biology and Biochemistry, Brunel University

D J Tweats BSc PhD CBiol FIBiol FRCPath
Director of Preclinical Safety Sciences, Glaxo Wellcome Research & Development Ltd

S Venitt BSc PhD
Institute of Cancer Research, Haddow Laboratories

SECRETARIAT

R J Fielder BSc PhD Dip RCPath (Scientific)
K N Mistry (Administrative)

J M Battershill BSc MSc

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

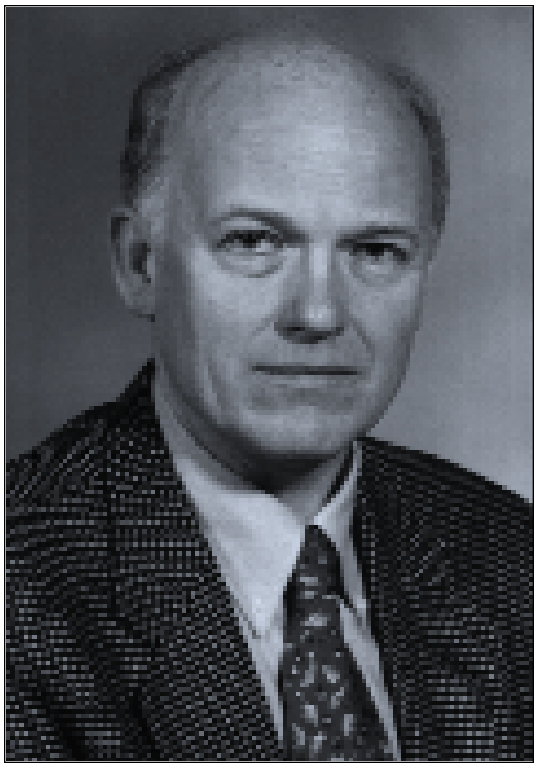
DECLARATION OF INTEREST DURING THE PERIOD OF THIS REPORT

	Personal Interest		Non-Personal Interest	
Member	**Company**	**Interest**	**Company**	**Interest**
Prof J M Parry (Chairman)	Albright and Wilson Compass Catering Jib Insurance National Power Powergen SmithKline Beecham	Share Holder Share Holder Share Holder Share Holder Share Holder Consultant	Boehringer Pfizer Welsh Water Glaxo Wellcome BAT Astra	Grant Grant Grant Grant Grant Grant
Prof J Ashby	Zeneca	Employee,Salary, Share option	NONE	NONE
Dr P Bryant	British Gas	Share Holder	Nuclear Electric Wertlakes Research Institute	Grant Grant
Prof C Cooper	Halifax Norwich Union	Share Holder Share Holder	NONE	NONE

Member	Personal Interest		Non-Personal Interest	
	Company	**Interest**	**Company**	**Interest**
Prof D S Davis	ICI plc ML Laboratories plc Schering Plough, USA Zeneca plc	Share Holder Non Executive Director and Share Holder Consultancy Share Holder	Astra Draco Bayer plc Boehringer Mannheim Bristol Myers Squibb Genentech Glaxo Wellcome Hoechst Marion Roussel Japan T Pharmaceutical Knoll Pharmaceutical Kyowa Hukko Kog Yo Co Leo Pharmaceutical Lilly Research E Merck Merck, Sharpe &Dohme ML Laboratories plc Orion-Farmos Park Davis Pfizer Pharmakopius Procter & Gamble Prodesfarma Rhone-Poulenc Rorer Roche Sanofi Winthrop Servier Shire Pharmaceuticals Zambon Zeneca	Fellowship, Research & Meeting Support Research & Meeting Support Research Support for Meeting Advisor Advisor Research & Studentship Research & Meeting Support Research Research & Meeting Support Research Meeting Support Research Research & Meeting Support Research Fellowship & Research Research & Advisor Research & Support for Meeting Research & Meeting Support Advisor Advisor Research Advisor Research & Meeting Support Research Research & Support for meetings Research Research Research
Prof M Green	BTR Dixons Foreign and Colonial Eurotrust HSBC Holdings Marks & Spencer National Westminster Second Alliance Investment Trust Shell Transport TR Property Investment Trust	Share Holder Share Holder Share Holder Share Holder Share Holder Share Holder Share Holder Share Holder Share Holder	MRC Pension Scheme	Trustee Director

Member	Personal Interest		Non-Personal Interest	
	Company	**Interest**	**Company**	**Interest**
Prof R F Newbold	NONE	NONE	NONE	NONE
Dr D J Tweats	Glaxo Wellcome Halifax Co-operative Bank plc	Salary Employee Share Option Holder Share Holder Share Holder PEP Holder	NONE	NONE
Dr S Venitt	Abbey National plc	Share Holder	NONE	NONE

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



Professor P Blain (Chairman)
BMedSci MB PhD FRCP(Lond) FRCP(Edin) FFOM CBiol FIBiol

PREFACE

The COC evaluates chemicals for their human carcinogenic potential at the request of the Department of Health and other Government Departments. There have been several major pieces of work undertaken by the Committee this year. I would like to highlight the statement to the Scientific Committee on Tobacco and Health (SCOTH) on passive smoking and cancer where the COC advice was crucial to SCOTH in reaching their conclusion on this subject. I wish to thank Members for their considerable efforts in evaluating the data and drafting the statement. The Committee considered a new publication which claimed an association between childhood cancer and drinking water quality in the South West of England. Members concluded that this study was inadequate and provided no evidence to support the authors claims. The Committee considered another epidemiological investigation where an association between childhood cancer and residence near to sources of environmental pollution and agreed that this particular investigation was also inadequate and did not support the claims made by the authors. The Committee also concluded that there was insufficient epidemiological evidence to associate paternal smoking with childhood cancer but noted that the COM considered such an effect was biologically plausible and therefore agreed to keep this subject under review. Regarding tests strategies and evaluation, the Committee commented on the proposal by the International Conference on Harmonisation of the Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) that the carcinogenicity assessment of human medicines required only one long-term carcinogenicity bioassay in a rodent species (usually the rat) in place of the current requirement for carcinogenicity bioassays in two species (usually the rat and the mouse). Part of this work involved a large review of the published literature on mouse carcinogenicity bioassays and a commentary on the new short-term carcinogenicity tests in transgenic mice (which have been proposed as replacement tests for long-term carcinogenicity bioassays in the mouse). Members agreed to publish the Committee's views on these tests in a relevant scientific journal.

This year has also seen the retirement of Professor Richard Carter CBE who served this Committee for many years as chairman and as an expert clinical histopathologist. His guidance and advice will be considerably missed by the Committee and Secretariat.

PETER G BLAIN

Environmental Tobacco Smoke (ETS) and lung cancer

3.1 The Department of Health established the Scientific Committee on Tobacco and Health (SCOTH) in 1994 to review the medical, scientific and behavioural information relating to the harmful effects of tobacco smoking on health. As part of their review, SCOTH considered a submission of data from the Tobacco Manufacturers Association (TMA) regarding exposure to ETS and the occurrence of lung cancer. The COC was asked to provide advice on this submission. The COC statement for SCOTH is reproduced in full below and also in the SCOTH report (published by the Stationary Office, London, 1988, ISBN 0-11-322124-X) . A list of the references cited in the COC statement has been published in the SCOTH report and can also be obtained from the COC secretariat.

Statement by the committee on the carcinogenicity of chemicals in food, consumer products and the environment to scoth on environmental tobacco smoke (ets) and lung cancer

3.2 The Committee reviewed the data on environmental tobacco smoke and lung cancer and agreed the following statement:

Introduction

(i) We have been asked by the Scientific Committee on Tobacco and Health (SCOTH) to review a submission from the Tobacco Manufacturers Association (TMA) comprising a meta-analysis of epidemiological data and supporting references, and a separate meta-analysis paper prepared by Dr A Hackshaw and Professor N Wald (a member of SCOTH). The data provided by the TMA comprised three volumes of reviews and references originally received by the SCOTH secretariat in 1994, and updated in February 1995. A key part of the TMA submission was a meta-analysis of epidemiological studies prepared by Mr P N Lee which was updated in December 1996. The TMA recently submitted 3 additional supplements dated January 1997 dealing with; misclassification bias, dose response (with and without exposed groups), and use of cotinine as a biomarker for exposure to ETS[6]. We considered all of the

[6] Some abbreviations used throughout this statement. ETS = Environmental Tobacco Smoke, MS = mainstream smoke, SS = side stream smoke, TSNA = Tobacco Specific Nitrosamine. NNK = 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone. NNN= N-nitrosonornicotine. NB: Throughout this statement the terms “exposure to ETS” and “passive smoking” have been used interchangeably.

submitted information at two meetings in 1997. A further meta-analysis report prepared by an ad-hoc European Working Group was also considered.We have also considered additional published literature on the formation and composition of ETS, the results obtained in animal experiments involving exposure to surrogates of ETS, and information regarding investigations to evaluate the potential genotoxicity and biological interactions of ETS in humans published up to June 1997.

(ii) Smoking tobacco is the predominant cause of lung cancer with approximately 90% of lung cancer deaths in Western populations attributable to cigarette usage. A lower percentage of lung cancer deaths may be attributed to tobacco smoking in developing non-Westernised populations. A number of epidemiological assessments undertaken by national regulatory agencies have reported a small but statistically significantly elevated relative risk for lung cancer in passive smokers of between 1.1 to 1.3 , whereas other reviewers concluded that the observed association is due to uncontrolled confounding and biases in these analyses. However, since many individuals within the population are exposed to ETS, it is important to resolve the scientific issues particularly as only a small increase in risk would be associated with many hundreds of deaths due to lung cancer per year.

(iii) Regarding the structure of our review, it was agreed to consider firstly the nature and composition of ETS followed by information on exposure and uptake of genotoxic components (eg adduct studies) with particular reference to the lung as the target organ. Finally to critically review the submitted epidemiological meta-analyses. All of the available information has been evaluated in accordance with our guidelines and also with regard to the criteria proposed by Sir Austin Bradford-Hill. These latter criteria, which are listed below, are generally regarded as being valuable in the consideration as to whether or not an association between an outcome (in this case lung cancer) and a putative risk factor (passive smoking) is causal. A specific reference to each of these criteria in respect of passive smoking and lung cancer has been included in our discussion.

Bradford-Hill criteria

Strength
Consistency
Specificity
Temporality
Biological gradient
Plausibility
Coherence
Experiment
Analogy

Composition of ETS

(iv) An essential part of our evaluation concerned the chemical composition of ETS and a comparison of this information with data on the composition of mainstream smoke (MS). There is extensive literature on the presence of chemicals in smoke from cigarettes and other tobacco products and many reviews of this information are available. ETS consists predominantly of aged diluted sidestream smoke (SS) and some exhaled MS with each type of smoke comprising both a particulate and vapour phase. MS is derived from direct inhalation of smoke from the mouth end of a cigarette whilst SS is the material released directly into the air from the burning tip of the cigarette plus that which diffuses through the cigarette paper. The physical and chemical characteristics of ETS are dynamic and differ significantly from MS and fresh SS. The size of ETS particles decreases rapidly with time due to evaporation of volatile constituents and thus ETS particles are usually smaller than MS particles (ETS particles are approximately 0.1-0.25 μm MMAD whereas MS are approximately 0.1-0.9 μm MMAD). The chemical composition of ETS also changes rapidly with aging and dilution. Nicotine, which is tobacco specific, is present predominantly in the vapour phase of ETS (ca 95%) with a relatively small amount in the particulate phase (ca 5%). Concentrations of ETS particulate nicotine rapidly reduce due to evaporation from particles whilst the concentration of nicotine in vapour may reduce due to adsorption onto surfaces.

(v) MS has been the subject of extensive investigation and approximately 4,000 chemicals have been identified to date comprising about 95% of the MS weight. About 10% of these chemicals have been quantified in both MS and SS and these include a lengthy list of known human carcinogens such as

2-naphthylamine, 4-aminobiphenyl, arsenic, hexavalent chromium, vinyl chloride, benzene and a number of genotoxic animal carcinogens that are regarded as potential human carcinogens such as certain polycyclic aromatic hydrocarbons (PAHs, eg benzo(a)pyrene) and nitrosamines (including the Tobacco Specific Nitrosamines (TSNAs) NNK and NNN). Yields per cigarette of some carcinogens have been reported to be greater in SS compared to MS as shown in the table below which presents some selected data from the United States National Research Council (NRC) review. Yields of some individual chemicals including a number of carcinogens present in SS have been reported to be relatively constant between different commercial brands including filter and non filter brands of cigarettes.

Phase	**Amount MS**	**Ratio SS/MS**
Vapour		
Benzene	12-48 µg	2.5-4.7
N-nitrosodimethylamine	10-40 ng	20-100
N-nitrosodiethylamine	ND-25 ng	<40
N-nitrosopyrrolidine	6-30 ng	6-30
Particulate		
2-Naphthylamine	1.7 ng	30
4-Aminobiphenyl	4.6 ng	31
Benzo(a)anthracene	20-70 ng	2-4
Benzo(a)pyrene	20-40 ng	2.5-3.5
N-nitrosonornicotine	100-3,000 ng	0.5-3
NNK	100-1,000 ng	1-4

(vi) One research group documented evidence that the use of filters reduced MS emissions from cigarettes but had little effect on SS emission of a number of carcinogens. Thus some reviewers consider that it is misleading to place too much emphasis on MS/SS ratios. However we consider it important to note that the data suggest that all three types of smoke MS, SS and ETS contain the same carcinogens and although there will be quantitative differences in composition between different types of smoke, it is likely that the exposure of active and passive smokers to carcinogens will be qualitatively similar. A critical review of the available exposure data on ETS with particular consideration of derived doses of carcinogens in the lung (the target tissue) is given below.

Exposure to carcinogens present in ETS

(vii) We have considered the available exposure data with particular consideration of the potential exposure of the lung to ETS particles and carcinogens adsorbed to these particles. Several reviews of ETS exposure studies are available. The majority of these studies have involved either static or personal monitoring of exposure to carbon dioxide, nicotine, total or respirable particles or ETS particles (estimated by UV or florescence light techniques, or as solanesol particulate material; solanesol is a tobacco leaf constituent). There are a number of recent examples of both static monitoring studies and personal monitoring studies. Fewer investigations have reported data on actual exposures to carcinogens present in ETS in field studies (ie under prevailing ambient conditions without manipulating either smoking or environmental conditions). However, there are data to show increased concentrations of carcinogens in indoor air either during or following smoking in respect of benzene, polycyclic aromatic hydrocarbons and nitrosamines thus providing some data on exposure to carcinogens from ETS in field studies. Some reviewers have commented on the poor control for extraneous non tobacco related sources of carcinogens in the available field studies of indoor air. Many of the carcinogens which can be found in indoor air such as benzene, polycyclic aromatic hydrocarbons and some volatile nitrosamines can be derived from several sources other than ETS. Exposure to these chemicals will vary depending on location (ie at home, work, or at public venues, during transportation or resulting from leisure activities), local environmental conditions such as cooking of foods and ventilation, and air pollution. A limited number of exposure studies have reported increased concentrations of TSNAs in ETS, or in SS. One report has documented increased air concentrations of TSNAs (NNK and NNN) derived from ETS in a variety of situations including restaurants, bars and trains.

(viii) Quantifying exposure to the carcinogens in ETS and in particular dose levels in the lung is complicated particularly as the chemical composition of ETS rapidly changes depending on factors controlling the levels of MS and SS such as the number of smokers present, the building or room occupation density, size of building/room, number of cigarette or other tobacco products smoked over a given period, individual smoking patterns (puff rate, inhalation volume and duration) and factors controlling losses such as degradation/modification of vapour and particulate ETS constituents through chemical reaction or UV light, and the dilution of ETS constituents due to ventilation, mixing of components (ie homogeneity of ETS) and/or absorption and desorption from surfaces in the room. To illustrate the high potential for variation in air levels of ETS, the United States National Research Council (NRC) modeled air levels of respirable particles (RSPs, <2.5 μm) for a range of conditions expected to be encountered in private residences with one smoker consuming 1-2 cigarettes per hour and found RSP levels varied by two orders of magnitude from approximately 17-5,000 $\mu g/m^3$.

(ix) A large number of the carcinogens associated with ETS are present in the particulate phase. The fraction of ETS particles deposited in the respiratory tract during passive smoking was reported to be 11 ±4%, ie lower than the fraction of MS particles deposited in the respiratory tract of active smokers (47% ± 13%). Data from the ICRP66 Lung model reported in the Department of Health Committee on Medical Effects of Air Pollutants report on non-biological particles and health suggest that approximately 42% of 0.05 μm particles and 29% of 0.2 μm particles are deposited in the respiratory tract with a significant proportion of these particles reaching the alveoli. These data suggest that ETS particles (ca 0.1-0.25 μm) will penetrate to all regions of the respiratory tract. One group of investigators has calculated that a higher deposition of ETS particles compared to MS particles will occur in the terminal bronchioles and alveoli of the lung. The respiratory epithelium of the human lung contains cells with appropriate metabolising capacity to activate carcinogens associated with ETS particles (for example polycyclic aromatic hydrocarbons, and tobacco specific nitrosamines such as NNK).

(x) Overall we consider that there are sufficient data to conclude that passive smoking results in an increased dose of genotoxic carcinogens to the respiratory tract including the alveolar region of the lung. In the following section we review the available studies which have investigated the biological properties of ETS particles.

Biological properties of ETS

(xi) ETS particles contain adsorbed genotoxic carcinogens. The following section presents a review of the biological properties of ETS particles and in particular an assessment of the mutagenic potential of urine samples obtained under field conditions and an evaluation of studies in animals and individuals exposed to ETS. In considering the available studies of the biological properties of ETS, we have paid particular attention to information which is important in assessing whether passive smoking results in exposure to and activation of genotoxic carcinogens in the lung. We have compared exposure data reported in these investigations with published information from field studies in order to evaluate degree of exposure to ETS, although we note that only a limited assessment of exposure is possible.

Mutagenic chemicals adsorbed to particles

(xii) Several research groups have used air sampling techniques in field studies to collect ETS particles and similar methods to collect SS particles during exposure studies. Solvent extracts made from these particles tested in bacterial mutagenicity tests showed the presence of adsorbed mutagenic chemicals which were active in both the presence and absence of an exogenous metabolising fraction. It is difficult to compare the results of the field studies in view of differing methods used in these investigations, the results of which depend heavily on the rate of smoking, sampling methods, number of particles collected by filters, solvent extraction methods, the mutagenicity test methods adopted and the possible influence of confounding sources of air particles containing adsorbed mutagenic substances. Although data regarding objective measures of actual exposures to ETS in these studies were incomplete, we conclude that the weight of evidence supports the view that exposure to mutagenic particles present in ETS occurs under a wide range of field conditions and therefore it is likely to occur under all conditions of passive smoking.

Studies in animals

(xiii) Exposure of mice to very high levels of fresh SS is clastogenic inducing micronuclei in polychromatic erythrocytes and exposure of rats to very high levels of either aged diluted or fresh SS induces DNA adducts in a variety of tissues such as the heart, lung, larynx and bladder. It has been established that MS is carcinogenic in hamsters and rabbits exposed by inhalation or following

the application of MS condensates to the skin of mice and rabbits or intrapulmonary injection in rats. MS condensates may also act as tumour initiators and promoters in animals. SS is carcinogenic in rats when implanted into the lung or in mice following skin application. The results of the skin painting studies in mice have also suggested that on a gravimetric basis the carcinogenic potential of SS condensate exceeds that of MS condensate. These data show that whole MS and its condensate and SS condensates are carcinogenic in animals and hence we consider it is likely that ETS will also be carcinogenic to animals. However, we note that there are no appropriate life-time bioassays using ETS available to confirm this. We consider that the recent inhalation study where a carcinogenic response was documented in strain A mice exposed to extremely high levels of SS reinforced with some MS was of very limited value and cannot be used to predict hazards to humans. Evidence of reversible hyperplasia and squamous metaplasia in the nasoturbinates accompanied by active chronic inflammation have been documented in short term inhalation studies of aged diluted SS, but the relevance of these findings to the potential carcinogenicity of ETS is unclear.

Studies in humans

(xiv) The biological effects of exposure to ETS have been examined in studies involving the measurement of metabolites of carcinogens and the presence of mutagenic substances in urine from exposed individuals. Other relevant studies have investigated chromosomal aberrations and markers of DNA damage (SCEs) in blood lymphocytes and the detection and quantification of carcinogen adducts with DNA and proteins such as haemoglobin or albumin. It was reported in the previous section of this statement (see paragraphs vii-x) that exposure to ETS occurs by inhalation and ETS particles are deposited throughout the respiratory tract which has the necessary metabolic capability to activate carcinogens present in ETS. We therefore consider that the presence of carcinogens and/or their adducts in blood or urine provides clear evidence of exposure of the lung to the ultimate genotoxic carcinogens.

(xv) There is evidence of a small increase in the concentration of mutagenic substances in urine samples taken from passive smokers in a number of investigations where small groups of individuals were exposed to high levels of ETS for periods of 5-8 hours. Only one of these studies included partial control for dietary confounding which has been reported to affect the excretion of mutagens in the urine of active smokers. A further exposure study where a small group of subjects were maintained on controlled diets did not find a significant increase in the excretion of urinary mutagens following exposure to

high levels of ETS for 8 hours. Limited evidence of increased urinary excretion of mutagenic substances following exposure to ETS has been documented in a small survey of waiters and waitresses and in a small survey of blood donors. No evidence for an increase in chromosome aberrations in peripheral lymphocytes was documented in one study involving waiters exposed to ETS in restaurants or in a number of investigations which considered sister chromatid exchanges in blood lymphocytes.

(xvi) Some more recent studies examined carcinogen DNA or protein adducts in passive smokers. No increase in ^{32}P-postlabelling of DNA was noted in blood monocytes taken from volunteers exposed to high levels of ETS for 8 hours. However, we considered that sampling of blood monocytes was not the most appropriate technique for monitoring exposure to tobacco smoke carcinogens, even in heavy smokers. Protein adducts can serve as surrogates for DNA adducts, particularly at low exposure doses and thus most recent attention has therefore been focused on the measurements of protein adducts. A short resume of the main results from three critical studies is presented below.

(xvii) Crawford et al found a statistically significant increase in protein adducts of polycyclic aromatic hydrocarbons using albumin as a marker in children whose mothers smoked compared to children whose mothers did not smoke. We note that elevated plasma cotinine was also found in children whose mothers smoked and consider that this study was adequately performed. MaClure et al measured adducts of 4-aminobiphenyl (4-ABP)and 3-aminobiphenyl (3-ABP) with haemoglobin following hydrolysis to release these aromatic amines. For 4-ABP adducts there was substantial variability in the results limiting the conclusions that could be drawn. Adducts of 3-ABP were more significantly associated with passive smoking. Hammond et al used the same assay as MaClure et al to examine the levels of 4-ABP haemoglobin adducts in pregnant women. Among non-smokers the levels of 4-ABP adducts increased with exposure to ETS. We have considered the results of this study and the subsequent correspondence relating to it and consider that the investigation was adequately conducted and results obtained were valid. It has been demonstrated that 4-ABP exposure in individuals with no history of occupational exposure to this chemical is predominantly derived from tobacco smoking and thus the results obtained by Hammond et al provide good evidence that low level exposure to ETS can result in the absorption of genotoxic carcinogens. In a separate study Hecht et al found increased excretion of urinary 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL), a specific marker for exposure to the tobacco specific carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) in a small group of 5

individuals exposed to a high level of fresh SS smoke for 3 hours. This study provides good evidence to support the view that passive smoking results in exposure of the respiratory tract to tobacco specific carcinogens.

(xviii) Exposure to ETS over a wide range of exposure levels, including those normally encountered in homes, at work and in public places can lead to the inhalation and delivery of genotoxic carcinogens to all parts of the respiratory tract. Furthermore such compounds will be in contact with cells capable of metabolic activation to produce the proximate carcinogens. These data give rise to concern regarding an increased risk of lung cancer in passive smokers. The available information on passive smokers is consistent with that reported for current cigarette smokers where elevated levels of DNA adducts have been documented in samples of lung tissue. The COC advice on genotoxic carcinogens is to make the prudent assumption that any exposure may be associated with some increased health detriment. This policy is further supported in this specific instance by the approximately linear dose-response relationship between daily consumption of cigarettes by active smokers and lung cancer risk which we consider is consistent with a lack of a threshold. Thus exposure to ETS may be associated with an increased risk of lung cancer, but it is not possible on the basis of these data to make any estimate of the putative increased risk.

Assessment of submitted meta-analysis reports

(ixx) The epidemiological problems regarding passive smoking are common to the evaluation of the potential association between a low level risk factor and disease. These include the requirement for large numbers of individuals in epidemiological investigations in order for the statistical power of these studies to be acceptable with regard to identifying potential associations, the potential for significant confounding by other risk factors for lung cancer, the possibility of publication bias and misclassification bias, and finally the adequacy of exposure estimation. Some research groups have considered it appropriate to evaluate relative risks of lung cancer in passive smokers by undertaking meta-analyses of the available epidemiological data. We note that the two meta-analyses prepared specifically for SCOTH and reviewed in this statement considered essentially the same studies and that the criteria for inclusion and exclusion were clearly stated. We concur with the specific analyses of publication bias which have concluded this is unlikely to be a factor in passive smoking studies, but the potential for dietary confounding, misclassification and measurement error need to be considered. We also note from the meta-analysis reports that there were geographical variations in the rate of misclassification that need to be considered.

(xx) We have previously considered the role of meta-analysis in the evaluation of cancer epidemiology at a COC symposium involving invited epidemiologists in February 1994 where participants agreed that meta-analysis was an improvement on conventional reviews, although the statistical analysis alone was not helpful without an associated qualitative review (unpublished report). The overall conclusion of the meeting was that a report of a meta-analysis must include 3 stages - a full narrative review, the statistical analysis (random effects and mixed models considered most useful) and careful interpretation of the results. These conclusions are in accordance with published reviews on the conduct, reporting and evaluation of meta-analysis of epidemiological studies of carcinogenesis. We have looked at the meta-analysis reports submitted to us in accordance with these criteria.

Meta-analysis prepared for TMA by PN Lee.

(xxi) The authors considered 44 studies, (39 case-control, 5 prospective). Three case-control studies were of a nested design. Data on smoking were available for 6 categories; husband (n=42), wife (n=13), workplace (n=16), childhood (n= 18), social (n=6), and total ETS exposure (n=15). There was a total of 5220 female non-smoking lung cancer patients and 388 male non-smoking patients considered. The authors noted that almost 80% of studies had not found a statistically significant effect. Meta-analysis was performed using a fixed effects model and the published RR estimates. A significant association was found in the 42 studies which evaluated lung cancer in non-smoking women living with husbands who smoked RR = 1.19 (95% CI 1.10-1.27) unadjusted and 1.16 (95% CI 1.08-1.24) based on published adjusted results. Additional calculations were performed using a random effects model which gave essentially the same estimates RR = 1.22 (95% CI 1.11-1.36) unadjusted and 1.24 (95% CI 1.11-1.39) for adjusted data. The analyses were not adjusted for misclassification bias because the authors noted that this varied considerably between investigations according to culture and situation in which questions were asked. A follow-up analysis which adjusted for misclassification of ever smokers as non-smokers (2.5% for US, Western or European and 10% for Asian studies) was conducted with data from 39 studies and reported that the overall risk estimate was not significant when a concordance ratio of 3 was assumed (RR = 1.06, 95% CI 0.98-1.14). Evidence for a dose response was reported for 10/39 case-control studies. The authors found significant heterogeneity between studies (including geographical, time of study, size of study, and quality of study). Although no heterogeneity by type of study (prospective versus case-control) was reported, the authors did report heterogeneity by type of control group in the case

control studies: investigations with hospital or decedent controls had an RR of 1.33 (95% CI 1.16-1.52) compared to an RR of 1.06 (95% CI 0.96-1.16) for studies with general population controls. The authors proposed that misclassification, uncontrolled confounding, publication bias, recall bias, and inconsistencies between cases and controls could explain the weak association and dose response for lung cancer found in non-smoking women living with men who smoked. The authors also noted that there was no statistically significant association between lung cancer and any other exposure index for ETS (ie exposure during childhood or at work). The authors also concluded that "When all the results are considered, and even when meta-analysis is used, the epidemiological data do not support an inference of causality or even genuinely elevated risk."

(xxii) We consider that the meta-analysis prepared for the TMA provides a limited narrative review of the studies included, and note that appropriate sensitivity analyses were not undertaken and also that the overall assessment has been based on meta-analyses using the fixed effects model rather than the preferred random effects model. However, we agree that there is little difference between the results obtained in this paper using either model in respect of the data on smoking by the husband. We note that the results obtained for smoking by the husband [unadjusted RR = 1.19 (95% CI 1.10-1.27) and adjusted RR = 1.16 (95% CI 1.08-1.24)] and smoking by the spouse [unadjusted RR = 1.19 (95% CI 1.11-1.28) and adjusted RR = 1.17 (95% CI 1.09-1.25)] were consistent with the meta-analysis conducted by the US Environmental Protection Agency (EPA). There was generally good consistency of the data when all information on spousal smoking was considered and that geographical heterogeneity may have been introduced by one particular paper from China which reported an implausible protective effect of passive smoking, but the appropriate analyses to investigate this possibility were not available. The meta-analysis report identified potential sources of confounding and bias in epidemiological studies of passive smoking but the only adjustment undertaken was for misclassification bias in a supplementary report. We note that no assessment of the dose-response was undertaken in the meta-analysis and consider that the assessment of dose-response by evaluation of the statistical significance of results obtained in individual investigations was not appropriate as many of these studies had limited statistical power. We also note that the authors had not considered under-estimation bias which would attempt to adjust for the exposure of referent groups to ETS.

Meta-analysis prepared for SCOTH by Hackshaw and Wald

(xxiii) A meta-analysis of 38 studies was undertaken using a random effects model. The reasons for excluding other studies cited in the paper submitted to SCOTH by the TMA were clearly stated. The authors noted that this meta-analysis included 25 more studies that the previous paper on ETS by the same group which was published in 1986. The authors considered that the assessment of ETS exposure in childhood had not been validated and that none of the available epidemiological studies of childhood exposure reported risk of cancer stratified by whether spouse smoked or not. Regarding exposure to ETS at work, the authors noted this varied considerably between different work environments and had not been well defined and was also difficult to quantify in questionnaires. We concur with the authors decision to base their meta-analysis on spousal studies. An interim analysis based on 34 studies reported an overall risk estimate of 1.24 (95% CI 1.11-1.38). In the submitted meta-analysis there were 5 cohort studies and 33 case-control studies. Odds ratios (ORs) were calculated for 30/33 of the case-control studies, the published odds ratio was used for the remaining 3 case-control studies. The authors commented that the calculated ORs were similar to the adjusted relative risk estimates in the published papers. Age-adjusted relative risk estimates were taken from the cohort studies. The majority of results reported in the meta-analysis document considered the 36 studies which presented data on non-smoking women (91% of cohort). There were 4340 lung cancer cases . Nine studies presented separate data for men (263 cases). The pooled RR from the 36 studies was 1.25 (95% CI 1.13-1.38) and was not significantly altered (ie RR = 1.24) if the data for men and for the two studies reporting men and women combined were included. Further meta-analyses using dose response data from 16 of the studies provided evidence of a dose-response based on number of cigarettes smoked per day by spouse and number of years women lived with a spouse who smoked. The findings remained statistically significant after adjustment for misclassification bias using an estimate derived from UK data (7%), underestimation bias (due to some exposure of the reference group to ETS) and dietary confounding.

(xxiv) This paper presents a narrative review of the methods used and the inclusion criteria, but not of the individual studies. We agree that the methods of analysis were acceptable, based on the random effects model. The overall relative risk estimate of 1.25 (95% CI 1.13-1.38) was consistent with the result obtained by the EPA. No evidence of heterogeneity was reported when one particular study (discussed in paragraph 22 above) was excluded from the analysis. The results of this meta-analysis were subject to careful interpretation with appropriate meta-analysis of dose-response undertaken, adjustment for misclassification bias by current and former smokers and for potential dietary confounding.

A sensitivity analysis was performed which suggested that the risk estimate would remain significant, even if a more extreme misclassification rate was assumed. One aspect of the correction for misclassification of ever smokers as non-smokers is of some concern since the data used to make the correction are from the UK. Whether these data are applicable to other, particularly non-westernised, populations is not known. However, we recognise that the best available information was used to adjust for misclassification bias.

(xxv) The authors commented that the excess risk in non-smoking women living with men who smoked is consistent with the calculated level of cotinine (and nicotine) in these individuals compared to active smokers (ca 1%). Preliminary information from a new analysis based on data provided for the 1994 Health Survey for England suggest that the level of cotinine in adults exposed to spousal ETS is about 0.6-0.7% of the level in active smokers. We consider that cotinine is a useful general indicator of recent exposure to ETS but cannot be used to quantify accumulated doses of carcinogens attributable to passive smoking. However the risk analysis in this report based on cotinine was generally supportive of the meta-analysis of the epidemiological data. The authors also stated that the concentrations of major tobacco smoke carcinogens in the blood and urine of passive smokers were higher than in unexposed individuals which is consistent with our evaluation of these data reported in paragraph 17 above.

(xxvi) The report also comments that pathological indicators of lung cancer (examples quoted included basal cell hyperplasia and squamous cell metaplasia) were more prevalent in women living with men who smoke compared to unexposed women. Overall we consider that results of this study and the most recent follow up report support the view that lung tissues from non-smoking women living with smoking men were more likely to show morphological abnormalities in the bronchial epithelium and mucus glands than similar tissues from non-smoking women living with non-smoking men.

Ad-hoc European Working Group report

(xxvii) We also considered a report from an ad-hoc European Working Group. The meta-analysis was only briefly described, for example, there was no narrative explanation of the methods and the account of the criteria for inclusion of studies and evaluation of results was limited. An overall RR of 1.16 (95% CI 1.08-1.25) unadjusted and 1.08 (1.00-1.16) adjusted was reported. The authors subdivided the studies according to regional groups as follows: USA, Europe, China, HongKong and Japan, and considered that there were significant differences in the results between the groups. It is unclear whether

a formal test of heterogeneity was undertaken. We concluded, on inspection of the data provided, that there was unlikely to be significant heterogeneity, but that among studies from China there were some which showed no evidence of any effect. This may well be due to confounding risk factors such as indoor air pollution, particular to those studies. The authors reviewed the composition of ETS and presented an analysis of the components of ETS commenting on the likely exposure to genotoxic carcinogens present in ETS. However, the relevant published adduct studies regarding exposure to ETS or fresh SS reviewed in paragraph 17 above were not considered by the ad-hoc European Working Group in their report. Overall the analysis of the epidemiological data was similar to that presented in the report commissioned for the TMA and hence the Committee agreed that this report did not add any new relevant information to the consideration of passive smoking and lung cancer.

Consideration of submitted meta-analysis reports

(xxviii) Both the submitted meta-analysis reports (prepared for the TMA and for SCOTH) document similar risk estimates that are consistent with the value reported by the EPA in 1992 of between 1.1-1.3. There were however, considerable differences in interpretation of the results. We consider that the Hackshaw and Wald meta-analysis presents a more thorough consideration of the epidemiological data. In particular this document reports a meta-analysis of dose response data and uses the best available data to adjust for dietary confounding, misclassification bias and under estimation of exposure.

Discussion and Conclusion

(ixxx) The consideration as to whether passive smoking is causally related to lung cancer starts from the standpoint that active smoking is recognised as a major cause of lung cancer. There is an approximately linear dose-response between daily consumption of cigarettes by active smokers and lung cancer risk, which we consider is consistent with a lack of a threshold. Moreover active smoking is associated with elevated levels of carcinogen DNA adducts in a number of tissues including the lung and with elevated levels of protein adducts including 4-ABP and TSNA haemoglobin adducts. We conclude that there are sufficient data available to show that ETS contains known genotoxic carcinogens and that exposure to ETS results in increased doses of genotoxic carcinogens to the lung with increased carcinogen-protein adducts also documented in passive smokers. A tabulated summary of the available evidence regarding passive smoking and lung cancer using the criteria established by Sir Austin Bradford-Hill is given overleaf:

Criterion	Evidence regarding passive smoking	Comments
Strength	No	Only a weak effect would be predicted from the nature of exposure. Three separate meta-analyses reviewed in this statement produced a similar overall relative risk of approximately 1.25.
Consistency	Yes	Most of the published meta-analysis studies and the meta-analyses considered in this report show consistent evidence of a small increase in relative risk. No heterogeneity was reported by Hackshaw and Wald when data from one Chinese study was excluded.
Specificity	Not assessed.	Evidence for tumours at other sites has not been considered in this review.
Temporality	Yes	The few prospective studies that were included in the meta-analyses where exposure was assessed prior to diagnosis of lung cancer had results which were consistent with those of retrospective studies.
Biological gradient	Yes	Demonstrated in meta-analysis submitted to SCOTH by Hackshaw and Wald.
Plausibility	Yes	Evidence of exposure of the lung to genotoxic carcinogens present in ETS, data from published reports of increased carcinogen-protein adducts in passive smokers and the linear nature of the dose response for lung cancer in active smokers, indicate it is plausible that ETS induces lung cancer.
Coherence	Yes	The available information on passive smoking (epidemiological, biological monitoring studies for exposure using cotinine, and evidence from protein adduct investigations) are coherent.
Experiment	Limited	Possible only in animals. Limited evidence that SS which predominantly forms ETS is carcinogenic in animals. Sufficient evidence that MS is carcinogenic in animals. No adequate study with ETS.
Analogy	Yes	An association between passive smoking and lung cancer is consistent with the known causal association for active smoking and lung cancer.

(xxx) Taking all the supporting data into consideration we conclude that there is evidence to satisfy six of the nine criteria and limited evidence for one of the remaining criteria (experiment) established by Bradford-Hill to assess the causality of an exposure-disease association. We would not anticipate the strength criteria to be fulfilled, given the low level of exposure, and there are no data with which to assess specificity. In fulfilling the other criteria, we conclude that passive smoking in non-smokers exposed over a substantial part of their life is associated with a 10-30% increase in the risk of lung cancer which could account for several hundred lung cancer deaths per annum in the UK.

(xxxi) Thus in summary our conclusions regarding passive smoking and lung cancer are;

Composition /Exposure

(a) MS, SS and ETS contain the same carcinogens and although there will be quantitative differences in composition between different types of smoke, it is likely that the exposure of active and passive smokers to carcinogens will be qualitatively similar **(Paragraph vi)**. We consider that there are sufficient data to conclude that passive smoking results in an increased dose of genotoxic carcinogens to the respiratory tract and including the alveolar region of the lung **(Paragraph x).**

Biological properties

(b) Exposure to mutagenic particles present in ETS occurs under a wide range of field conditions and is likely to occur under all conditions where passive smoking occurs **(Paragraph xii).**

(c) Whole MS and its condensate and SS condensates are carcinogenic in animals which suggests that ETS will also be carcinogenic to animals. However we note that there are no appropriate life-time bioassays using ETS available **(Paragraph xiii).**

(d) Exposure to ETS over a wide range of exposure levels, including those normally encountered in homes, at work and in public places can lead to the inhalation and delivery of genotoxic carcinogens to all parts of the respiratory tract. Furthermore such compounds will be in contact with cells capable of activation to produce the proximate carcinogens. This gives rise to concern regarding increased carcinogenic risk of lung cancer, although it is not possible to make any quantitative estimate of

risk from these particular data. The COC advice on genotoxic carcinogens is to make the prudent assumption that any exposure may be associated with some increased health detriment, in this case a risk of lung cancer **(Paragraph xviii).**

Submitted meta-analysis reports

(e) Both the submitted meta-analysis reports (prepared for TMA and for SCOTH) document similar risk estimates that are consistent with the value reported by the EPA in 1992 of between 1.1-1.3. However there are considerable differences in interpretation of the results. We are of the view that the paper prepared for SCOTH by Hackshaw and Wald presents a more thorough consideration of the epidemiological data. In particular this document reports a meta-analysis of dose response data and uses the best available data to adjust for dietary confounding, misclassification bias and under estimation of exposure (**Paragraph xxviii).**

Overall conclusion

(f) Taking all the supporting data into consideration we conclude that passive smoking in non-smokers exposed over a substantial part of their life is associated with a 10-30% increase in the risk of lung cancer which could account for several hundred lung cancer deaths per annum in the UK **(Paragraph xxx).**

Leukaemia and Drinking Water in South West England

3.3 In 1996, the Committee advised the Department of Health on a possible association between the occurrence of leukaemia in three children who attended the same class at the Sir James Smith School in Camelford, North Cornwall, and the Lowermoor water contamination incident in July 1988. The Committee concluded that ".. there was no epidemiological or toxicological evidence to support the hypothesis that short term exposure to the chemical contaminants identified in drinking water in the Camelford area following the Lowermoor Incident in 1988, was involved in the aetiology of the three cases of childhood leukaemia under investigation". The Committee agreed to keep this aspect of the Lowermoor incident under review. In this respect the Committee was asked by the Department of Health to review and comment on the recent publication by Foster AM, Prentice AG, Copplestone JA, Cartwright RA and Ricketts C in the European Journal of Cancer Prevention, **6**, 11-19, 1997 entitled "The distribution of leukaemia in association with domestic water quality in South West

England". The authors of this paper had claimed that the standardised incidence ratios of some haematological malignancies differed between geographical areas of water supply in South West England, and that the evidence suggested that this variation might be associated with variation in water quality indicators.

3.4 The Committee concluded that the paper by Foster et al had a number of limitations particularly regarding the quality of exposure data and in respect of the interpretation of the results by the authors. The Committee concluded that the study by Foster *et al* did not alter the COC's previous conclusion regarding the three cases of childhood cancer identified in Camelford, North Cornwall. The specific comments on the Foster *et al* paper were as follows:-

(i) The adequacy of the study was limited by the drawbacks inherent in all ecological studies but, in this instance, particularly with regard to the absence of any direct measure of individual exposures to chemical contaminants in tapwater.

(ii) There were reservations regarding the reliability and variability of the water quality data, and about their aptness as indicators of concentrations of chemical contaminants at the consumers' tap.

(iii) There were concerns regarding the potential inaccuracies in the allocation of patients to defined water supply areas.

(iv) There were reservations regarding the reliability of the statistical analyses used in the study.

(v) Members noted the absence of statistically significant associations when latency between exposure and diagnosis, and disparities in population size, were taken into account.

(vi) Members noted the lack of biological plausibility or other relevant toxicological or epidemiological evidence presented to support the conclusions proposed by the authors.

3.5 The Committee concluded that the study by Foster *et al* did not indicate that copper, fluoride, trihalomethanes or aluminium affected the incidence of leukaemia and related conditions. Members agreed a number of conclusions in respect of the individual chemicals which were considered in the Foster *et al* paper as follows:-

Copper

3.6 The Committee had previously found no evidence for genotoxic or carcinogenic effects of copper in animals, although only limited data were available. Members noted that the concentrations of copper in the present work were low and would account for less than half the daily dietary intake. Foster's study had measured concentrations of copper in the distribution system but, since the use of copper water-pipes in the household was common, it was doubtful that the measurements were a reliable indicator of an individual's exposure from tapwater. The reported association was not found in analyses which attempted to allow for a latent period between exposure and diagnosis. For this reason, the Committee considered that it was improbable that copper in drinking water was a risk factor in myeloproliferative disorders.

Fluoride

3.7 The Committee noted that many toxicological and epidemiological studies of fluoride in relation to mutagenicity and cancer had been published but they provided no evidence of a relationship between ingested fluoride and leukaemia or related conditions. In this study, the associations reported, for the low concentrations of fluoride which occurred naturally in this study, did not persist through all of the analyses, including those which attempted to allow for a latent period between exposure and diagnosis. For these reasons, the Committee considered that the associations did not indicate that fluoride had any effect on the incidence of the conditions concerned.

Trihalomethanes (THMs)

3.8 The Committee noted that many toxicological and epidemiological studies of THMs, chlorination by-products and chlorinated drinking water in relation to mutagenicity and cancer had been published but they provided no evidence of a relationship between these and leukaemia or related conditions. The levels of THMs reported by Foster et al were within the range typically found in chlorinated water supplies in the UK and other countries, and data from these earlier investigations were thus relevant to the situation in question. In the present study, the reported associations did not persist through all of the analyses, and there were insufficient data to permit analyses attempting to allow for a latent period between exposure and diagnosis. For these reasons, the Committee considered that the associations did not indicate that THMs or chlorination had any effect on the incidence of the conditions concerned.

Aluminium

3.9 The Committee considered its conclusions of October 1996, on aluminium in the context of the diagnoses of acute leukaemia in three school children in Camelford, North Cornwall, seven years after the Lowermoor incident in which the drinking water was contaminated with very high concentrations of aluminium sulphate. The levels of aluminium reported by Foster et al were within the range typically found in water supplies in the UK and other countries, and accounted for less than half of the daily dietary intake. The concentrations were far smaller than the peak concentration following the Lowermoor incident. Foster *et al* found no association between levels of aluminium (or any of the other chemicals studied), and acute lymphoblastic leukaemia, the diagnosis in two of the three Camelford school children. No association was found between aluminium and leukaemia and related conditions in their analysis which attempted to allow for a latent period between exposure and diagnosis, and for disparities in population sizes in the water supply areas. In view of these limitations, and because there was no evidence that aluminium salts were carcinogenic or mutagenic (using data from adequately conducted studies), the Committee considered that the results reported in the study did not indicate that aluminium was a risk factor in acute myeloid leukaemia and myelodysplastic syndromes.

Childhood Cancer and paternal smoking

3.10 The Committee was asked by the COM to review three epidemiological papers.[7,8,9] Two of these reported an association between paternal smoking (but not maternal smoking) and an increase in the relative risk for total cancers.[7,82] The third paper documented evidence for an elevated risk for some specific and some groups of cancers (acute lymphoblasitc leukaemia, lymphoma, and brain tumours) in children aged 5 years or less whose fathers had smoked for at least 5 years prior to conception.[9] The COM had been considering the potential mechanisms whereby paternal exposure to mutagens could induce specific groups of cancers in the offspring but agreed that epidemiological advice was required in order to further assess the available data.

3.11 Members agreed the following conclusions.

(i) The Committee considered three recent publications which reported epidemiological investigations regarding the potential association between childhood cancer and paternal smoking.

(ii) Two of the studies were retrospective in design and had been published by one group of investigators from the Institute of Occupational Health and the Department of Public Health and Epidemiology at the University of

Birmingham.[7,8] Cases for these two studies had been ascertained from the Oxford Survey of Childhood Cancers (OSCC) which collated information on childhood cancer deaths (aged 16 years or less) in the UK between 1953-1984. The Committee agreed that the OSCC was a good database to use in analyses of childhood cancer but noted that analyses of paternal smoking was only presented for two periods, 1953-1955 and 1977-1981. The methods used in these two studies were similar and were considered to be adequate, but a number of difficulties in the assessment of retrospective studies applicable to the papers under consideration were evident, namely; unreliability of cancer diagnoses based on death certificates, poor response rate amongst cases, the likelihood of biased recall of smoking status by case parents, and uncertainty regarding the accuracy of data on the smoking habits of fathers particularly with respect to smoking prior to conception. The third study was a large case-control investigation based on cancer registry data from Shanghai, China.[9] The Committee queried the rationale used to categorise children into one of three age groups.

3.12 The Committee subsequently became aware of a further paper by Sorahan et al which was published towards the end of 1997.[10] This new study provided information on paternal smoking for an additional 2587 cases of childhood cancer registered with the OSCC between 1971-1976 and brought the total number of case-control pairs available for study up to 5777. The following conclusion was agreed with respect to this additional paper.

(iii) The Committee agreed that the comments made regarding the two previous studies by Sorahan were also relevant to this new study, although the authors had adjusted these analyses for social class and mobility. The results were consistent with the previous studies by this research group indicating an association between paternal smoking and childhood cancer. A combined analysis of all 5777 case-control pairs reported an overall risk estimate of 1.29 (95% CI (1.19-1.41)).

---

7 Sorahan T, Lancashire R, Prior P, Peck I and Stewart A. Childhood cancer and parental use of alcohol and tobacco. Annals of Epidemiology, **5** 354-359, 1995.

8 Sorahan T, Lancashire, Hulten MA, Peck I and Stewart AM (1997). British Journal of Cancer, **75** 134-138, 1997

9 Ji BT et al (1997). Paternal cigarette smoking and the risk of childhood cancer among offspring of nonsmoking mother. Journal of the National Cancer Institute, **89** 238-244 1997.

10 Sorahan et al (British Journal of Cancer, **76**, 1525-1531, 1997.

3.13 The Committee reached a number of conclusions following a consideration of all the epidemiological data.

(iv) The quality of the diagnostic pathology in these particular studies was uncertain. The statistical analyses were confined to assessment of all cancers or groups of cancers rather than the usual approach of assessing specific cancers.

(v) The Committee noted that evidence of an association between childhood cancer and paternal smoking had been recorded in all of these retrospective studies. However, it considered that no definite conclusions could be drawn particularly in view of the methodological problems noted above. A further concern was the absence of data to support a mechanism by which paternal smoking prior to conception could induce cancer at several sites in the offspring without resulting in other effects on reproduction. Regarding the analysis of specific types of tumours, the Committee agreed that there was some evidence for an association between paternal smoking and CNS tumours and lymphoma in children, but agreed that no definite conclusions should be made since both of these consist of a heterogeneous mixture of tumour types.

(vi) The Committee agreed that further comments should be sought from the COM regarding the mechanisms which could result in the effects documented in these reports.

(vii) The Committee agreed that this subject should be kept under review.

Hazard proximities of childhood cancers in Great Britain (from 1953 to 1980)

3.14 The Committee was asked by the Department of Health to review the methods and results used in the paper by Knox EG and Gilman EA (1997) entitled "Hazard proximities of childhood cancers in Great Britain from 1953-80". Journal of Epidemiology and Community health, vol 51, 151-159. The authors of this paper had claimed that childhood cancers were geographically associated with certain types of industrial atmospheric effluent namely; petroleum derived volatiles and kiln and furnace smoke and gases and effluents from internal combustion engines.

3.15 In outline, the study considered all 22458 deaths from leukaemia or other cancers in children aged 0-15 years in England, Scotland and Wales, between 1953 and 1980. The authors examined the relationship between the birth and death addresses of these children, on a postcode basis, and the location of industrial sites, railway lines and

motorways ("potential environmental hazards") as determined from business directories, with map coordinates obtained from OS maps. Numbers of deaths (and births) at successive radial distances from the "hazards" were counted and compared with expected numbers. Expected numbers were based on a count of all postcodes at similar distances. From the observed and expected results, the authors calculated a parameter called the Standard Density Ratio (SDR) for each successive circle around each hazard type. This analysis was repeated for different hazard types and the results were tested for evidence of systematic centrifugal case density gradients.

3.16 The authors report relative excesses of leukaemias and of solid cancers within 5 kilometres of a fairly disparate range of industrial processes involving the production, refining or use of petroleum fuels and volatile petroleum products, processes using high temperature kilns and furnaces, and near airfields, railways, motorways and harbours. A stronger association was claimed with birth addresses than death addresses. The authors conclude that childhood cancers are geographically associated with two main types of industrial atmospheric effluent: (1) petroleum derived volatiles and (2) kiln and furnace smoke and gases, and effluents from internal combustion engines.

3.17 The Committee agreed the following conclusions:

(i) We note a number of major limitations in the methodology used which we believe prevent any conclusions being drawn from these data. These are listed below:

a. the absence of any reliable 'denominator' data for comparison purposes ie the total number of children at risk in each post code area. This database suggests that the dose-response analysis to examine whether there was a significant decline in incidence with distance from the "hazard", is questionable.

b. the method for identification of 'potential environmental hazards' was imprecise being based on local business directories and map co-ordinates, with no confirmation and no information on potential chemical exposures.

c. it is not appropriate to consider total cancer mortality alone (with no consideration of specific malignancies and their incidence).

(ii) In addition we question the authors suggestion that the results of this study confirm previously published findings with respect to childhood leukaemia and exposure to petroleum fumes since no analysis is carried out of leukaemia incidence on the present study. Further, more appropriate data in regard to the

incidence of lympho-haematopoeitic cancer in the vicinity of major oil refineries will be available from a SAHSU[11] study which will be completed in due course.

(iii) We conclude that it is not possible to draw any conclusions from this study by Knox and Gilman in view of the limitations noted above and we do not consider that any further work is warranted.

Test Strategies and evaluation

3.18 The Committee provided advice on strategies for assessing carcinogenic potential in respect of the ongoing discussions of the Genotoxicity Working Party of the International Conference on Harmonisation of the Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH). The Committee also considered the available information on the use of short-term tests in transgenic mice as potential replacement(s) for the conventional long-term carcinogenicity bioassay in mice. As part of this work, the Committee reviewed the published data on carcinogenicity bioassays in mice using the Gold Carcinogenicity Potency Database (CPD) to identify 'genuine' mouse-specific carcinogens and assess the significance to human health of these chemicals.

ICH guideline on testing for carcinogenicity of pharmaceuticals

3.19 The International Conference on Harmonisation of the Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) is a joint regulatory/industry project to improve, through harmonisation, the efficiency of the process of developing and registration of new human medicines in Europe, Japan and USA. Areas for harmonisation are selected by a Steering Committee, In the context of carcinogenicity assessment the ICH has produced three guidelines which consider the need for carcinogenicity tests, dose selection in carcinogenicity tests and the strategy for testing carcinogenicity of pharmaceuticals. The Committee considered, in detail, the latter of these three guidelines which was entitled ICH harmonised tripartite guideline for the testing for carcinogenicity of pharmaceuticals (S1B). Although, these guidelines specifically dealt with the testing requirements of pharmaceuticals, there were several generic issues raised in the guideline specifically concerning the strategy for the carcinogenicity evaluation of chemicals considered to be mutagenic *in-vivo*, the utility

[11] Small Area Health Statistics Unit, Imperial College School of Medicine, London.

of routinely testing for carcinogenicity in only one species and the use and interpretation of the newly proposed short-term supplementary *in-vivo* tests. The Committee agreed the following conclusions.

(i) The scientific arguments advanced in the document were not sufficiently rigorous to form the basis of a regulatory guideline and it was possible that the ICH guideline could result in increased costs in terms of time and animals used.

(ii) The Committee confirmed its earlier advice that *in-vivo* mutagens should be regarded as potential carcinogens and thus it would not be appropriate to test these chemicals in a long-tern carcinogenicity bioassay.

(iii) The Committee agreed that the choice of the rat as the first test species for carcinogenicity testing was often based on pragmatic considerations. The arguments developed to support the use of the rat as the species of choice in the absence of clear evidence favouring any other species were not adequate. In particular, the statement that the rat was more "sensitive" to carcinogenic compounds than the mouse based on the surveys of pharmaceutical data was based on limited surveys of pharmaceuticals submitted for registration and could not be used to draw conclusions on the species of choice in long-term bioassays. In addition the Committee considered that the claim in the ICH document that there were more background information on carcinogenic mechanisms and metabolism of pharmaceuticals in the rat could not be used as part of the argument to advance the choice of the rat as a test species in preference to the mouse.

(iv) The Committee agreed that there were insufficient data to concluded that the available short-term tests in transgenic mice and the neonatal mouse assay had been validated to a sufficient degree where they could provide additional valuable information regarding carcinogenicity which could not be obtained from a long- term bioassay. The Committee noted that the COM had previously considered the cell transformation assay in detail and had concluded that little value could be attributed to these results in the absence of appropriate supporting mechanistic data.

3.20 Overall the Committee concluded that the ICH guideline on testing of carcinogenicity for pharmaceuticals lacked appropriate scientific rigour to justify its use as a working document in the provision of information to support regulatory decisions. The short/medium term tests could be used as useful research tools in evaluating specific carcinogens but were not currently sufficiently well developed for use in regulatory screening for potential human carcinogens.

Consideration of short-term transgenic mouse models

3.21 The Committee was asked by the Department of Health to consider the available literature from three research groups (namely NTP/NIEHS, ILSI/HESI and CIEA*) on three proposed short-term *in-vivo* test models for assessment of potential carcinogenic activity carcinogenicity in mice (namely Heterozygous $p53^{+/-}$ deficient, Tg.AC model, and Tg Hras 2 model). The International Conference on the Harmonisation of Technical Requirements for the Registration of Pharmaceuticals for human use (ICH) had recently agreed that bioassay data from only one species, (ie the rat), supported by appropriate mutagenicity and pharmacokinetic data and also information from these new proposed short term *in-vivo* screening tests for potential carcinogenicity could be used for the licensing of human medicines (see section 3.19 above). Any decision regarding pharmaceuticals could have significant implications for other categories of chemicals (eg food additives, pesticides, industrial chemicals etc).

3.22 A summary of the Committee's conclusions is given below.

Models considered

(ii) The COC agreed that it was essential to first consider the mechanistic rational underlying the use of individual short term tests in the assessment of chemical carcinogenicity. The following comments were agreed regarding the models considered in detail:

Heterozygous $p53^{+/-}$ deficient: There is a mechanistic rationale for assuming that this model could identify genotoxic chemical carcinogens but there are insufficient data on the mechanism of chemically induced tumours in these mice. For example there is scant information on the effects of different genetic backgrounds on the susceptibility to chemical carcinogens, and data on the status of the remaining p53 gene in such mice are scarce.

Tg.AC model; There is a mechanistic rationale which could potentially support the use of this model to identify chemical carcinogens and also tumour promoters. The skin of these mice exhibit the features of genetically initiated skin since the topical application of tumour promoters or full thickness wounding rapidly induces skin papillomas. This model therefore appears to be very sensitive to skin promoters and to adventitious skin damage, for example shaving mice before application of the test chemical. There

are insufficient data regarding the mechanism of tumour induction in this model to assess the biological significance of such tumours.

Tg Hras 2 model: There is uncertainty about the significance of the integration of five or six copies of the c-Ha-*ras* gene into the CB6F1 mouse in respect of the relevance of the model to the carcinogenic process in humans. There is, at present, no information available on the genetic changes in tumours in the Tg Hras 2 model. It is possible that the Tg H*ras* 2 model could be unduly sensitive. There is no acceptable mechanistic rationale for the use of this model to screen for potential human carcinogens.

(iii) Regarding other models which were briefly considered, it was concluded that there were insufficient data to draw any conclusions regarding the $XPA^{-/-}$ mouse model. Regarding the SHE assay, the Committee on Mutagenicity (COM) had previously expressed concerns at the lack of mechanistic data underlying the relationship between cell transformation in the SHE assay and the carcinogenic process.

(iv) Overall the COC agreed that much of the current research effort had been placed on the evaluation of the three short-term animal model tests systems reviewed in ii) above but little interest had been devoted to the underlying mechanistic basis for these tests and also to the most appropriate transgenic animal model for screening for potential human carcinogens. The Committee agreed that many transgenic models were likely to be developed over the next few years which might be applicable to specific areas of interest, such as the identification of tumour promoters. This was an area to keep under review.

Consideration of validation exercises

(v) The COC considered that it was important to be clear as to mechanistic rationale for the test models under scrutiny as well as he purpose of the validation exercise (for example validation as a screen for potential mouse carcinogens or assessing carcinogenic hazard to humans). Appropriate selection of chemicals was a key aspect of any validation exercise and this has been comprehensively reviewed by Ashby and Paton in Mutation Research, **331**, 27-38, 1995. These authors grouped key chemicals into one of six categories in order to provide a core set of chemicals for the evaluation of the proposed short-term tests in transgenic animals using known carcinogens and non-carcinogens. Members agreed that it was appropriate to compare the approach used by each of the research groups and in particular the chemicals

selected for validation with the information in this paper and then to comment on the results obtained to date. In general the Committee agreed that there was relatively little use of a systematic approach regarding chemical selection as had been suggested by Ashby and Paton. Conclusions regarding the approach used by each research group are given below:

National Institute of Environmental Health Sciences, USA: The selection of chemicals for evaluation in this model is relatively limited and has been restricted to a few carcinogens and non-carcinogens. It is not currently possible to draw any conclusions regarding the evaluation of the heterozygous $p53^{+/-}$ and Tg.AC models. In particular, it is notable that one of the test chemicals used to date in the $p53^{+/-}$ model namely, *N*-methylolacrylamide should be regarded as an *in-vivo* mutagen. The negative findings in the heterozygous $p53^{+/-}$ mouse model with this chemical appear to be inconsistent with the predicted response based on the available mutagenicity data and thus further studies with this chemical are warranted.

International Life Science Institute/Health and Environmental Sciences Institute, USA: This group has used a logical rationale for selecting chemicals in a programme of work which was designed to validate an extensive range of short-term tests in mice as screening tests for the identification of human carcinogens. It is notable that the number of chemicals studied is relatively small and is confined to pharmaceuticals. No results are currently available from this group.

Central Institute for Experimental Animals/National Institute of Health Sciences, Japan: A wide range of chemicals has been selected based on carcinogenic response in one or two rodent species and mutagenic activity in *Salmonella typhimurium* but testing has been limited to the Tg H*ras* 2 model. A more rapid onset and higher incidence of tumours (predominantly lung adenocarcinomas) has been reported for a range of genotoxic carcinogens compared with non-transgenic mice. The mechanism of carcinogenesis in transgenic Tg H*ras* 2 mice and its relevance to the carcinogenic process in humans need to be identified before the significance of these findings to hazard identification can be ascertained.

(vi) The Committee felt that there were several issues which needed to be addressed before definite conclusions could be drawn regarding the validation of these models and before they could be used to support regulatory decisions. These included interlaboratory validation for robustness and reliability using

internationally recognised criteria for validation of alternative assays, for example as recommended by the European Centre for the Validation of Alternative Methods (ECVAM). To this end the evaluation of carcinogen/non-carcinogen pairs using coded chemicals would be of value.

Potential uses and overall conclusions

(vii) The Committee agreed that there was a need for urgent harmonisation between the collaborating research groups to clarify the purposes of the validation exercises and the potential end uses of such tests. Members were concerned that the three transgenic models considered appeared to be highly sensitive to genotoxic carcinogens and questioned whether data from such tests would add much to the information which could be derived from a well conducted *in-vivo* evaluation of mutagenic potential. Dose-response data from tests in transgenic animals might be useful but at present there is no way of interpreting these data and extrapolating them to humans. The Committee considered that the further development and validation of short-term *in-vivo* models to evaluate non-genotoxic carcinogenesis and tumour promoters may be valuable. However, it was likely that there would be less scope for the use of the proposed short-term animal models for other categories of chemicals, such as pesticides and industrial chemicals, where the available supporting information, such as the results of metabolism studies, is likely to be more limited. Hence the development of short-term carcinogenicity tests for these chemicals needed to be considered carefully. The Committee concluded that, in view of the lack of appropriate validation data, it would not be appropriate to use the data from short-term transgenic bioassays considered in this statement to support regulatory decisions at the present time.

[A separate paper giving further information has recently been published] Mutation Research **403,** 259-263, 1998.]

Utility of carcinogenicity bioassays in the mouse

3.23 Animal carcinogenicity bioassays in two species (namely the rat and mouse) have been traditionally used for over two decades as the critical scientific data required to identify potential human carcinogenic chemicals in the absence of adequate epidemiological studies. The International Conference on the Harmonisation of Technical Requirements for the Registration of Pharmaceuticals for human use (ICH) has agreed that bioassay data from only one species, (ie the rat), supported by appropriate mutagenicity and pharmacokinetic data and also information from new (unvalidated)

short term *in-vivo* screening tests for potential carcinogenicity could be used for the licensing of human medicines. The main objective of the COC review was to consider the published literature in order to identify and evaluate the prevalence of definite mouse-specific carcinogens and to present hazard assessments for these chemicals. The Gold Carcinogenicity Potency Database (CPD) was used to identify potential mouse specific carcinogens but databases specific to pharmaceuticals were not considered since these had already been reviewed. The Committee began by considering the advantages and disadvantages of using the CPD and the potential for publication bias in this work (ie are there relevant unpublished carcinogenicity bioassays). The Committee agreed that although publication bias was likely to be a factor, valuable information should emerge from this review since the CPD was the largest published database available (with carcinogenicity data on 1298 chemicals).

3.24 The main conclusions from this review are summarised below.

(i) 76 potential mouse specific carcinogens were identified through the Gold Carcinogenicity Potency Database (CPD). A total of 10 chemicals were excluded from further consideration (three were multispecies carcinogens, five were considered to be non-carcinogenic in the mouse, and the data for two were uninterpretable). The review focused on 66 chemicals. There was equivocal evidence of carcinogenicity to the rat for 28 chemicals and inadequate data for a further 23 chemicals. 15 convincing mouse specific carcinogens were identified (see Table 1).

Table 1: Mouse-specific carcinogens

GROUP	CHEMICALS/ TARGET ORGANS
Mouse-specific: Genotoxic	2,6-Dichloro-p-phenylenediamine (liver M/F) N-methylolacrylamide (Liver, lung, Harderian gland M/F, ovary F)
Mouse-specific: Equivocal evidence of mutagenicity (* based on formation of DNA adducts in target tissue)	2,4-Diaminophenol (kidney M), Dipyrone (Liver) Ozone (Lung M) Vinylidene chloride (lung) Zearalenone* (liver F, pituitary M/F)
Mouse-specific: Mutagenicity data inadequate.	Benzaldehyde (forestomach M/F), Piperonyl sulphoxide (Liver M), Ripazepam (Liver M),
Mouse-specific: Not genotoxic	2-Aminobiphenyl (Vascular M/F), Captan (Duodenum M/F), Dieldrin (Liver M/F), Diethylhexyladipate (Liver M/F), Probenicid (Liver F).

(ii) It was suggested that the two genotoxic mouse carcinogens **(2,6-Dichloro-p-phenylenediamine and N-methylolacrylamide)** would have been considered as potential carcinogens in the absence of a mouse bioassay. Regarding the five non-genotoxic mouse specific carcinogens; three induced tumours in mouse liver only and were considered as being of low potential hazard to human health. The remaining 2 chemicals would have been missed in the absence of a mouse bioassay (**2-aminobiphenyl and captan)**. Overall it is suggested that these four chemicals would represent good candidates for evaluation in the short term bioassays in transgenic mice currently being validated.

(iii) The potential health hazards of the mouse-specific carcinogens with equivocal or inadequate mutagenicity data could not be fully evaluated particularly in view of the need for adequate mutagenicity data in addition to adequate carcinogenicity data. The Committee noted new data which reported specific mutations in lung tumours from mice exposed by inhalation to ozone and asked the COM for further advice on this chemical. The Committee was also made aware of new data reporting DNA adducts in mice dosed with zearalenone and asked the COM for further advice on this chemical. The COC

agreed to further consider ozone and zearalenone when appropriate advice from the COM was available. For the purposes of the current review it was agreed to include both ozone and zearalenone in a separate group of "potential" *in-vivo* genotoxins.

(iv) Regarding the 28 mouse-carcinogens that gave an equivocal response in long-term carcinogenicity bioassays in the rat, it was noted that many of the chemicals in this group induced liver tumours in the mice probably through a non-genotoxic mechanism and thus may be considered of relatively low significance to humans. There were nine chemicals with evidence for mutagenicity. For these nine chemicals, both the mouse and rat studies provide useful information with regard to assessing the potential significance of these chemicals to human health. In the event that a mouse bioassay were not available, it is probable that the structure of these chemicals and/or mutagenicity data would have indicated that the equivocal response in the rat should be considered as a significant evidence of carcinogenic activity.

(v) Other general conclusions of interest included:

a) The selection of database (ie inclusion criteria for chemicals evaluated), the quality of available carcinogenicity and other toxicological data, and the criteria for defining negative, equivocal or positive responses in carcinogenicity bioassays can all influence the identification of convincing mouse specific carcinogens.

b) In view of the potential for publication bias and the large variation in quality of carcinogenicity data, it was not possible to accurately predict the overall concordance between mouse and rat bioassays from the currently available databases.

c) A key outcome of the review was the lack of adequate published mutagenicity data for 55/76 of the potential mouse-specific carcinogens identified from the Carcinogenicity Potency Database. This is surprising given the clear value of such data in interpreting bioassay data and the much greater resources required for carcinogenicity bioassays.

Overall conclusion on mouse-specific carcinogens.

(vi) The key public health issue is whether the omission of the mouse bioassay from the present convention of carcinogenicity testing in the rat and the mouse

would result in a reduction in the quality of decisions on human health hazard assessment. The COC review identified only 15 convincing mouse-specific carcinogens out of 559 chemicals originally identified with published two-species carcinogenicity data. Of these 15, a potential human health hazard is possible for the two mouse-specific carcinogens that were definitely genotoxic *in-vivo*, whereas on the available data, the human health hazards for the remaining 13 chemicals are minimal or at most equivocal. These conclusions must be viewed within the limitations of the current review and the regulatory context within which long-term mouse carcinogenicity studies may be requested. The limited quality and quantity of published carcinogenicity and mutagenicity data have been noted. It is possible that data held in regulatory files or by industry could identify further mouse-specific carcinogens, some of which might be potentially significant for human health. The COC review was undertaken in the context of the proposals by the ICH for regulatory testing of human medicines. Under these guidelines, there would be a considerable amount of supporting data available to assist in hazard assessment of human medicines which might not be available for other categories of chemicals. It would thus be valuable to consider further information from the results of carcinogenicity bioassays on different chemical categories such as pesticides, and industrial chemicals before a definitive answer regarding the value of mouse carcinogenicity can be derived.

[A detailed paper summarising the working documents used by the Committee has subsequently been published in Human and Experimental Toxicology April 1998, volume 17, pp 193-205.]

Topics Under Consideration

3.25 The following topics, which were discussed by the Committee at meetings held in 1997, are still under review:

Alcohol and breast cancer.
IARC monograph on 2,3,7,8-tetrachloro-dibenzo-p-dioxin.
Ozone
Paternal smoking and childhood cancer.
Zearalenone

1997 Membership of the Committee on Carcinogencity of Chemicals in Food, Consumer Products and the Environment

CHAIRMAN

Professor P G Blain BMedSci MB PhD FRCP(Lond) FRCP(Edin) FFOM CBiol FIBiol
Head of Department of Environmental and Occupational Medicine, University of Newcastle

MEMBERS

Professor C E D Chilvers BSc(Econ) DSc Hon MFPHM
The Division of Public Health Medicine & Epidemiology, Queens Medical Centre, Nottingham

Professor C Cooper BSc PhD DSc
Head of Molecular Carcinogenesis Section, Institute of Cancer Research, Haddow Laboratories

Professor A D Dayan BSc MD FRCPath FFOM FFPM CBiol FIBiol
Director of DH Toxicology Laboratory, St Bartholomew's Hospital Medical College

Professor P B Farmer MA DPhil CChem FRSC
Professor of Chemistry, MRC Toxicology Unit, University of Leicester

Professor D Forman BA PhD Hon MFPHM
Professor of Cancer Epidemiology, Centre for Cancer Research, University of Leeds

Dr Sandra Jane Kennedy BSc PhD FRCPath CBiol FIBiol
Director of Pathology, Quintiles England Limited

Professor R F Newbold BSc PhD CBiol FIBiol
Professor of Cancer Genetics, Dean of Faculty of Science and Head of Department of Biology and Biochemistry. Brunel University

Professor J M Parry BSc PhD DSc
Professor of Genetics, School of Biological Sciences, University of Wales, Swansea

I F H Purchase OBE BVSc MRCVS PhD FRCPath CBiol FIBiol
Director, Zeneca Central Toxicology Laboratory

Professor A G Renwick BSc PhD DSc
Professor of Biochemical Pharmacology, University of Southampton

S Venitt BSc PhD
Institute of Cancer Research, Haddow Laboratories

Professor G T Williams BSc MD FRCP FRCPath
Department of Pathology, University of Wales College of Medicine

SECRETARIAT

J M Battershill BSc MSc (Scientific)
K N Mistry (Administrative)

R J Fielder BSc PhD Dip RCPath

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

DECLARATION OF INTEREST DURING THE PERIOD OF THIS REPORT

Member	Personal Interest		Non-Personal Interest	
	Company	**Interest**	**Company**	**Interest**
Prof P G Blain (Chairman)	NONE	NONE	Unilever plc	Research Studentship
Prof C Chilvers	ICI plc Tomkins Morgan Crucible Lloyds TSB British Telecom UTD Assurance Group Hardy's & Hanson Glaxo-Wellcome Shell	Share Holder Share Holder Share Holder Share Holder Share Holder Share Holder Share Holder Share Holder Share Holder	Merck, Sharpe & Dohme	Research Support
Prof C Cooper	Halifax Norwich Union	Share Holder Share Holder	NONE	NONE
Prof A D Dayan	British Petroleum Glaxo-Wellcome Schering Plough SmithKline Beecham TI	Share Holder Pension Consultant Consultant Share Holder	NONE	NONE
Prof P B Farmer	British Gas National Power Powergen Centrica Chiroscience Abbey National plc Woolwich Alliance & Leicester	Share Holder Share Holder Share Holder Share Holder Share Holder Share Holder Share Holder Share Holder	Glaxo SmithKline Beecham	Research Studentship & Research Support Collaborative - Studentship
Prof D Forman	Halifax Woolwich	Share Holder Share Holder	NONE	NONE
Dr S J Kennedy	Unilever Quintiles	Share Holder Share Holder	NONE	NONE
Prof R F Newbold	NONE	NONE	NONE	NONE
Prof J M Parry	Albright and Wilson Compass Catering JIB Insurance National Power Powergen SmithKline Beecham	Share Holder Share Holder Share Holder Share Holder Share Holder Consultant	Boehringer Pfizer Welsh Water Glaxo - Wellcome BAT Astra	Grant Grant Grant Grant Grant Grant
Dr I F H Purchase	Celltech ICI plc Zeneca British Gas British Telecom	Share Holder Consultant Employee Share Holder Share Holder	NONE	NONE

Member	Personal Interest		Non-Personal Interest	
	Company	**Interest**	**Company**	**Interest**
Prof A G Renwick	International Sweeteners Association	Consultant	Hoffmann-La Roche Unilever SmithKline Beecham Pfizer FEMA	Research Support Research Support Research Support Grant Grant
Dr S Venitt	Abbey National plc	Share Holder	NONE	NONE
Prof G T Williams	Abbey National plc Norwich Union plc AMP Ltd	Share Holder Share Holder Share Holder	NONE	NONE

Annex 1

TERMS OF REFERENCE

To advise at the request of:

Department of Health
Ministry of Agriculture, Fisheries and Food
Department of the Environment, Transport and the Regions
Department of Trade and Industry
Health and Safety Executive
Medicines Control Agency: Section 4 Committees and the Licensing Authority
Committee on the Medical Aspects of Food Policy
Home Office
Scottish Office Agrculture, Environment and Fisheries Department
Scottish Office Health Department
Welsh Office
Northern Ireland Office Health and Social Services Department
Other Government Departments

1. To assess and advise on the toxic risk to man of substances which are:

   a. used or proposed to be used as food additives, or used in such a way that they might contaminate food through their use or natural occurrence in agriculture, including horticulture and veterinary practice or in the distribution, storage, preparation, processing or packaging of food;

   b. used or proposed to be used or manufactured or produced in industry, agriculture, food storage or any other workplace;

   c. used or proposed to be used as household goods or toilet goods and preparations;

   d. used or proposed to be used as drugs, when advice is requested by the Medicines Control Agency, Section 4 Committee or the Licensing Authority;

   e. used or proposed to be used or disposed of in such a way as to result in pollution of the environment.

2. To advise on important general principles or new scientific discoveries in connection with toxic risks, to co-ordinate with other bodies concerned with the assessment of toxic risks and to present recommendations for toxicity testing.

Annex 2

DECLARATION OF INTERESTS: A CODE OF PRACTICE FOR MEMBERS

INTRODUCTION

1. This Code of Practice is intended to act as a guide for members of these three Committees as to the circumstances in which they should declare an interest in the chemical industry.

2. The advice of these Committees concerns matters which are connected with the chemical industry and it is therefore desirable that members should have a good understanding of the work of the industry. It is also desirable that some members should have practical experience of the scientific problems of product development. To avoid any public concern that commercial interests might affect the advice of the Committees it has been decided that the arrangements which govern relationships between members and the chemical industry and information on significant and relevant interests should be on public record.

DEFINITIONS

3. In this code, 'the chemical industry' means

   a. companies, partnerships or individuals who are involved with the manufacture, sale or supply of products subject to the following legislation:-

      The Food Safety Act 1990

      The Medicines Acts 1968 and 1971

      The Food and Environmental Protection Act 1985

      The Consumer Protection Act 1987

      The Cosmetic (Safety) (Amendment) Regulations 1987

      The Notification of New Substances Regulations 1982

b. trade associations representing companies involved with such products,

c. companies, partnerships or individuals who are directly concerned with research, development or marketing of a product which is being considered by the Committees on Toxicity, Mutagenicity or Carcinogenicity of Chemicals in Food, Consumer Products and the Environment.

4. In this code 'the Secretariat' means the Secretariat of the relevant Committee.

DIFFERENT TYPES OF INTEREST

5. There are a number of different types of interests and the following is intended only as a guide.

A **personal interest** involves payment to the member personally. The main examples are:-

Consultancies: any consultancy, directorship, position in or work for the chemical industry, which attracts regular or occasional payments in cash or kind.

Fee-Paid Work: any work commissioned by the chemical industry for which the member is paid in cash or kind.

Shareholdings: any shareholding in or other beneficial interest in shares of the chemical industry. This does not include shareholdings through unit trusts or similar arrangements where the member has no financial management.

A **non-personal interest** involves payment which benefits a department for which a member is responsible, but is not received by the member personally. The main examples are:-

Fellowships: the holding of a fellowship endowed by the chemical industry.

Support by industry: any payment, other support or sponsorship by the chemical industry which does not convey any pecuniary or material benefit to a member personally but which does benefit their position or department, for example:-

i. a grant from a company for the running of a unit or department for which a member is responsible;

ii. a grant or fellowship or other payment to sponsor a post or a member of staff in the unit for which a member is responsible. This does not include financial assistance for students;

iii. the commissioning of research or other work by, or advice from, staff who work in a unit for which the member is responsible.

Trusteeship: where a member is a trustee of a charity with investments in the chemical industry, the Secretariat can agree with the member a general declaration to cover this interest rather than draw up a detailed portfolio.

6. Members are under no obligation to seek out knowledge of work done for or on behalf of the chemical industry within departments for which they are responsible if they would not normally expect to be informed.

7. Members should inform the Department in writing when they are appointed of their *current personal* and *non-personal* interests. Only the name of the company and the nature of the interest is required; the amount of any salary, fee, shareholding, grant etc need not be disclosed to the Department. An interest is current if the member has an on-going financial involvement with the chemical industry, eg if he or she holds shares in a chemical industry, has a consultancy contract, or if the member or the department for which he or she is responsible is in the process of carrying out work for the chemical industry. Members are asked to inform the Department at any time of any change in their *personal* interests, and will be invited to complete a declaration form once a year. It would be sufficient if changes in *non-personal* interests are reported in the annual declaration form following the change. (Non-personal interests involving less than £1000 from a particular company in the previous year need not be declared to the Department.)

8. Members are required to declare relevant interests at Committee meetings, and to state whether they are personal or non-personal interests and whether they are specific to the product under consideration or non-specific.

a. A member must declare a *personal specific* interest if he or she has *at any time* worked on the product under consideration and has personally received payment for that work, in any form, from the chemical industry. If the interest is no longer current, the member may declare it as a *lapsed personal specific* interest. The member may then only take part in the proceedings at the Chairman's discretion.

b. A member must declare a *personal non-specific* interest if he or she has a *current* personal interest in the company concerned which does not relate

specifically to the product under discussion. The member may then only take part in the proceedings at the Chairman's discretion.

c. A member must declare a *non-personal specific* interest if he or she is aware that the department for which he or she is responsible has at any time worked on the product but the member has not personally received payment in any form from the industry for the work done. The member may then take part in the proceedings unless the Chairman should decide otherwise.

d. A member must declare a *non-personal non-specific* interest if he or she is aware that the department for which he or she is responsible is *currently* receiving payment from the company concerned which does not relate specifically to the product under discussion. The member may then take part in the proceedings unless the Chairman should decide otherwise.

9. If a member is aware that a product under consideration is or may become a competitor of a product manufactured, sold or supplied by a company in which the member has a *current personal* interest, he or she should declare the interest in the company marketing the rival product.

10. A member who is in any doubt during a meeting as to whether he or she has an interest which should be declared, or whether to take part in the proceedings, should ask the Chairman for guidance. The Chairman has the power to determine whether or not a member with an interest shall take part in the proceedings.

11. If the Chairman should declare an interest of any kind he or she should stand down from the chair for that item and the meeting should be conducted by the Deputy Chairman.

RECORD OF INTERESTS

12. A record is kept in the Department of names of members who have declared interests to the Department on appointment, as the interest first arises or through the annual declaration, and the nature of the interest.

13. It is the responsibility of individual members to declare all relevant interests. The Secretariat does not check whether members have done so. However, members can seek advice from the Secretariat if they have any doubts as to whether or not an interest should be declared.

Annex 3

GLOSSARY OF TERMS

ACUTE Describes a disease of rapid onset, severe symptoms and brief duration.

ADDUCT A chemical grouping which is covalently bound (strong bond formed by the sharing of a pair of electrons) to a large molecule such as DNA (qv) or protein.

ADENOCARCINOMA A malignant tumour arising from the epithelia (qv) (see 'tumour').

ADI Acceptable daily intake, defined as 'An estimate of the amount of a food additive, expressed on a body weight basis, that can be ingested daily over a lifetime without appreciable health risk'.

Ah RECEPTOR The Ah (Aromatic hydrocarbon) receptor protein regulates gene expression. The identity of the natural endogenous chemical which bind to the Ah receptor are unknown. A range of chemical such as chlorinated dibenzodioxins and polychlorinated biphenyls bind to Ah receptor. The available research suggests that binding to the Ah receptor is an intrical part of the toxicological mechanism of these compounds.

ALANINE AMINOTRANSFERASE An enzyme that, when elevated activity is detected in serum, may indicate damage to certain organs.

ALLELES Alternative forms of a gene found at the same locus on homologous chromosomes.

AMES TEST *In vitro* (qv) assay for bacterial gene mutations (qv) using strains of Salmonella typhimurium developed by Ames and his colleagues.

ANEUGENIC Inducing aneuploidy (qv).

ANEUPLOIDY The circumstances in which the total number of chromosomes within a cell is not an exact multiple of the normal haploid (see 'polyploidy') number. Chromosomes may be lost or gained during cell division.

ASPARTATE AMINOTRANSFERASE An enzyme that, when elevated activity is detected in serum, may indicate damage to certain organs.

BIAS An inference which at any stage of an epidemiological investigation tends to produce results that depart systemically 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.

ß-ISOMER Isomers are two or more chemical compounds with the same molecular formula but having different properties owing to a different arrangement of atoms within the molecule. The ß-isomer of alitame is formed when the compound degrades and the atoms within the molecule are rearranged.

BIOAVAILABILITY A term referring to the proportion of a substance which reaches the systemic circulation unchanged after a particular route of administration.
BIOMARKER A readily measurable biological concentration or similar quantity which acts as a surrogate for a biological effect.

BOLUS DOSE When all the test substance is administered at the same time ie as a single dose.

BRONCHIAL Relating to the air passages conducting air from the trachea (windpipe) to the lungs.

CARCINOGENICITY BIOASSAY Tests carried out in laboratory animals, usually rats and mice, to determine whether a substance is carcinogenic. The test material is given, usually in the diet, throughout life to groups of animals, at different dose levels.

CARCINOGENIC POTENCY The magnitude, with respect to dose, of the carcinogenic activity of a chemical in the species under consideration. Inherent in this definition are a dose descriptor (defined here as a dose needed to induce tumours) and other elements bearing on carcinogenic potency including dose-response relationships, site/species/strain/gender activity and degree of malignancy, mechanisms including genotoxicity, other mechanistic information relevant to humans and toxicokinetic properties of the substance.

CARCINOGENESIS The origin, causation and development of tumours. The term applies to all forms of tumours, benign as well as malignant (see 'tumour') and not just to carcinomas (qv).

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 *genotoxic* (qv) carcinogens which have been shown to react directly with and mutate DNA, and *non-genotoxic* 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 activated 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.

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').

CELL TRANSFORMATION ASSAY See 'Transformation'.

CENTRILOBULAR HYPERTROPHY Enlargement of cells surrounding the central vein in a liver lobule

CHROMOSOMAL ABERRATION Deviation from the normal structure of chromosomes (qv) (see 'clastogen').

CHRONIC Describing a disease of long duration involving very slow changes. Such disease is often of gradual onset. The term does not imply anything about the severity of the disease.

CLASTOGEN An agent that produces chromosome breaks and other structural aberrations such as translocations (qv). 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.

COHORT STUDY (Synonyms - follow-up, longitudinal, prospective study) The method of epidemiological study in which subsets of a defined population can be identified who may be exposed to a factor or factors hypothesized to influence the probability of occurrence of a given disease. An essential feature of the method is observation of the population for a sufficient number of person-years to generate reliable incidence or mortality rates in the population subsets. This generally implies study of a large population and/or study for a prolonged period of time.

CONGENER Compounds varying in chemical structure but with similar biological properties

COVALENT The type of binding formed by the sharing of an electron pair between two atoms. Molecules are combinations of atoms bound together by covalent bonds.

CYTOGENETIC Concerning chromosomes, their origin, structure and function.

CYTOGENETICS ASSAY An assay for detecting damage to the genome of a cell

DELETION Usually a chromosome aberration in which a proportion of a chromosome is lost.

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, each nucleotide consisting of 3 elements: a pentose sugar, a phosphate group and a nitrogenous base derived from either purine (adenine, guanine) or pyrimidine (cytosine, thymine).

DOMINANT LETHAL ASSAY See 'Dominant Lethal Mutation'.

DOMINANT LETHAL MUTATION A dominant mutation that causes death of an early embryo.

ELECTRON MICROSCOPY A type of microscopy with higher magnification than is possible with the ordinary light microscope.

EMULSIFIER A compound or preparation that facilitates the formation of emulsions

ENDOCRINE ACTIVITY Affecting the bodies response to, or formation of, hormones

EPIDEMIOLOGY Study of the distribution and, in some instances, the causal factors of disease in communities and populations. Originally confined to infectious diseases -epidemics - but now increasingly applied to non-infectious conditions such as cancer.

EPITHELIA The tissue covering the outer surface of the body, the mucous membranes and cavities of the body.

EPITHELIUM The tissue covering the outer surface of the body, the mucous membranes and cavities of the body.

EPOXYRESINS A type of plastic

ERYTHROCYTE Red blood cell.

EXOGENOUS Arising outside the body.

F1 First filial generation - offspring resulting from the (specified) parental generation.

FORESTOMACH See 'glandular stomach'.

GENE The functional unit of inheritance: a specific sequence of nucleotides along the DNA molecule, forming part of a chromosome.

GENETICALLY MODIFIED ORGANISM An organism which has had genetic material from another species inserted into its cells.

GENOMIC INSTABILITY Describes cells or tissues where the controls which maintain the integrity of the genome (DNA and chromosomes) have failed leading to changes in structure or quantity of DNA in the cell. Tumour cells frequently show genomic instability.

GENOMIC IMPRINTING Describes changes in genes that occur during passage through sperm or egg with the result that the paternal and maternal alleles have different properties. Maybe caused by different methylation patterns of DNA.

GENOTOXIC The ability of a substance to cause DNA damage, either directly or after metabolic activation (see also 'carcinogens).

GERM CELL Reproductive cell eg spermatid.

GLANDULAR STOMACH The stomach in rodents consists of two separate regions - the fore stomach and the glandular stomach. The glandular stomach is the only area directly comparable to human situations.

GUT MICROFLORA The colony of micro-organisms found in the gastrointestinal tract.

HAEMATOPOIETIC Concerning the production of formed elements - red cells, white cells and platelets - in the circulating blood.

HAEMOGLOBIN A pigment contained within red blood cells which is the medium by which oxygen is transported within the body.

HEPATOCARCINOGEN A chemical, or other agent or factor, causing cancer of the liver.

HEPATOCELLULAR Relating to the cells of the liver.

HEPATOCYTE The principal cell type in the liver, possessing many metabolizing enzymes (see 'metabolic activation').

HYDROLYSIS The breakdown of a chemical by water into simpler products.

HYPERPLASIA An increase in the size of organs and tissues due to an increase in the total numbers of the normal cell constituents.

HYPERTROPHY An increase in the size of cells or tissues.

HYPOTHYROIDISM Clinical condition in which the activity of the thyroid is low.

INTRAPERITONEAL Within the abdominal cavity.

IN VITRO A Latin term used to describe effects in biological material outside the living animal.

IN VIVO A Latin term used to describe effects in living animals.

ISOMERS See 'ß-isomer'.

LEUKAEMIA A group of neoplastic disorders (see tumour) affecting blood-forming elements in the bone marrow, characterised by uncontrolled proliferation and disordered differentiation (qv) or maturation (stage which forms final cell types). Examples include the lymphocytic leukaemias which develop from lymphoid (qv) cells and the myeloid leukaemias which are derived from myeloid cells (producing red blood cells, mainly in bone marrow).

LIPOPHILIC 'Lipid liking' - a substance which has a tendency to partition into fatty materials.

LOAEL Lowest Observed Adverse Effect Level, the lowest administered dose at which a toxic effect has been observed.

LYMPHOCYTE Type of white blood cell.

LYMPHOID TISSUE Tissue which produces lymphocytes (qv), it is widely distributed in the body eg spleen, lymph nodes.

LYMPHOMA Malignant tumours arising from lymphoid tissues. They are usually multifocal, involving lymph nodes, spleen, thymus and sometimes bone marrow and other sites outside the anatomically defined lymphoid system. (See also 'tumour').

MALIGNANCY See 'tumour'.

META-ANALYSIS A statistical procedure to summarise quantitative data from several different epidemiological studies. It is most commonly used in summarising epidemiological evidence with respect to disease incidence.

METABOLIC ACTIVATION Conversion by enzymes of a chemical from one state to another, for example by chemical reactions such as hydroxylation, epoxidation or conjugation. The term is used in a more narrow sense to describe the addition of a mammalian cell free preparation from livers of rats pre-treated with a substance which stimulates production of metabolising enzymes. These preparations are added to *in vitro* short term tests to mimic the metabolic activation typical of mammals.

METABOLITE Product formed from the original compound by enzymic reactions in the body/cell.

METALLOTHIONEIN A protein capable of binding ions of metals, e.g. zinc or cadmium.

METAPHASE Stage of cell division (mitosis and meiosis) during which the chromosomes are arranged on the equator of the nuclear spindle (the collection of microtubule filaments which are responsible for the movement of chromosomes during cell division). As the chromosomes are most easily examined in metaphase, cells are arrested at this stage for microscopical examination for chromosome aberrations (qv) - known as metaphase analysis.

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 (qv). 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'.

MITOSIS The type of cell division which occurs in somatic cells when they proliferate. Each daughter cell has the same complement as the parent cell.

MONOCYTE A type of white blood cell.

MONOMER A single unit (as opposed to a polymer which is built up by the repeated union of many monomer molecules) which when polymerised forms a plastic.

MOUSE LYMPHOMA ASSAY An *in vitro* assay for gene mutation in mammalian cells using a mouse lymphoma cell line L5178Y, which is heterozygous for the gene (carries only one functional gene rather than a pair) for the enzyme thymidine kinase ($TK^{+/-}$). Mutation of that single gene is measured by resistance to toxic trifluorothymidine. Mutant cells produce two forms of colony - large, which represent mutations within the gene and small, which represent large genetic changes in the chromosome such as chromosome aberrations. Thus this assay can provide additional information about the type of mutation which has occurred if colony size is scored.

MUTATION A permanent change in the amount or structure of the genetic material in an organism which can result in a change in the characteristics of the organism. The alternation 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.

MYCOTOXIN Toxic compound produced by a fungus.

MYELODYSPLASTIC SYNDROME Aquired clonal and progressive pancytopenclas associated with hypercellular bone marrow and ineffective haematopotesis.

NEONATE A newborn.

NEPHROTOXICITY Toxicity to the kidney.

NEUROBEHAVIOURAL Of behaviour determined by the nervous system.

NEUROTOXICITY Toxicity to the nervous system.

NOAEL No Observed Adverse Effect Level, the highest administered dose at which no toxic effect has been observed.

NON-GENOTOXIC See ‘carcinogens’.

OESTROGEN ACTIVITY Hormonal activity of the female steroid hormone oestrogen or its analogues.

P32 POSTLABELLING A sensitive experimental method designed to quantitatively measure low levels of DNA adducts induced by chemical treatment.

PARENTERAL The word applied to the administration of substances to an animal or to man by any route other than by the mouth or by the bowel.

PERIPORTAL VACUOLATION Development of vacuoles in cells adjoining the portal tracts of a liver lobule.

PEROXISOME PROLIFERATION Increased formation of the certain organelles in the cell.

PLASTICISER A substance which increases the flexibility of certain plastics.

POLYCARBONATE A form of plastic.

POLYMER A very large molecule comprising a chain of many similar or identical molecular sub units (monomers) joined together (polymerized).

POLYMERIC A form of chemical structure, e.g. a plastic, made up of joined monomers (qv).

POLYPLOIDY Having three or more times the haploid (single set of unpaired chromosomes as found in germ cells) number of chromosomes. Somatic cells from animals generally contain a diploid set of chromosomes, with pairs of equivalent chromosomes, so that twice the haploid number are present.

POSTNATAL The period after birth.

PRESERVATIVE A substance which prolongs the shelf life of foodstuffs by protecting them against deterioration caused by micro-organisms.

PREVALENCE The number of cases of a disease that are present in a population at one point in time.

RECEPTOR A small, discrete area on the cell membrane or within the cell with which specific molecules interact to initiate a change in the working of a cell.

REFERENCE NUTRIENT INTAKE (RNI). An amount of the nutrient that is enough, or more than enough, for most (usually at least 97%) of people in a group. If the average intake of a group is at the RNI, then the risk of deficiency in the group is very small.

RENAL Relating to the kidney.

SCF The European Commission's Scientific Committee on Food.

SERUM The fluid remaining after blood has clotted.

SISTER CHROMATID EXCHANGE (SCE) Exchange of genetic material between two sub-units of a replicated chromosome.

SOLVENT EXTRACTS Separation of constituents of a mixture by means of selective solubility of constituents in various solvents.

SOMATIC Occurring in cells of the body other than germ cells (see 'mutation').

SQUAMOUS EPITHELIA A type of epithelium consisting of square shaped cells. Examples are the skin and the lining of the oesophagus. (see also 'epithelia').

STABILISER A compound or material that stabilises emulsions against separation or compounds against degradation.

STANDARDIZED INCIDENCE RATIO The ratio of the incident number of cases of a specified condition in the study population to the incident number that would be expected if the study population had the same incidence rate as a standard or other population for which the incidence rate is known; this ratio is usually expressed as a percentage.

TDI See 'Tolerable Daily Intake'.

TOLERABLE DAILY INTAKE (TDI)

TERATOGEN A substance which, when administered to a pregnant woman or animal, can cause congenital abnormalities (deformities) in the baby or offspring.

TERATOLOGY The study of development abnormalities and their causes.

THRESHOLD The lowest dose which will produce a toxic effect and below which no toxicity is observed.

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. Systems have been published for chlorinated dibenzodioxins and dibenzofurans and for polycylic aromatic hydrocarbons.

TOXICOKINETICS The description of the fate of chemicals in the body, including a mathematical account of their absorption, distribution, metabolism and excretion.
TRANSAMINASE An enzyme removing amino groups from a compound.

TRANSFORMATION The process by which a normal cell acquires the capacity for neoplastic growth. Complete transformation occurs in several stages both *in vitro* and *in vivo*. One step which has been identified *in vitro* is 'immortalisation' by which a cell acquires the ability to divide indefinitely in culture. Such cells do not have the capacity to form tumours in animals, but can be induced to do so by extended passage *in vitro*, by treatment with chemicals, or by transfection with oncogene DNA. The transformed phenotype so generated is usually, but not always, associated with the ability of the cells to grow in soft agar and to form tumours when transplanted into animals. It should be noted that each of these stages of transformation can

involve multiple events which may or may not be genetic. The order in which these events take place, if they occur at all, *in vivo* is not known.

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 *in-vivo* effects such as mutagenicity in transgenic mice containing a recoverable bacterial gene (lacZ or *lac 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 (qv) -, or an activated form of the *ras* oncogene which may enhance their susceptibility of these mice to certain types of carcinogenic chemicals.

TRANSLOCATION The transfer of a region of one chromosome to another chromosome.

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 (qv). 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 recognizable 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 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.

UDS See 'Unscheduled DNA Synthesis'.

UMU TEST The *umu* test (essentially similar to the SOS-chromotest) is based on *Salmonella typhimurium* TA 1535/pSK1002 and measuring induction of the *umu* C operon by DNA damage using ß- galactosidase activity as an indicator. The *umu* test has not been considered by the OECD and has not been validated for regulatory purposes and is not suited to the mutagenicity assessment of complex mixtures of chemicals in water samples.

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.

VITAMERS Interconvertible forms of a vitamin.

WHO-ECEH/IPCS The World Health Organization's European Centre for Environment and Health and the WHO's International Programme on Chemical Safety.

XENOESTROGENS A 'foreign' compound, i.e. not natural to the body, with oestrogenic activity.

Annex 4

INDEX TO SUBJECTS AND SUBSTANCES CONSIDERED IN PREVIOUS ANNUAL REPORTS OF THE COMMITTEES ON TOXICITY, MUTAGENICITY AND CARCINOGENICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT

Annex 5

INDEX OF CONSIDERATIONS BY THE COMMITTEE ON TOXICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT OF MAFF FOOD SURVEILLANCE PAPERS

Intakes of Intense and Bulk Sweeteners in the UK 1987-88	29	48	1990
Plasticisers: Continuing Surveillance	30	27-32	1990
Di-2-ethylhexyl adipate		28-29	
Acetyl tributyl citrate		29-30	
Polymeric plasticisers		30	
Epoxidised soya bean oil		30-31	
Azelates		31	
Phthalates		31	
Baby foods		31	
Dioxins in Food	31	46-50	1992
Nitrate, Nitrite and N-Nitroso Compounds in Food: Second Report	32	39-43	1992
Veterinary Residues and Animal Products 1986-90	33	54-59	1992
Stibines		54-55	
Growth-promoting hormones		55	
Sulphonamides		55	
Chloramphenicol		55	
Furazolidone		56	
Tetracyclines		56	
Other antimicrobial agents		56	
Clenbuterol		56	
Benzimidazoles		56-57	
Levamisole		57	
Nitroxynil		57	
Lasalocid		57-58	
Oxolinic acid		58	
Oxytetracycline		58	
Report of the Working Party on Pesticides Residues: 1988-90	34	83-88	1992
Mycotoxins: Third Report	36	59-63	1993
Aflatoxins		59	
Ochratoxin A		59-60	
Moniliformin		60	
Patulin		61	

Aluminium in Food	39	49	1993
Naturally occurring Toxicants in Food	42	50-54	1994
Aquatic biotoxins		50	
Non-licensed herbal preparations and other selected foods		51	
Pyrrolizidine alkaloids in herbal products and other selected foods		51-52	
Other alkaloids in herbal products		52	
Ethyl carbamate		52-53	

Annex 6

PUBLICATIONS PRODUCED BY THE COMMITTEES ON TOXICITY, MUTAGENICITY AND CARCINOGENICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT

- 1991 Annual Report of Committees on Toxicity, Mutagenicity and Carcinogenicity of Chemicals in Food, Consumer Products and the Environment. HMSO ISBN 0 11 321529 0 Price £9.50.

- 1992 Annual Report of Committees on Toxicity, Mutagenicity and Carcinogenicity of Chemicals in Food, Consumer Products and the Environment. HMSO ISBN 0 11 321604-1 Price £11.70.

- 1993 Annual Report of Committees on Toxicity, Mutagenicity and Carcinogenicity of Chemicals in Food, Consumer Products and the Environment. HMSO ISBN 0 11 321808-7 Price £11.95.

- 1994 Annual Report of Committees on Toxicity, Mutagenicity and Carcinogenicity of Chemicals in Food, Consumer Products and the Environment. HMSO ISBN 0 11 321912-1 Price £12.50.

- 1995 Annual Report of Committees on Toxicity, Mutagenicity and Carcinogenicity of Chemicals in Food, Consumer Products and the Environment. HMSO ISBN 0 11 321988-1 Price £18.50.

- 1996 Annual Report of Committees on Toxicity, Mutagenicity and Carcinogenicity of Chemicals in Food, Consumer Products and the Environment. The Stationery Office ISBN 0 11 322115-0 Price £19.50.

- Guidelines for the Testing of Chemicals for Toxicity DHSS Report on Health and Social Subjects 27 HMSO ISBN 0 11 320815 4 Price £4.30.

- Guidelines for the Evaluation of Chemicals for Carcinogenicity DH Report on Health and Social Subjects 42 HMSO ISBN 0 11 321453 7 Price £7.30.

- Guidelines for the Testing of Chemicals for Mutagenicity DH Report on Health and Social Subjects 35 HMSO ISBN 0 11 321222 4 Price £6.80.

- Guidelines for the Preparation of Summaries of Data on Chemicals in Food, Consumer Products and the Environment submitted to DHSS Report on Health and Social Subjects 30 HMSO ISBN 0 11 321063 9 Price £2.70.

Produced by Department of Health
14327 1.5k 1P Dec 98 (CWP)
CHLORINE FREE PAPER