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Quantitative aspects of enhanced liver tumour formation in CF-1 mice by dieldrin

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The dose-response characteristics of dieldrin-mediated enhancement of liver tumour formation in CF-1 mice were analysed, using existing tumour data from chronic feeding studies at six levels of continuous exposure, involving a total of >1500 animals. The dose-response relationship can be expressed as: $D_x \times T_x = D_0 \times T_0$ = constant, where T_0 = the median liver tumour induction period in control CF-1 mice, T_x = the median liver tumour induction period in dieldrin-treated mice at a dose level D_x, D_0 = the background dose equivalent for the induction of 'spontaneous' liver tumours, D_x = the sum of background dose (D_0) and actual dieldrin dose (δ_x). The relationship, which is a Druckrey equation ($D \times T^n$ = constant) where n = 1, indicates that: (i) the velocity of liver tumour development is proportional to the daily dose level (D_x), (ii) the total tumourigenic dose is constant across all doses, (iii) the effects of dieldrin on the neoplastic process in mouse liver are essentially irreversible and cumulative, and (iv) there is no evidence for a threshold level. However, when $\delta_x \ll D_0$, the actual contribution of dieldrin to tumour formation is expected to be negligible.

Introduction

A variety of xenobiotic compounds are known to induce characteristic changes in the livers of laboratory animals. These changes include: (i) liver enlargement, usually as a result of cell enlargement, polyploidisation or cell replication, (ii) induction of drug-metabolising enzymes, and (iii) proliferation of the smooth endoplasmic reticulum (1–6). Such changes are not usually accompanied by evidence of liver damage, and are reversible upon withdrawal and elimination of the compound (5,6). Consequently, this phenomenon is likely to be an adaptive response of liver to increased functional demands. However, chronic exposure of various strains of mice to microsomal enzyme inducers, such as dieldrin, phenobarbitone, 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane (DDT)* and hexachlorocyclohexane (HCH)-stereoisomers, may cause an increase in the incidence of liver tumours (7–12). Phenobarbitone, DDT, butylated hydroxytoluene (BHT) and α-HCH have also been shown to promote the formation of rat liver tumours from lesions previously initiated by hepatocarcinogens (13–16). By analogy, it has been suggested that microsomal enzyme inducers do not exert an intrinsically carcinogenic effect on mouse liver, but function by enhancing the effect of a pre-existing oncogenic factor, which may be of environmental or genetic origin (5,7).

It is conceivable, in principle, that enhancers of carcinogenesis and intrinsically carcinogenic compounds exhibit different dose-response characteristics. The dose-response characteristics of chemical carcinogens, in single-dose and chronic-exposure experiments, have been elucidated by Druckrey and his associates (17–22):

$$D \times T^n = \text{constant} \quad (1)$$

where D = daily dose, T = the median tumour induction period, and n = an exponent, always >1.

Thus, carcinogenic response is defined as the median time period required for a constant end-point of the carcinogenic process. Equation (1) describes a quantitative relationship between the median velocity of tumour formation (or tumour-associated death) and the dose level of the carcinogen (H.Druckrey, personal communication). Velocity is measured in units of reciprocal time and, accordingly, the most appropriate form of Druckrey's relationship is:

$$(1/T)^n = (\text{constant}) \times D \quad (2)$$

or

$$1/T = (\text{constant}) \times D^{1/n} \quad (3)$$

The dose-response characteristics of putative carcinogenesis enhancers have been studied with various model compounds, particularly with phenobarbital (23–27), but no mathematical equivalent has emerged so far. We have recently reported a non-linear relationship between the logarithm of daily dieldrin dose and the logarithm of the median liver tumour induction period in CF-1 mice (25). This non-linearity contrasted with equation (1). However, in the dieldrin study, the likelihood is that the velocity of tumour formation was determined by multiple factors, i.e., by combination of dieldrin dose and background factors (responsible for liver tumour development in untreated control CF-1 mice). In mathematical terms:

$$1/T_x \sim D_0 + \delta_x \quad (4)$$

where T_x = median liver tumour induction period at a dieldrin dose level δ_x and D_0 = a background dose equivalent for liver tumour development in control CF-1 mice (when $\delta_x = 0$), the velocity of which will be denoted as $1/T_0$. Accordingly, dieldrin dose related to the *increase* in the velocity of liver tumour formation only, i.e., to:

$$(1/T_x) - (1/T_0) \quad (5)$$

The aim of the present analysis was to elucidate a possible quantitative relationship between dose and response, as defined above.

Materials and methods

Details of animal experiments, liver pathology and statistical procedures have been reported previously (8,9,25), but some important aspects are briefly reviewed. Treatment of CF-1 mice with dieldrin commenced at the age of 3 weeks. The animals were palpated weekly as from 16 weeks of treatment to detect the presence of intra-abdominal masses, and killed when the enlargement was considered to be detrimental to health. The treatment period up to that point was referred to as the liver tumour induction period. Liver tumours were classified as adenomas (nodular growths of solid cords of parenchymal cells) or carcinomas (papilloform and adenoid growth with cells proliferating in confluent sheets with necrosis,

*Abbreviations: DDT, 1,1,1-tricholoro-2,2-bis(*p*-chlorophenyl)ethane; HCH, hexachlorocyclohexane; BHT, butylated hydroxytoluene; 4-DAB, 4-dimethylaminoazobenzene.

Table I. Liver tumour data from long-term feeding studies with dieldrin in CF-1 mice (conducted by A.I.T.Walker and E.Thorpe at Shell Toxicology Laboratory, Sittingbourne, Kent)

Dieldrin (p.p.m.)	Initial number of mice	Number of mice with liver adenomas	Number of mice with liver carcinomas	Median tumour induction period (weeks)	Median total dose[a] (mg/kg)	Carcinoma induction period (10% incidence) (weeks)
0	586	84	11	131 (128–139)[b]	0	120 (>116)
0.1	244	48	10	122 (120–133)	8.5	119 (>117)
1	228	52	16	117 (115–121)	81.9	112 (>107)
2.5	58	27	4	108 (106–115)	189	–[c]
5	60	36	8	93 (91–99)	325	–[c]
10	324	119	190	66 (65–67)	462	60 (59–62)
20	38[d]	8	12	44 (41–48)	616	39 (<44)

[a]Calculated on the basis of an average daily food intake of 100 g/kg bodyweight.
[b]Numbers in parentheses indicate 95% confidence intervals.
[c]Carcinoma data considered too scant to warrant analysis.
[d]Sixteen animals in this treatment group died from acute intoxication within the first 13 weeks of treatment.

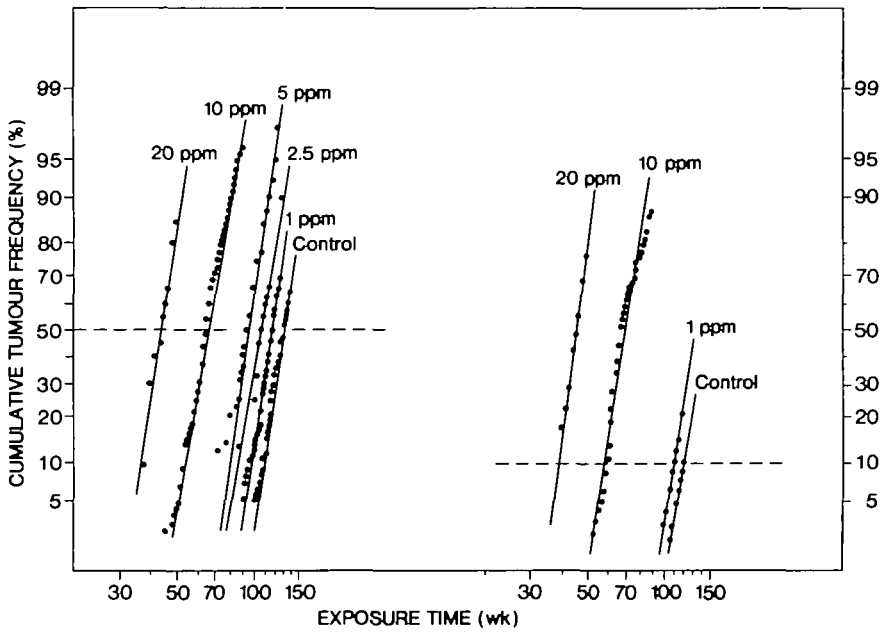

Fig. 1. Left: cumulative proportion of CF-1 mice with liver tumours versus dieldrin exposure time, at indicated dose levels (log-probit). The exposure time interval up to 50% liver tumours was designated as the median liver tumour induction period. **Right:** cumulative proportion of CF-1 mice with liver carcinomas versus dieldrin exposure time, at indicated dose levels (log-probit). The exposure time interval up to 10% liver carcinomas was designated as the liver carcinoma induction period.

increased mitosis, and sometimes associated metastases to the lungs). No significant sex difference in the tumourigenic response of liver to dieldrin could be detected (25), and tumour data for males and females within groups were combined. Details of statistical procedures have been described previously (25).

Results

Most of the liver tumours observed in control CF-1 mice and in treatment groups up to the level of 5 p.p.m. dieldrin in the diet were classified as liver adenomas (Table I). At higher dose levels, i.e., in the 10 and 20 p.p.m. treatment groups, ~2/3 of the liver tumours were classified as carcinomas (Table I). A substantial proportion of the animals exposed to 20 p.p.m. dieldrin died from acute intoxication within the first 3 months of experimentation. As with any non-liver tumour bearing animal, these early losses were classified as incidental deaths. The cumulative incidence of liver tumours (adenomas or carcinomas) as well as the cumulative incidence of liver carcinomas (only) is shown in Figure 1. Both data sets showed an excellent fit to the log-normal distribution, and linear regressions were virtually parallel. The median liver tumour induction period was defined as the treatment interval up to a 50% incidence of liver tumours. Median liver carcinoma induction periods could not be established in any but two dose groups (10 and 20 p.p.m., respectively). Instead, the time interval up to a 10% incidence of liver carcinomas was used as an indicator of the carcinoma induction period (Table I). The relationship between dieldrin dose and the acceleration of tumour formation was analysed with median liver tumour induction periods as well as with liver carcinoma induction periods. Both indicators of neoplastic response yielded similar results (see below).

Acceleration of liver tumour formation and dieldrin dose

The velocity of liver tumour formation and of liver carcinoma formation versus dieldrin dose is shown in Figure 2. Tumourigenic response was found to be linearly related to dose:

$$(1/T_x) = (1/T_0) + K \times \delta_x \quad (6)$$

The proportionality factor of dose (K), which is the tangent of an angle is defined as the ratio of the velocity of tumour development in control mice ($1/T_0$), and the background dose equivalent

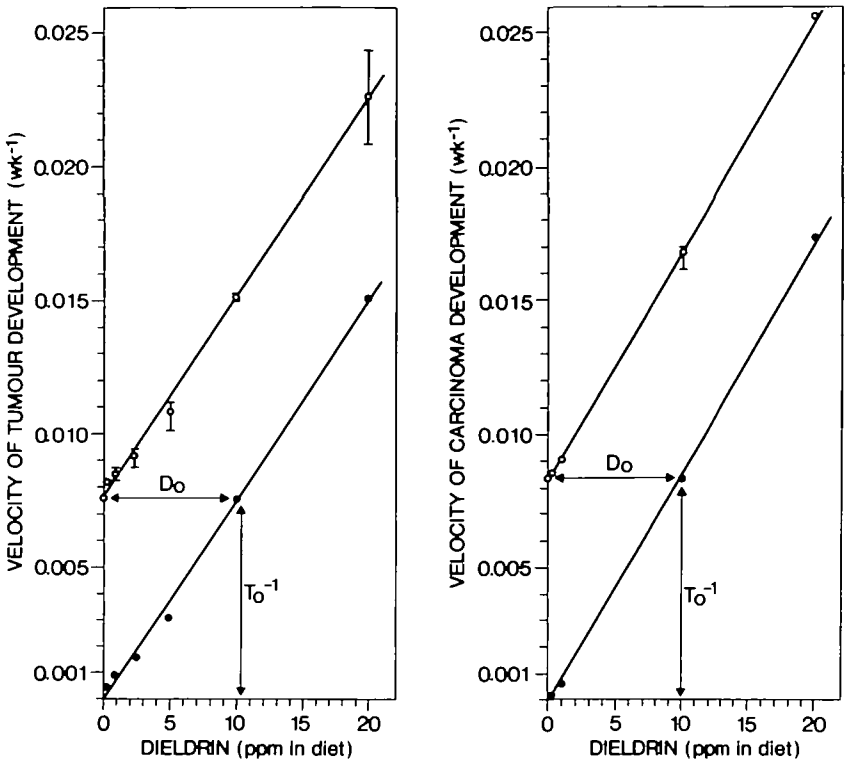

Fig. 2. Left: the reciprocal median liver tumour induction period (-○-) (= the velocity of tumour development) versus dieldrin dose. The dieldrin-associated increase in the velocity of tumour development (-●-), as defined in the text, versus dieldrin dose is also shown. The proportionality factor of dieldrin dose, which is the tangent of the angle, is defined as the ratio of the reciprocal median liver tumour induction in untreated CF-1 mice ($1/T_0$) and the background dose (D_0), which is equipotent to a level of 10.2 p.p.m. dieldrin in the diet. **Right:** the reciprocal liver carcinoma induction period (-○-) (= the velocity of carcinoma development) versus dieldrin dose. The dieldrin-associated increase in the velocity of carcinoma development (-●-) versus dieldrin dose is also shown. The proportionality factor of dose was defined as described above. The background dose (D_0) for carcinomas was found to be equipotent to a level of 10.0 p.p.m. dieldrin in the diet.

(D_0), i.e.

$$K = \frac{1/T_0}{D_0} = [1/(D_0 \times T_0)] \quad (7)$$

D_0 was found to be equipotent to a level of ~10 p.p.m. dieldrin in the diet (Figure 2). Equations (6) and (7) lead to:

$$(1/T_x) = (1/T_0) + [\delta_x/(D_0 \times T_0)] \quad (8)$$

or

$$(1/T_x) = (1/T_0) \times [(D_0 + \delta_x)/D_0] \quad (9)$$

The sum of background dose (D_0) and dieldrin dose (δ_x) will be denoted as D_x. This modification leads to the equation:

$$D_x \times T_x = D_0 \times T_0 = \text{constant} \quad (10)$$

Accordingly, there is a linear relationship between the negative logarithm of the sum of background dose and actual dieldrin dose ($-\log D_x$) and the logarithm of the median liver tumour induction period ($\log T_x$), as is shown in Figure 3. These results demonstrate that the kinetics of liver tumour formation in dieldrin-treated CF-1 mice are consistent with the Druckrey relationship, i.e., with equation (1).

However, in the dieldrin study, the value of the exponent of time (n) equals 1, i.e., there is no time-associated acceleration of the neoplastic process in CF-1 mouse liver. The implication is that, in contrast to carcinogens ($n > 1$), the total tumourigenic dose ($= D_x \times T_x$) is constant across all doses. Dieldrin's actual contribution to the total tumourigenic dose is, however, dependent on the *acceleration* of liver tumourigenesis. Equation (10) can be modified to read as:

$$(D_0 + \delta_x) \times T_x = D_0 \times T_0 \quad (11)$$

or

$$\delta_x \times T_x = D_0 \times T_0 - D_0 \times T_x \quad (12)$$

or

$$\delta_x \times T_x = D_0 \times (T_0 - T_x) \quad (13)$$

In words, the shorter the median liver tumour induction period in dieldrin-treated mice, i.e., the higher the daily dieldrin dose level, the greater will have been dieldrin's contribution to the total tumourigenic dose (Table I). This relationship is illustrated in Figure 4.

Discussion

The analysis yielded a simple relationship to describe the dose-response characteristics of enhanced liver tumour formation in dieldrin-treated CF-1 mice. The total tumourigenic dose, which is defined as the product of the sum of daily background and dieldrin dose (D_x) and the median liver tumour induction period (T_x), was shown to be constant across all doses.

The constancy of the product of concentration and time (needed to produce a specific effect) was first established for the action of a number of drugs by Clarke in a remarkable monograph published half a century ago (28). A theoretical explanation for 'c.t. = constant' was provided by Druckrey and Küpfmüller in 1949 (18).

These authors inferred that dose-response relationships are essentially determined by two processes (Table II), i.e., (i) the reversibility of binding to specific receptors in target cells, and (ii) the reversibility of the *effect* of receptor binding (pharmacological action or toxicity). The reversibility of any process is indi-

cated by time constants (in this case, T_R for binding to specific receptors, and T_r for the effect of receptor binding) (Table II).

When both of these time constants approach zero, i.e., when both processes are quickly reversible, the effect will be strictly dose-dependent ('Konzentrationsgift') (Table II). However, when one of two processes is irreversible, the effect will depend on the dose as well as on the duration of treatment. This has now been demonstrated to be the case for the neoplastic response of mouse liver to dieldrin [equation **(10)**]. Presumably, the sustained but essentially reversible interaction of dieldrin with critical receptors in CF-1 mouse hepatocytes results in irreversible progression (read: acceleration) of an ongoing neoplastic process. Finally, Druckrey and Küpfmüller predicted that the irreversibility of both processes would lead to time-associated acceleration of the effect ('Verstärkerwirkung') (Table II). Such dose-response relationships were subsequently established for chemical carcinogens of different organotropy [equation **(1)**] (19–22). The interaction of ultimate carcinogen(s) with critical receptors in the genome ('initiation') as well as the adverse effects of such receptor binding on the regulation of cell divisioin and cytodifferentiation ('expression of neoplastic potential') are likely to be irreversible processes. Thus, there would appear to be a fundamental difference in the nature of receptor binding between the non-genotoxic carcinogenesis enhancer dieldrin (29–31) and liver carcinogens, such a diethylnitrosamine, where n = 2.3 (19), or diethanolnitrosamine, where n = 4.7 (22). It is interesting to note, in this context, that the dose-response relationship for dieldrin is very similar to that observed for 4-dimethylaminoazobenzene (4-DAB), where n = 1.1 (17,19). There is evidence to indicate that 4-DAB is a very poor initiator compared with diethylnitrosamine or diethanolnitrosamine (32).

Druckrey's equation **(1)** holds for single-dose as well as chronic-exposure experiments with chemical carcinogens, and identical n-values (2.3) have been observed in single-dose experiments with N-nitroso-N-ethylurea (21) and in chronic-exposure experiments with diethylnitrosamine (19). The 'initiation' of carcinogenesis is immediate, i.e., almost timeless in comparison with the latent period of tumours. The implication is that the second process, i.e., the expression of neoplastic potential, may well determine the kinetics of tumour development. Sharply delineated stages of functional development of hepatocytes, e.g., at birth and at the 'late suckling' period (33), have been found to be associated with high susceptibility to a single dose of a liver carcinogen (34,35). Apparently, neoplastic potential is more readily expressed when, due to drastic changes in the animals' environment, initiated hepatocytes are committed to embark on a major process of functional development. Likewise, microsomal enzyme inducers, such as dieldrin, might enhance tumourigenesis by

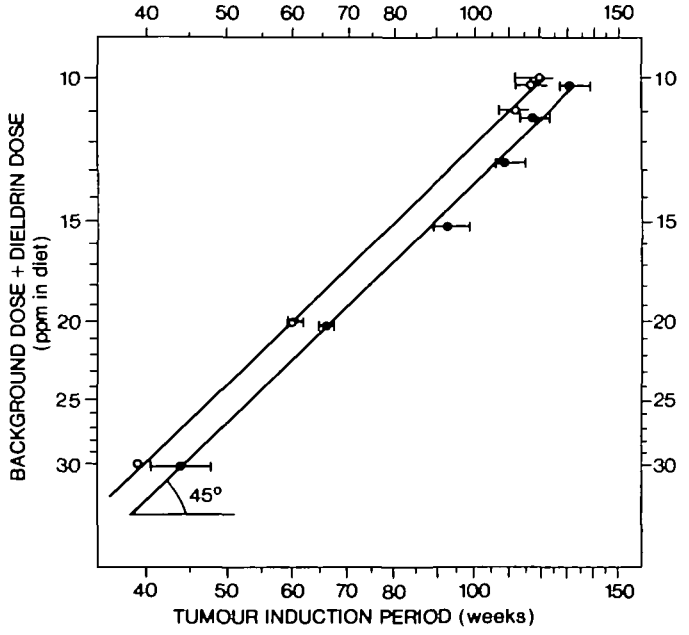

Fig. 3. The sum of background dose and dieldrin dose versus the median liver tumour induction period (-●-), and the liver carcinoma induction period (-○-), respectively, on logarithmic coordinates. The tangent of the angle (45°) is 1, in both cases. Linearity leads to equation **(10)**.

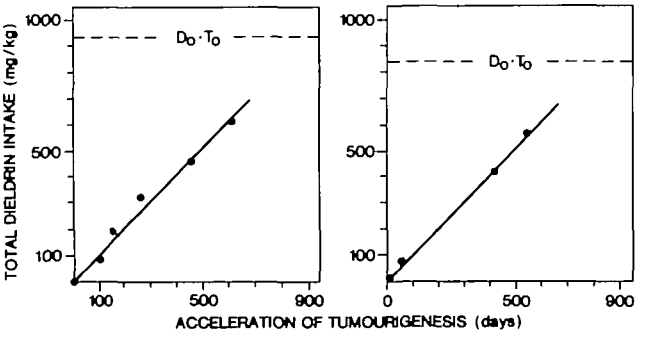

Fig. 4. Total dieldrin intake ($\delta_x \times T_x$), as a function of the acceleration of tumourigenesis ($T_0 - T_x$). **Left:** liver tumours (50%). **Right:** liver carcinomas (10%). Linearity leads to equation **(13)**. Broken line denotes the total tumourigenic (carcinogenic) dose, which is constant, irrespective of the daily dieldrin dose level.

Table II. The theoretical basis of dose-response relationships, according to H.Druckrey and K.Küpfmüller (1949)[a]

Reversibility of receptor binding	Receptor binding in relation to compound concentration	Reversibility of the effect	Effect in relation to receptor binding	Effect in relation to compound concentration	Dose-response characteristics
$T_R - 0$	$C_R \sim c$	$T_r - 0$	$E \sim C_R$	$E \sim c$	Dose-dependent ('Konzentrationsgift')
$T_R - 0$	$C_R \sim c$	$T_r - \infty$	$E \sim \int C_R\, dt$	$E \sim \int c\, dt$	Dose- and time-dependent ('c.t.-Gift')
$T_R - \infty$	$C_R \sim \int c\, dt$	$T_r - 0$	$E \sim C_R$	$E \sim \int c\, dt$	Dose- and time-dependent ('c.t.-Gift')
$T_R - \infty$	$C_R \sim \int c\, dt$	$T_r - \infty$	$E \sim \int C_R\, dt$	$E \sim \iint c\, dt$	Dose- and time-dependent time-associated acceleration ('Verstärkerwirkung')

[a] $T_R -$ = time constant for the reversibility of receptor binding; $T_r -$ = time constant for the reversibility of the effect; c = compound concentration; C_R = concentration of receptor binding; E = effect.

creating a higher level of functional commitment in their target cells ('functional stress').

The results of a previous study (36) indicated that dieldrin is unlikely to exert its tumourigenic action by exacerbating the effect of a potent environmental carcinogen. No difference in liver tumour incidence was observed between CF-1 mice bred, reared and maintained on a semi-synthetic diet and filter-paper bedding, and those exposed to a conventional diet and sawdust bedding. Dieldrin was found to be equally tumourigenic in both environments (36). These experimental data suggest that the origin of background tumours may be genetically-linked and transmitted from one generation to the next. If so, this neoplastic potential is only slowly expressed, under normal circumstances. The median liver tumour induction period in untreated control CF-1 mice (T_0) is 2.5 years, which exceeds the average lifespan of the animals by ~6 months. The background dose (D_0) was found to be equipotent to a level of ~10 p.p.m. dieldrin in the diet.

It is conceivable that D_0 reflects a certain level of naturally occurring or endogenous substances, which express intrinsic neoplastic potential in mouse hepatocytes in the course of time. Equation **(10)** is consistent with the view that the effects of background tumorigens and dieldrin are additive, and that there is no threshold level for the tumour-promoting action of dieldrin. However, when the actual level of dieldrin in the diet is very low in comparison with the level of background tumorigens, dieldrin's contribution to liver tumour development is expected to be negligible. Accordingly, there may be a rationale for a practical 'no effect' level of carcinogenesis enhancers, such as dieldrin.

Dieldrin has been found to be non-tumourigenic in experimental species which are less susceptible to spontaneous development of liver tumours (37,38). The strain differences observed in the tumourigenic response of mouse liver to dieldrin (E.Thorpe, unpublished observations) and to phenobarbital (39) would also seem to be related to a different genetic predisposition to spontaneous tumourigenesis. Such observations are consistent with the view that pre-existing levels of tumour susceptibility in human populations could be critically important in defining virtually safe levels of tumour-promoting agents.

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