The Impact of Genetic Factors and Physiological Variables in Chronic Low-dose Toxicity

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A B S T R A C T

For non-carcinogenic chemicals it is commonly accepted that 1/100th of a dose which causes no adverse effect in laboratory animals is likely to be without toxicity for man. This factor allows for a 10-fold difference in susceptibility between the test species and man and a 10-fold intra-species range of susceptibility in man. This simple rule-of-thumb, though useful, can easily be shown to be misleading insofar as both inter-species and intra-species genetically-determined differences in metabolism, pharmacokinetics and immunobiology sometimes greatly exceed the 10-fold margins incorporated in the formula. Furthermore, the formula does not cover the possibilities of interactions between the test substance and concomitant exposure to pharmaceutical agents, alcohol, dietary items, environmental chemicals, etc.

For carcinogenic chemicals, it is necessary, first, to distinguish between agents which are themselves genotoxic, agents which can be converted to genotoxins by metabolic activation and non-genotoxic carcinogens which predispose to cancer indirectly via a wide variety of mechanisms. Prudence dictates that no level of exposure to genotoxic carcinogens should be regarded as safe unless there is a clear understanding of why a threshold exists. Since many non-genotoxic carcinogens are demonstrably species-specific and often strain-specific and sex-specific, and since many such agents are of natural origin, and/or are chemicals in widespread use, there is no justification for assuming that no dose can be safe.

In this paper the possible risks to man of toxicity or carcinogenicity because of chronic low-dose exposure to 4 air pollutants: formaldehyde, acetaldehyde, dimethylnitrosamine and ethanol are discussed in the light of the above considerations.

Introduction

Toxicology as a scientific discipline has never got really to grips with the need to take genetic variation into account in relation to the prediction of safety or toxic risk of chemicals for man. This is particularly so in the case of weak and slowly acting toxins and that of low-level chronic exposure to known toxins. Laboratory tests require the prolonged observation of large numbers of animals if weak effects are to be detected and this, in practice, means that it is impractical to use species other than small rodents.

Inter-species differences in metabolism and pharmacokinetics, etc., mean that observations in rats and mice cannot be simply extrapolated to man and the idea, sometimes voiced by biochemists, that one should select an animal species which mimics man in how it metabolises the chemical to be evaluated, rarely overcomes the difficulty. This is firstly because the costs of identifying such a species in terms of money and time may be considerable. Secondly, it may be that no such species exists. And thirdly, the identified species may be of such a body size and/or so long-lived that no meaningful study is possible.

Intra-species variation has also to be considered. Different laboratory strains of rats and mice vary widely in the spectra of diseases to which they are prone and in how they respond to potential toxicants. A test carried out in one inbred strain may give a very different result from a

similar test carried out in another strain, and there may be no way of telling which, if either, of the results is more relevant to man. It may well be that neither is relevant. As an aid to overcoming this problem, some toxicologists have recommended the use of outbred as distinct from inbred strains of rats and mice in the pious hope that at least some of the animals will be genetically good models for man. However, this approach far from resolves the difficulty, firstly because it is likely to be difficult to identify the sub-population which is the best model for man and, secondly, there may well be too few members of such a sub-population to satisfy the requirements of statisticians responsible for analysing the data derived from such studies.

Prediction of toxicity/safety for man

It is often said that in order to study the toxic potential of a chemical for man, the best approach is to study its effects in man. This is all very well in the case of proposed new drugs where the hopes of benefit can be balanced against the risks of toxicity in carefully planned and conducted clinical trials. However, for chemicals of other kinds, deliberate exposure of volunteers may be deemed unethical and the study and follow-up of populations of inadvertantly exposed individuals is made difficult because the information about exposure dose is unreliable because it is impossible to control for confounding variables, and because there is rarely any way of taking intra-species genetic variation into account. Despite these difficulties seemingly reliable results have been obtained for heavy exposure to some chemicals in some work-place situations and to ionizing radiation, etc. However, epidemiology is rarely able to throw useful light on whether long-term exposure to a low dose of even a known toxicant is safe or harmful.

In the case of a chemical which is not suspected of being carcinogenic in the light of its chemical structure, its lack of genotoxic activity and the results of long-term toxicity studies in laboratory rodents, regulatory bodies have adopted a rule-of-thumb approach whereby chronic exposure to a daily dose which is less than 100th of a dose demonstrated to be without toxicity in laboratory animals, is deemed to be safe for man. This overall 100-fold safety factor allows for a 10-fold difference between the sensitivity of man and the most susceptible animal species used for the laboratory tests and a further 10-fold factor to cover the intra-species genetically-determined variation in susceptibility in man.

This rule-of-thumb approach, based on practicality, caution and common sense rather than on observation, has for the most part stood the test of time in relation to pesticide and other agrochemical residues in food, food additives, cosmetic ingredients, etc., but is of little or no value in relation to the safety regulation of food ingredients where the daily intake is measured in whole-number percentages of the total food ingested. Also, the 100-fold safety factor does not protect individuals with genetically-determined inborn errors of metabolism or acquired senitivities to particular chemicals.

Prediction of carcinogenic risk for man

It used to be assumed that there could be no safe dose for man of any agent found to increased the incidence of any form of neoplasm in laboratory animals. This assumption was based on the further assumption that any agent that increases tumour incidence does so by directly causing genetic damage or because it is converted, by the process known as 'metabolic activation', to a genotoxin. During recent years it has become clear that there are ways in

which tumour incidence may be increased by agents which are not themselves genotoxic. The key to understanding how non-genotoxic agents can give rise to cancer is the realization that during normal physiological processes involved in the conversion of ingested foodstuffs to energy, genotoxins capable of damaging cellular proteins and DNA are produced in abundance within all the cells of the body (Ames, 1983). In normal circumstances, DNA repair enzymes effectively repair almost all the damage these endogenously produced genotoxins do whilst damaged intracellular proteins are similarly replaced by newly synthesised ones. However, DNA-repair is hampered during the short phase of the cell-replication cycle when the DNA is single stranded. Hence any stimulus to increased cell turnover favours the accumulation of cells with unrepaired DNA damage (ie mutant cells). Thus, hormones or irritants which, for different reasons, stimulate cell division can predispose to the accumulation of mutations and consequently to the risk of cancer development (Ames and Gold, 1990; Cohen and Ellwein, 1990). Conversely, caloric restriction which leads to reduced cell-turnover rates (Heller et al, 1990; Lok et al, 1990) is associated with highly significantly reduced cancer risk in almost all body sites (Roe et al, 1995).

The relationships between chronic toxicity, ageing and carcinogenicity

A particular difficulty in the case of low-dose toxicology is that it may be difficult to distinguish between the effects of a toxicant and the onset and progression of an ageing process. Ageing can be a feature of individual cells or of whole organs or tissues. Furthermore, the ageing of one tissue may impact on the functioning of another. The predominant manifestations of ageing vary from species to species. In many strains of rat, the commonest manifestations are progressive chronic

nephrosis, myocarditis, degenerative changes in the lower lumbar spinal cord and corda equina, and increasing incidence of neoplasms of many organs including particularly the pituitary and mammary glands and other endocrine and hormone-responsive tissues. In Westernized humans, atherosclerosis, which may give rise inter alia to coronary artery occlusion, cataracts, degenerative changes in hip and knee joints, Alzheimer's disease and cancers of many different sites are seemingly the commonest manifestations.

The idea that cancers should not be included within the spectrum of ageing diseases either in the rat or in man stemmed from the theory that virtually all cancers can be attributed to exposure to environmental carcinogens with just a few being predetermined by genetic inheritance. The realization that mutagens are produced endogenously during ordinary metabolic processes opened the way to understanding how cancers can be a part of the spectrum of ageing-related diseases as has long been proposed by researchers in the field of gerontology (e.g. Weindruch and Walford, 1988).

Almost inevitably studies in the field of low-dose, low-risk epidemiology involve the collection of data on middle-aged or elderly persons in whom manifestations of ageing are common. This is, of course, particularly true where the anticipated form of toxicity involves atherosclerosis, reduced lung function, the progressive loss of neurones in the brain, or one of the common forms of neoplasia.

In the sections that follow the role of genetic physiological and anatomical factors as determinants of response to inhaled acetaldehyde, formaldehyde, dimethylnitrosamine and ethanol vapour are reviewed in the light of the above considerations. Other speakers at this seminar will be considering carbon monoxide, trace metals,

benzene and environmental tobacco smoke in a similar context.

Inhaled formaldehyde and acetaldehyde and cancers of the nose and larynx

Some 15 years ago there was great consternation when it was found that laboratory rats and mice exposed to formaldehyde vapour in the concentration range of 2 to 14.3 ppm for 6 hours per day on 5 days per week, developed severe inflammatory changes and squamous cancers of the nasal epithelium (Swenberg et al, 1980). Rats were far more sensitive to these effects than mice, and in both species exposure to 2 ppm give rise to no nasal neoplasms. The nasal tumours seen in response to higher concentrations of formaldehyde arose mainly in the anterior part of the nose of affected rodents.

In the case of inhaled acetaldehyde vapour similar responses have been seen in the noses of rats and hamsters exposed to concentrations in the range of 1000 to 5000 ppm (Feron et al, 1982; Woutersen and Feron, 1987). In hamsters, exposure to 2500 ppm acetaldehyde also gives rise to tumours of the larynx (Feron et al, 1982).

Insofar as both formaldehyde and acetaldehyde have given positive results in some laboratory tests for genotoxicity (IARC, 1982; IARC 1985), it is tempting to assume that both agents should be classified as genotoxic carcinogens for which no level of human exposure should be regarded as safe. However, the results of epidemiological studies of humans exposed to formaldehyde at work and the fact that both formaldehyde and acetaldehyde are formed endogenously during normal metabolic processes, point to there being no real risk of any form of neoplasia in humans exposed to either acetaldehyde or formaldehyde (Wood and

Roe, 1992). An explanation of the tumour-inducing properties of the two agents advanced by Woutersen et al (1986) offers a resolution of the dilemma. These investigators suggested that the genotoxicity of formaldehyde depends on its ability to react with single-strand DNA during cell division when DNA-repair is hampered. Normally the turnover of cells in intact nasal epithelium is low and very little irreparable DNA damage is caused unless exposure also leads to mucosal damage and regenerative hyperplasia involving increased cell-turnover rates. In other words, carcinogenesis by formaldehyde or acetaldehyde are probably examples of "mitogenesis increasing mutagenesis" (Ames and Gold, 1990).

In reality, the most serious basis for concern with regard to human exposure to formaldehyde is not its irritancy or potential carcinogenicity but the fact that it is a potent allergen capable of provoking serious skin and other reactions in sensitized subjects. In relation to the animal studies referred to above, it is interesting to point out that whereas individual humans vary widely in the levels of airborne formaldehyde that they can tolerate, concentrations of 4-5 ppm for even short periods of exposure are intolerable for most people.

Conclusion: The most serious manifestation of toxicity from exposure of humans to formaldehyde vapour, namely those of allergy in sensitive individuals, was not predicted by long-term inhalation studies in laboratory rodents. Furthermore, the occurrence of nasal and laryngeal tumours in rodents exposed over long periods to high concentrations of formaldehyde or acetaldehyde are not matched by any epidemiological evidence of a similar risk in man.

Dimethylnitrosamine (DMN), hepatotoxicity and primary cancer of the liver

Appreciable levels of dimethylnitrosamine (DMN) have in the past been found in factories and in the air in the vicinity of factories (Fine, 1978). It is also present in tobacco smoke (Klimish et al, 1976) and in the air of smoke-filled rooms (Brunnemann and Hoffmann, 1978). It is of interest, therefore, to see if exposure to low levels of airborne DMN gives rise to any toxicity of the kinds seen in animals heavily exposed to it via other routes.

Historically the hepatotoxicity of DMN was recognised before its carcinogenicity for the liver. However, the extent of species differences in its hepatotoxic potential were only realised later. Barnes and Magee (1954) found that rats fed on a diet containing 50 ppm DMN survived well for 40 weeks. By contrast we (Carter et al, 1969) found that exposure of mink to a diet containing only 2.5 ppm DMN rendered the animals sick in only 11 days and killed them in 32 days, the cause of death being veno-occlusive hepatotoxicity. The probable explanation of this more than 20-fold difference in toxicity is that in mink fed on a fish diet the enzyme needed for the metabolic activation of DMN to the proximate hepatotoxin (probably the unstable hydroxymethyl compound) is present in abundance whereas this is not so in rats fed on standard laboratory chow.

Peto et al (1984) undertook a long-term multi-species study especially designed to study the shape of the dose-response curve for the induction of tumours of the liver and other body sites in response to various carcinogenic nitrosamines. Included in this study were groups of 48 male and 48 female rats exposed to DMN at dose levels covering a 500-fold range from 0.033 to 16.9 ppm v/v

in the drinking water throughout life. Whereas results did not establish the existence of a threshold for the induction of tumours of the liver or of any other site in rats, the findings in the groups exposed to only very low doses are subject to difficulties in interpretation. One problem is that the lower the daily dose of a genotoxic carcinogen, such as DMN, the later in life tumours attributable to exposure to it are likely to appear. Consequently, the liver tumours that arose in the low dose groups in the Peto et al (1984) study did so against the background of an increasing incidence of liver tumours in untreated control animals. Another problem is that primary tumours of several different histological types can arise from kinds of cell in the liver, and the dose-response relationships are likely to be different for different kinds of tumour. For these reasons the Peto et al study could not confidently define the shape of the dose-response curve in the very low dose range. On the other hand, data of a quite different kind are consistent with there being a threshold. Thus, in two separate studies, despite the presence of DMN in cigarette smoke, no evidence of hepatotoxicity or increased incidence of liver tumours was seen in rats chronically exposed to inhaled tobacco smoke (Davis et al, 1975; Dalbey et al, 1980).

Conclusion: Since the liver is the main target for both toxicity and tumour induction by DMN in the rat, the fact that no pathological changes were seen in two studies involving the chronic exposure of different strains of rat to low doses of DMN in the form of inhaled tobacco smoke is consistent with the existence of a threshold dose for these manifestations of DMN toxicity.

Ethanol hepatoxicity, cirrhosis and atherosclerosis

The toxicity of ethanol is usually considered in the context of oral exposure to it. Nevertheless, appreciable exposure to ethanol by the inhalation route may occur and it is relevant therefore to consider whether such exposure has consequences in terms of toxicity.

Objective discussion of ethanol toxicity is rendered difficult because some religions do not permit any consumption of alcohol and because there is widespread experience of the social and personal health consequences of the abuse of alcohol. It is also difficult because alcohol is consumed in many different forms and concentrations and in most cases mixed with other chemicals. Yet another problem is that comparisons of pre-death clinical diagnoses with diagnoses based on autopsy point to high levels of misdiagnosis of cirrhosis of the liver, and of primary liver cancer with many cases being missed clinically and some clinical diagnoses not being confirmed at autopsy.

Against this background of religious scruples, prejudice, diagnostic inaccuracy and potential confounding with regard to exposure, there is presently a vociferous debate concerning the dose-response relationship between the average daily oral consumption of alcohol and longevity with particular reference to the risk from coronary heart disease. Marmot et al (1981) followed up 1422 male civil servants for over 10 years and found that moderate drinkers (0.1 to 34g alcohol per day) experienced a statistically significantly lower mortality from cardiovascular diseases than either non-drinkers or heavy drinkers. Although a greater proportion of heavy drinkers were smokers and had higher mean blood pressures, multivariate analysis showed the U-shaped relationship between alcohol consumption and

mortality from coronary heart disease to be independent of differences in smoking, blood pressure, plasma cholesterol or grade of employment within the civil service. Several years later Shaper et al (1988) reported similar findings but considered that the apparent protective effect of moderate or low intakes of alcohol on health resulted from flawed methodology. However, more recently other investigators (Jackson et al, 1991; Rimm et al, 1991; Doll and Peto (1994), confirmed the earlier conclusions of Marmot et al (1981). A possible mechanism whereby moderate alcohol consumption protects against coronary heart disease was recently suggested by Hendriks et al (1994). These investigators found that alcohol consumed with the evening meal reduced the risk of blood clotting.

IARC (1988) reviewed the evidence associating alcohol consumption with risk of developing cancer of the oral cavity, pharynx, larynx and liver and concluded that the consumption of alcohol per se increased cancer risk at these sites. In contrast, there is no clear evidence that cancers of the stomach, colon, rectum, pancreas, breast or lung are associated with alcohol consumption.

The metabolism of ethanol takes place mainly in the liver and proceeds in three basic steps. First it is oxidized by alcohol dehydrogenase to acetaldehyde. Next the acetaldehyde is converted to acetate. Finally the acetate is oxidized in various tissues to carbon dioxide, fatty acids and water. Factors such as hormones, medicines, foodstuffs and vitamin deficiencies which affect the activities of the enzymes involved in these metabolic steps influence the acute toxic and mood-changing effects of alcohol but whether they influence the long-term effects of consuming ethanol is not clear. Numerous investigators have studied modification by ethanol of the metabolism of medicines and foodstuffs etc., and claims have been made

that such modification may lead to, or increase, the production of carcinogenic metabolites from the medicines in question. However, the relevance of much of this research to real life situations is dubious.

Whereas there is no evidence that moderate oral alcohol consumption is beneficial in relation to liver function, there is likewise no evidence that, in the case of healthy subjects, that it is harmful (Sherlock, 1995). Women are more susceptible to liver damage from higher intakes than men. Deficient protein intake is associated with increased risk of hepatotoxicity in both humans and rats (Sherlock, 1995).

Conclusions: Although the pharynx and larynx are possible targets for carcinogenicity by orally consumed ethanol, the mechanisms involved are not clear and there is no evidence of any such risks from inhaled ethanol.

The U-shaped dose response for coronary heart disease in relation to alcohol consumption is a good example of the potential pitfalls involved in predicting toxicity from low doses on the basis of evidence for high doses.

Despite the clear evidence of toxicity of orally-consumed ethanol, there is seemingly no evidence either from studies on humans or from studies on animals that the inhalation of ethanol vapour gives rise to toxicity. Commonsense suggests that if such exposure poses any risk it is that the aroma of ethanol will arouse a desire to imbibe an alcoholic drink by a person who has been advised on health grounds not to drink.

General conclusions

The value of animal experiments in the prediction of the toxicity or safety of a chemical for man is inversely proportional to the period of exposure required to produce evidence of toxicity and to the exposure dose. Even in relation to acute and subacute toxicity, anatomical, physiological and genetic differences between species and between individuals of the same species renders prediction of safety or toxicity for man extremely inexact.

The late Gerhard Zbinden (1987) drew attention to how predictive value of animal studies could be improved through avoidance of what he called "the 10 deadly sins of toxicology" (see Table 1). However, even with strict avoidance of these sins it is unlikely that the prediction of risk to humans from chronic exposure to low doses of airborne chemicals will be reliable. Nor, alas, are the prospects that, given the imprecision of exposure dosage and duration, the inaccuracy in detecting the present of toxic effects and the lack of information concerning confounding variables, epidemiologists will be able to define risks to humans from chronic low dose exposure to potential toxicants.

In the case of the 4 airborne pollutants considered in the present paper, commonsense suggests (i) that humans should avoid exposure to levels of formaldehyde or acetaldehyde that irritate them, (ii) that whereas it would always be prudent for them to avoid exposure to carcinogenic nitrosamines, they need not live in fear that they are at measurable risk of developing liver disease or liver cancer because of exposure to low levels of dimethylnitrosamine, (iii) that there are almost certainly no risks to health associated with exposure to ethanol vapour, and (iv) that they may live longer if they consume a little ethanol from time to time.

Idiosyncratic sensitivity, either because of genetically-determined inborn errors of metabolism or because of allergy, can dramatically affect the safety/toxicity of particular substances for individual persons and for many of these idiosyncrasies there are no good animal models. For the most part these idiosyncrasies are manifest in the short term. On the other hand, genetically-determined conditions such as α_1-antitrypsin deficiency are highly relevant to the development of chronic progressive lung toxicity in the form of emphysema in response to the chronic inhalation of irritants such as nitrogen dioxide.

References

Ames BN : Dietary carcinogens and anticarcinogens. Oxygen radicals and degenerative diseases
Science 1983;221:1256-1264

Ames BN, Gold LS : Too many rodent carcinogens : mitogenesis increased mutagenesis
Science 1990;249:970-971

Barnes J : Research v. testing
Food Drug Cosmetic Law Journal 1960;March Issue:158-164

Barnes JM, Magee PN : Some toxic properties of dimethylnitrosamine
Brit J Indust Med 1954;11:167-174

Brunnemann KD, Hoffmann D : Chemical studies on tobacco smoke. LIX Analysis of volatile nitrosamines in tobacco smoke and polluted indoor environment in "Environmental Aspects of N.nitroso compounds" edited by E A Walker, M Castegnaro, L Griciute and R E Lyle
IARC Scientific Publications 1978;19:1-

Carter RL, Percival WH, Roe FJC : Exceptional sensitivity of mink to the hepatotoxic effects of dimethylnitrosamine
J Path 1969;1:79-88

Cohen SM, Ellwein LB : Cell proliferation in carcinogenesis
Science 1990;249:1007-1011

Dalbey WE, Nettesheim P, Griesemer R, Caton JE, Guerin MR : Chronic inhalation of cigarette smoke by F344 rats
J Natl Cancer Inst 1980;64:383-390

Davis BR, Whitehead JK, Gill ME, Lee PN, Butterworth AD, Roe FJC : Reponse of rat lung to inhaled tobacco smoke with or without prior exposure to 3,4-benzpyrene (BP) given by intranasal instillation
Brit J Cancer 1975;31:469-484

Doll R, Peto R : Mortality in relation to consumption of alcohol : 13 years observation of male British Doctors
Brit med J 1994;309:911-918

Feron VJ, Kruysse A, Woutersen RA : Respiratory tract tumours in hamsters exposed to acetaldehyde vapour alone or simultaneously to benzo(a)pyrene or diethylnitrosamine
Europ J Cancer Clin Oncol 1982;18:13-31

Fine DH : An assessment of human exposure to N-nitroso compounds in "Environmental Aspects of N-Niroso Compounds" edited by E A Walker, M Casteguaro, L Griciute and R E Lyle
IARC Scientific Publications 1978;19:1-?

Haseman JK, Huff JE, Zeiger E, McConnell EE : Comparative results of 327 chemical carcinogenicity studies
Environmental Health Perspectives 1987;74:229-235

Heller TD, Holt PR, Richardson A : Food restriction retards age-related histological changes in rat small intestines
Gastroenterology 1990;98:387-391

Hendriks HFJ, Veenstra J, Wierik EJMV, Schaafsma G, Kluft C : Effect of moderate dose of alcohol with evening meal on fibrinolytic activity
Lancet 1994;308:1003-1006

International Agency for Research on Cancer (IARC) : Some industrial chemicals and dye stuffs
Monogr Eval Carcinog Risk Chem Hum 1982;29:345-389

International Agency for Research on Cancer (IARC) : Alyl compounds, aldehydes, epoxides and peroxides
Monogr Eval Carcinog Risk Chem Hum 1985;36:101-131

International Agency for Research on Cancer (IARC) : Alcohol drinking
Monogr Eval Carcinog Risk Chem Hum 1988;44:1-416

Jackson R, Scragg R, Beaglehole R : Alcohol consumption and risk of coronary heart disease
Lancet 1991;303:211-215

Klimisch H-J, Stadler L, Brahm S : Quantitative Bestimmung fluchtiger Nitrosamine in Zigarettenrauchkondensat. Z Lebensmittel-Untersuch 1976;162:131-138

Lok E, Scott FW, Mongeau R, Nera EA, Malcolm S, Clayson DB : Calorie restriction and cellular proliferation in various tissues of the female Swiss Webster mouse Cancer Letters 1990;51:67-73

Marmot MG, Rose G, Shipley MJ, Thomas BJ : Alcohol and mortality : A U-shaped curve Lancet 1981;1:580-583

Peto R, Gray R, Branton P, Grasso P : Nitrosamine carcinogenesis in 5120 rodents : Chronic administration of 16 different concentrations of NDEA, NDMA, NPYR and NPIP in the water of 4440 inbred rats, with parallel studies on NDEA alone of the effect of age of starting (3, 6 or 20 weeks) and of species (rats, mice or hamsters) in N-nitrosocompounds : Occurrences, biological effects and relevance to human cancer edited by I K O'Neill, R C Von Barstel, C T Miller, J Long, H Bartsch IARC Scientific Publications 1984;57:627-665

Rimm EB, Giovannucci EL, Willett WC, Colditz GA, Ascherio A, Rosner B, Stampfer MJ : Prospective study of alcohol consumption and risk of coronary heart disease in man Lancet 1991;338:464-468

Roe FJC, Lee PN, Conybeare G, Kelly D, Matter B, Prentice D and Tobin G : The Biosure Study : Influence of composition of diet and food consumption on longevity, degenerative diseases and neoplasia in Wistar rats studied for up to 30 months post weaning Food and Chemical Toxicology 1995;Volume33 Supplement 1:1S-100S

Saunders JB, Beevers DG, Paton A : Alcohol induced hypertension Lancet 1981;2:653-656

Shaper AG, Wannamethee G, Walker M : Alcohol and mortality in British man : explaining the U-shaped curve Lancet 1988;2:1267-1273

Sherlock S : Alcoholic liver disease Lancet 1995;345:227-229

Swenburge JA, Kems WD, Mitchell RI, Gralla EJ, Pavkov KL : Induction of squamous cell carcinomas of the rat nasal cavity by inhalation exposure to formaldehyde vapor Cancer Res 1980;40:3398-3402

Weindruch RH, Walford RL : The retardation of aging and disease by dietary restriction
Charles C Thomas : Springfield Illinois, 1988:1-436

Wood D, Roe FJC : Acetaldehyde and formaldehyde : Is there a cancer risk for man
Indoor Environ 1992;1:8-15

Woutersen RA, Appelman LM, Van Garderon-Hoetmer A, Feron VJ : Inhalation toxicity of acetaldehyde in rats III Carcinogencity study
Toxicology 1986;41:213-231

Woutersen RA, Feron VJ : Inhalation toxicity of acetaldehyde in rats. Progression and regression of nasal lesions after discontinuation of exposure
Toxicology; 47:296-305 /1987

Zbinden G : Predictive value of animal studies in toxicology
Centre for Medicines Research, Woodmansterne Road, Carshalton, Surrey SM5 4DS CMR Annual Lecture;1987: pp1-12

Table 1 : Reasons for incorrect predictions from toxicity studies

From Zbinden, 1987

False negative responses

1. Effect not looked for
2. Use of inappropriate assay method
3. Improper timing of assay
4. Insufficient target organ exposure
5. Incorrect evaluation of an experimental finding
6. Failure to consider absence of pre-existing pathological condition
7. Inability to identify and measure adverse effect

False negative and false positive responses

8. Failure to consider differences in metabolic activation and detoxification
9. Disregard of anatomical and physiological differences between species
10. Inability of animals to express human-specific reaction patterns