

A Comparison of the Potency of the Mutagenic Effect of Chemicals in Short-term Tests with their Carcinogenic Effect in Rodent Carcinogenicity Experiments

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ABSTRACT

Short-term tests for carcinogenicity, particularly the *Salmonella* mutation assay of Ames, are used extensively for the qualitative identification of potential organic chemical carcinogens. Quantitative risk assessment requires a further step of estimating the carcinogenic potency of the chemical considered, a step which currently relies on epidemiological or long-term mammalian carcinogenicity studies. Attempts have been made to compare the potency of chemicals in mutagenicity assays with their carcinogenic potency in rodents. Nine such studies with the Ames test are reviewed and it is concluded that there is no good evidence to show that mutagenic and carcinogenic potencies are correlated. Data from two mammalian cell mutagenicity assays are insufficient to confirm a correlation.

Several fundamental differences between the *in vitro* mutagenicity assays and *in vivo* carcinogenicity assays mitigate the correlation, including the latency period between dosing and effect, the duration of dosing, the route of administration, the metabolic kinetics, the multitude of extraneous factors which modulate the responses and the inter-test and inter-laboratory variability.

The attempts to compare mutagenic and carcinogenic potency have not demonstrated a relationship which would be useful in risk assessment. Improvements may come from methods which give accurate estimates of dose at the site of action and better ways of expressing dose-response relationships.

1 INTRODUCTION

The development of short-term tests for carcinogenicity in the mid-1970s was due to societal concern to reduce exposure to chemical carcinogens together with technical methodological improvements culminating in a practical *Salmonella*-

based assay published by Ames and co-workers (1975). This assay, and some of many other tests developed in the last few years, performed well as regards qualitative identification of carcinogenic activity of chemicals so that the impression was created that the control of chemically induced cancer was within our grasp. The *Salmonella*/microsome assay had been very widely used in screening synthetic and natural chemicals and the results for over 2000 compounds were published by 1979 (Hollstein *et al.*, 1979). A large variety of synthetic chemicals, particularly those reactive chemicals used in industrial synthesis, were found to be positive as were large numbers of chemicals found in food, the environment, and even the contents of human gut. There was thus a pressing need to set priorities among carcinogens, and to concentrate on those which were likely to cause the most harm. Existing long-term animal studies had been used for this purpose but resource limitation once again encouraged the exploration of alternative techniques.

2 ASSESSMENT OF CARCINOGENIC POTENCY

In order to make a sensible, informed choice of chemicals which can be used and which should be controlled, it is necessary to be able to assess, among other things, the magnitude of any adverse effect they may have. Assessment of the adverse (carcinogenic) effect may consider one or all of the following criteria which taken collectively define the potency of a carcinogen:

- (1) *quantitation*. How many people are likely to develop cancer (the societal risk) or, put in another way, how likely is any particular individual to develop cancer (the individual risk)?
- (2) *severity*. How serious is the disease likely to be? The degree of malignancy and site of the neoplasm are two of the most critical issues. A skin cancer which is easy to control and treat may be viewed differently from a brain tumour which is life-threatening and non-treatable.
- (3) *time*. When is the neoplasm likely to occur? Will it be in 6 or 60 years?

The potency of the carcinogen is only one of the factors which must be taken into account in judging the likely effects that exposure to a carcinogen may produce; the duration, magnitude and route of exposure are equally important.

3 COMPARISONS OF THE MUTAGENIC AND CARCINOGENIC POTENCY OF CHEMICALS

All examples published to date attempt to compare the magnitude of response for a particular dose in one assay system with a similar measure at another dose in a second assay.

In some cases the carcinogenic potency takes time into account, but, not surprisingly, no attempt has been made to consider all aspects mentioned above

(e.g. quantitation, severity and time). Two short-term test systems have been compared extensively with rodent carcinogenicity: the Ames' *Salmonella* test and a point mutation assay in mammalian cells.

3.1 The *Salmonella*/Microsome Assay

Meselson and Russell (1977) provided the first serious attempt to compare mutagenic potency (in the *Salmonella* test) with carcinogenic potency (in rodents). Fourteen chemicals which had been reviewed by IARC and tested by McCann and co-workers (1975) were studied. Carcinogenic potency, K , is defined as $\ln 2/D_{1/2}$, where $D_{1/2}$ is the daily dose which gives 50% cumulative single-risk incidence of induced cancer in two years. Mutagenic potency (m) is expressed as micrograms of compound per 100 *Salmonella* revertants. The results from 10 of the chemicals studied is presented as their Figure 1; 4 chemicals had been omitted because the value of m/k was much less than 1, the value found for the other 10 chemicals. The graph of $\log D_{1/2}$ and $1/m$ is given for all 14 compounds in Figure 1.

As in all work of this type, it was necessary to normalize the carcinogenicity data to provide an estimate of dose which would produce 50% neoplasms at two years; this required a number of assumptions to be made, such as that proportion of unaffected animals developing neoplasms per unit time after exposure increases with the first power of the dose, D , and the third power of time, t .

The exclusion of the four nitroso compounds from the general relationship $m/k = 1$ was on the basis that values were much less than 1. For nitrosomethylurea, two estimates of $D_{1/2}$ were given which differed by more than 10^2 ; this was considered to be due to instability in solution. For the others, it was suggested that 'refinements in the *Salmonella* test system will bring these nitroso compounds into agreement with the relation suggested in the figure', $m/k = 1$.

One of the problems perceived by Bartsch *et al.* (1980) in establishing a quantitative relationship between potency in *Salmonella* and in animal carcinogenicity, was the major differences in metabolism between *in vitro* S-9 and *in vivo* experiments. They selected direct acting alkylating agents in order to avoid this problem although this does not obviate confounding factors of bacterial metabolism and different levels of nucleophilic macromolecules in the two systems. Their results are plotted on Figure 1 adjusting the mutagenicity index for different units ($\mu\text{g}/100$ revertants in Meselson and Russell's work; $\mu\text{g}/500$ revertants in Bartsch and co-worker's work) by dividing by 5. The index of carcinogenic potency (TD_{50}) is essentially equivalent to that used by Meselson and Russell.

On the basis of their own work, Bartsch and co-workers concluded that 'a quantitative relationship between carcinogenesis and mutagenesis in *Salmonella* is not sufficiently established to allow the confident prediction of carcinogenic potency'.

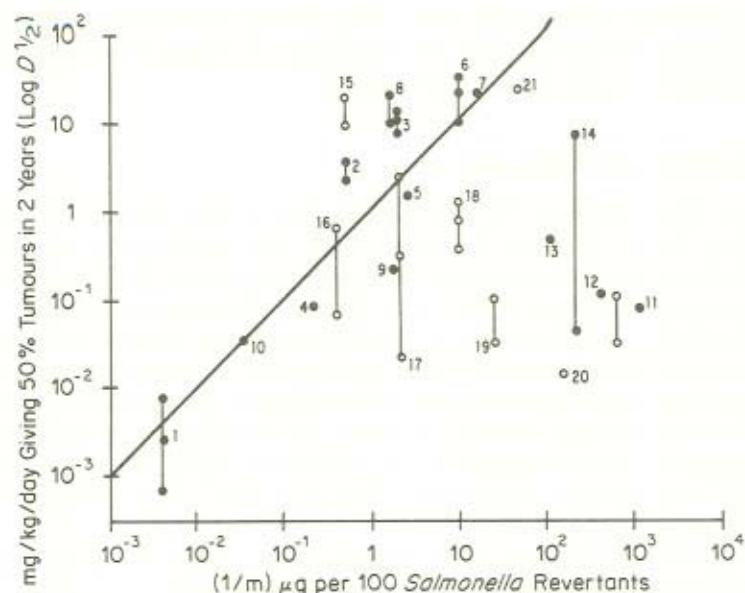


Figure 1 Mutagenic and carcinogenic potency. 1. Aflatoxin B₁. 2. 4-Aminobiphenyl. 3. Benzidine. 4. Benzo[*a*]pyrene. 5. Dibenz[*a,h*]anthracene. 6. 4-4'-Methylene-*bis*-2-chloroaniline (MOCA). 7. Methyl methanesulphonate (MMS). 8. 2-Naphthylamine. 9. 1,3-Propane sultone. 10. Sterigmatocystin. 11. Diethylnitrosamine (DEN). 12. Dimethylnitrosamine (DMN). 13. Di-*n*-butylnitrosamine. 14. *n*-nitroso-*N*-methyl urea (MNU). 15. Glycidaldehyde. 16. *N*-nitroso-*N*-methyl guanidine (MNNG). 17. Propane sultone. 18. β -Propiolactone. 19. *N*-nitroso-*N*-methyl urea (MNU). 20. *N*-nitroso-*N*-ethyl urea (ENU). 21. Epichlorhydrin. Chemicals 1–14 from Meselson and Russell (1977). Chemicals 15–21 from Bartsch *et al.* (1980). Values for mutagenic activity have been divided by 5 to convert from $\mu\text{g}/500$ revertants to $\mu\text{g}/100$ revertants. The line represents a value of $m/k = 1$

There is such a wide variety of chemical structures of carcinogens, that limiting the range tested might be expected to reduce the confounding factors. Ashby and co-workers (1981) selected eight derivatives of 4-dimethylaminoazobenzene which had been tested for carcinogenicity and subjected them to three variations of the *Salmonella* mutagenicity assay (plate incorporation, liquid preincubation and fluctuation assays). Their results are presented in Figure 2. The index of carcinogenicity used was that proposed by Miller and Miller (1953) and it suffers from the drawback that the range of potencies is limited to between 1 and 40; the method of Meselson and Russell is not strictly comparable but has potentially a much larger range (in this paper a range of 10^5 was noted). Nevertheless, no fixed relationship was observed and in particular the most potent mutagen was not

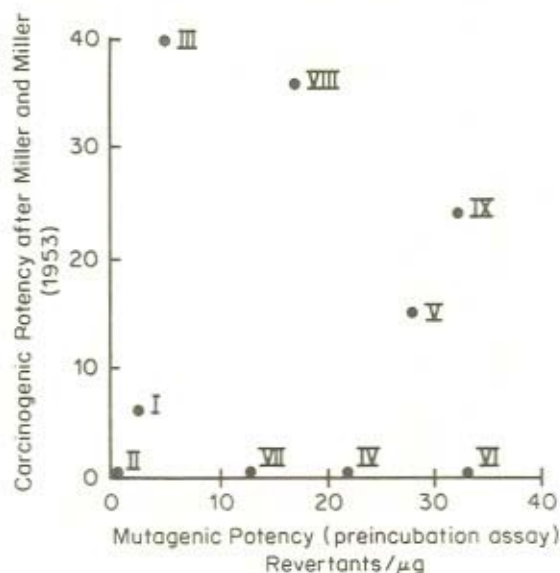


Figure 2 The relationship between carcinogenic potency and mutagenic potency for nine derivatives of 4-dimethylaminoazobenzene (after Ashby *et al.*, 1981)

carcinogenic and the most potent carcinogen was barely mutagenic.

Coombs *et al.* (1976) compared the potency of a series of polycyclic compounds in a standard skin carcinogenicity assay with that in the *Salmonella* assay. The units of mutagenic activity (revertant/nmole) are similar to those used by Meselson and Russell. The carcinogenic activity was expressed using arbitrary units ($= 100 \times \% \text{ tumour incidence/mean latent period in days}$) which suffer from the same drawbacks as those used by Ashby *et al.* (1981). Nevertheless, some indication of any relationship would be obtained by examining the data (Figure 3); there was no apparent relationship.

Glatt *et al.* (1979) studied the mutagenicity in *Salmonella* and carcinogenicity in mice of 43 heterocyclic compounds. Four strains of *Salmonella* were used with the plate incorporation protocol and, in order to overcome the problem of which set of data to use, an arbitrary mutation index was devised (mutagenicity index = revertants per nmole in TA100 + twice revertants per nmole in TA98 + four times revertants per nmole in TA1537) or the data from individual strains were used. The carcinogenicity studies were different from those used in the other studies reviewed in that three monthly injections of 0.6 mg of test compound were given to groups of 28 mice; the proportion of tumour-bearing animals observed provided the index of carcinogenicity. The authors concluded that there was no quantitative correlation between potency of the mutagenic and carcinogenic effects.

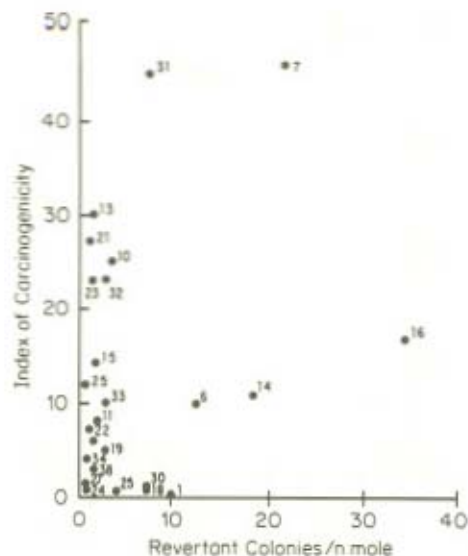


Figure 3 Relationship between carcinogenic and mutagenic potency. Data from Coombs *et al.* (1976). Numbers refer to polycyclic compounds in their Table 1

The mutagenic activity of five nitrosamines was studied in the standard *Salmonella*/microsome assay (Lagenbach *et al.*, 1980). There was no correlation with carcinogenic potency in that the least potent carcinogen was the most potent mutagen (Figure 4). A better correlation was obtained when a cell-mediated metabolic system was incorporated.

A series of seven polycyclic hydrocarbons was tested in strains TA1537 and TA1538 in the standard *Salmonella* assay and the results compared with the Iball index of carcinogenicity (Teranishi *et al.*, 1975). No good correlation was found with TA1537 but the results from TA1538 showed an increase in mutagenic potency with increasing carcinogenicity in that three compounds were most potent in both assays (Figure 5). Two compounds, dibenzo[*a,h*]anthracene and dibenzo[*a,e*]pyrene, had significant carcinogenic activity but no mutagenic activity. It is interesting to note the fairly substantial changes in mutagenicity produced by changes in the metabolic activation systems used.

Seven derivatives of 2-aminofluorene were tested by Bartsch *et al.* (1977) for carcinogenicity by subcutaneous injection and for mutagenicity in the *Salmonella* assay. Four of the derivatives were both carcinogenic and mutagenic but two potent carcinogens showed no mutagenic activity in TA1538 and TA98.

Bartsch *et al.* (1979) tested for mutagenicity of five polycyclic hydrocarbons and their related non-K-region dihydrodiols in strain TA100 and compared

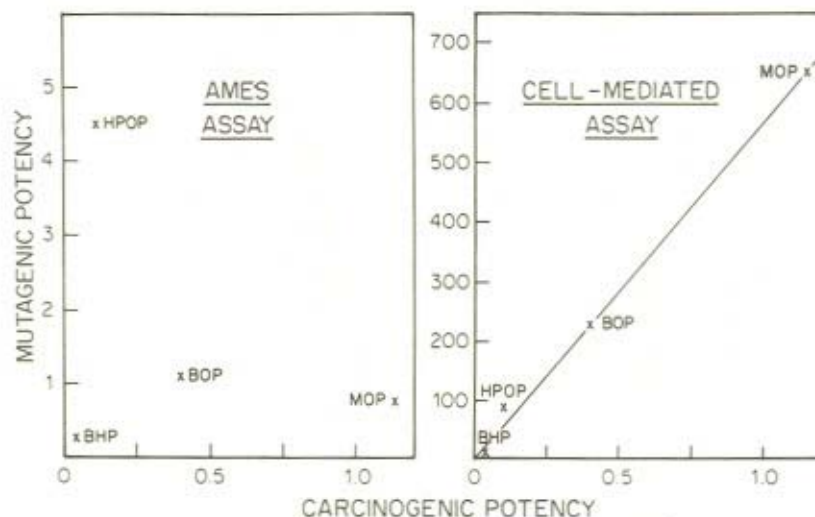


Figure 4 Relationship of carcinogenicity with mutagenicity in the two assays. Carcinogenic potency is expressed as the reciprocal of the lowest weekly dose tested which produced a tumour incidence of at least 60–70%: MOP, 0.875 mg/kg; BOP, 2.5 mg/kg; HPOP, 9.5 mg/kg; and BHP, 25 mg/kg. All compounds were administered by s.c. administration weekly in water to hamsters for life. Mutagenic potency in both assays is expressed as number of mutants (or revertants) per 10^6 survivors. For the Ames assay, results are taken at 10 mM; results at 1 and 100 mM would also indicate no correlation between mutagenic and carcinogenic potencies. Mutagenic potencies in the cell-mediated assay are taken at 0.7 mM, a concentration at which all compounds were tested. MOP = *N*-nitroso-methyl-2-oxopropylamine; BOP = *N*-nitrosobis(2-oxopropyl)amine; HPOP = *N*-nitroso(2-hydroxypropyl)(2-oxopropyl)amine; BHP = *N*-nitrosobis(2-hydroxypropyl)amine. Reproduced by permission of Cancer Research Inc. from Langenbach *et al.* (1980)

the results with the Iball index of carcinogenicity. There was a much closer association between the mutagenicities of the dihydrodiols and the carcinogenicities of the hydrocarbons from which they were derived than there was between the mutagenicities and carcinogenicities of the hydrocarbons themselves (Figure 6).

3.2 Mammalian Cell Mutation

There are far fewer data available for mammalian mutation tests *in vitro* than for the *Salmonella* test.

Huberman and Sachs (1976) tested a variety of polycyclic hydrocarbons for mutagenicity using three separate loci in Chinese hamster cells. Metabolizing

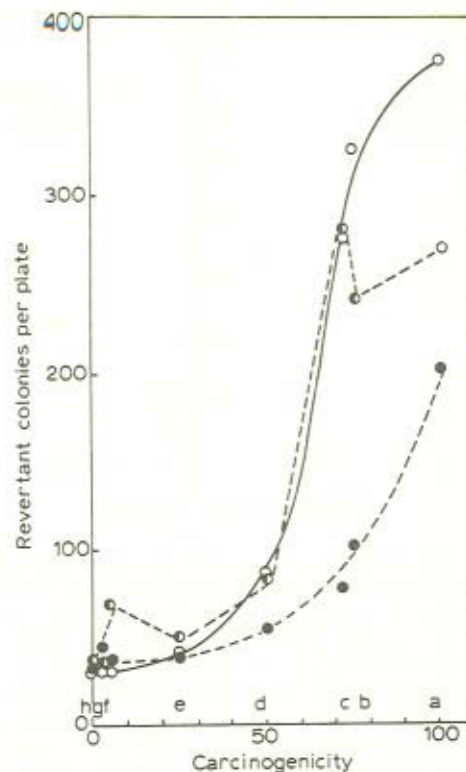


Figure 5 The correlation between carcinogenicity and mutagenicity in TA1538. Each chemical was used at a concentration of 50 $\mu\text{g}/\text{plate}$; a, 3-methylcholanthrene; b, dibenzo[*a,i*]pyrene; c, benzo[*a*]pyrene; d, dibenzo[*a,e*]pyrene; e, dibenz[*a,h*]anthracene; f, benz[*a*]anthracene; g, benzo[*e*]pyrene; h, dimethylsulphoxide (control). Each value was the average of duplicate plates. ● Liver homogenate from rats given drinking water containing 0.1% phenobarbital for 7 days. ○ Liver homogenate from rats given the PB-containing drinking water as above, and then injected intraperitoneally with 3-MC at 48 h before being killed (140 mg/kg). ○ Liver homogenate from rats given the PB-containing drinking water as above, and then injected intraperitoneally with DB[*a,h*]A at 48 h (140 mg/kg). Each value is the average of duplicate plates. Reproduced by permission of Elsevier/North Holland Biomedical Press from Teranishi *et al.* (1975)

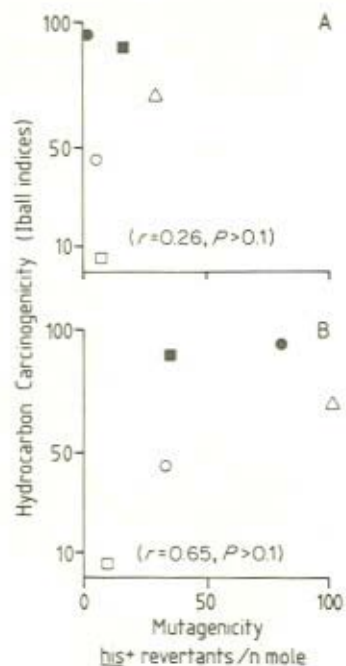


Figure 6 Relationships between the carcinogenic potencies of polycyclic hydrocarbons, assessed as Iball indices, and the mutagenic activities in microsome-mediated assays with *S. typhimurium* TA100 of: (A) these polycyclic hydrocarbons; (B) the related non-K-region dihydrodiols whose further metabolism would yield 'bay-region' vicinal diol-epoxides. The mutagenic activities have been taken from the linear, ascending portions of the dose-response curves. ○ Benz[a]anthracene; ○ 7-methylbenz[a]anthracene; ● 7,12-dimethylbenz[a]anthracene; ■ 3-methylcholanthrene; ▲ benzo[a]pyrene. Reproduced by permission of Elsevier Scientific Publishers Ireland Ltd from Bartsch *et al.* (1979)

but the most potent carcinogen, 7,12-dimethylbenz(a)anthracene, was the most mutagenic.

In an extensive study of the mouse lymphoma system, 25 chemicals of diverse structure were tested in the L5178Y/TK⁺ system and compared with published carcinogenicity data (Clive *et al.*, 1979). The carcinogenic potency was expressed as tumour-bearing animals/ μ mole/kg body weight and did not include the various corrections used by Meselson and Russell (1977). A reasonable correlation was found between the two indexes (Figure 7), bearing in mind that carcinogenic potency varied by as much as 10^4 (DES from 2×10^{-4} to 10^0) and

Table 1 Abbreviations for Figure 7

| Abbreviation | Chemical |
|----------------------------|---|
| 2-AP | 2-Aminopurine |
| 2-AAF | 2-Acetylaminofluorene |
| 4-AAF | 4-Acetylaminofluorene |
| B[a]P | Benzo[a]pyrene |
| B[e]P | Benzo[e]pyrene |
| ϵ -Cap | ϵ -Caprolactone |
| CP | Cyclophosphamide |
| DDE | <i>p,p'</i> -DDE |
| DEN | Diethylnitrosamine |
| DES | Diethylstilboestrol |
| DMN | Dimethylnitrosamine |
| DPN | Diphenylnitrosamine |
| EDB | Ethylene dibromide |
| EMS | Ethyl methanesulphonate |
| AF-2 | Furylfuramide |
| Hyc | Hycanthone methanesulphonate |
| Luc | Lucanthone hydrochloride |
| 1A-3 to 1A-6, SW-1 to SW-8 | 12 hycanthone and lucanthone analogues |
| Mtx | Methotrexate |
| MeI | Methyl iodide |
| MMS | Methyl methanesulphonate |
| MNNG | <i>N</i> -Methyl- <i>N</i> -nitro- <i>N</i> -nitrosoguanidine |
| MMC | Mitomycin C |
| Myl | Myleran ^R |
| Nat | Natulan |
| Proflavin | Proflavin sulphate |
| β -Prop; β -P | β -Propiolactone |
| Sacch | Saccharin, sodium |
| NaN ₃ | Sodium azide |
| Succ Anh | Succinic anhydride |
| UrMus | Uracil mustard |
| WSC | Whole-smoke condensate |

Reproduced with modification, from Clive *et al.* (1979) with permission.

mutagenicity by as much as 10^2 (4-AAF from 2×10^{-7} to 1×10^{-8}). It is also worth noting that some different protocols were used to optimize the results (DES without serum, *p,p'*-DDE without Aroclor-induced S-9). Nevertheless, an impressive overall relationship was presented.

3.3 Conclusions

The reports reviewed above examine a relatively small number of chemicals from selected classes. There are insufficient data on which to draw firm or definitive conclusions. From the evidence available, the following tentative conclusions may, however, be drawn.

- (1) Using the available methods there is no obvious correlation between mutagenic potency in *Salmonella* and carcinogenic potency. Meselson and Russell (1977) excluded four nitroso compounds from their graphical presentation of results as they considered them exceptional. Further studies lead to the view that there is no fixed relationship between k and m , even within homologous series of widely differing structural types, and that the 14 compounds originally used by Meselson and Russell were not unusual.
- (2) There are serious problems in expressing carcinogenic potency from the data presented in the published literature. Ames and his co-workers have mounted an extensive study of the published literature with a view to assessing carcinogenic potency of as many chemicals as possible.
- (3) There are some problems in expressing mutagenic potency. If data from more than one strain are available, should only one strain be used (as by Bartsch *et al.*, 1980), should the most sensitive strain for that chemical be used (as by Meselson and Russell, 1977) or should an arbitrary index (as by Glatt *et al.*, 1979) be used?
- (4) The obvious and important differences in metabolism between *in vitro* and *in vivo* studies had to be ignored, and this fact was considered by several authors to contribute to the lack of correlation.
- (5) The correlation described by Clive *et al.* (1979) for mutation in mouse lymphoma cells and carcinogenicity is quite similar to that originally described for *Salmonella* by Meselson and Russell (1977). It is an encouraging start but requires larger numbers of chemicals and repetition in other laboratories before its general applicability can be assessed.

4 PROBLEMS ENCOUNTERED IN EXPRESSING POTENCY FOR QUANTITATIVE ESTIMATION OF RISK

4.1 Hazard and Risk

It is convenient at this point to consider the overall process of quantitative risk estimation, because the separate steps involved influence the way in which

potency is assessed. To use the terminology defined below, potency is generally considered to be a component of hazard assessment. It is, however, difficult to express potency at the low levels of exposure normally being dealt with in the risk assessment stage and this influences the consideration of hazard.

4.1.1 Assessment of Carcinogenic Hazard

Hazard may be defined as the existence of a situation with a potential for harm to life. Thus the carcinogenic hazard of a chemical is the potential of the chemical to produce cancer. The structure of the chemical endows it with more or less potency and contributes to the determination of its site and time of action.

4.1.2 Quantitative Risk Assessment

Risk is defined as the probability of the realization of the potential of the hazard at any given level of exposure. Thus the cancer risk of a chemical is the probability that cancer will occur in a population exposed to a particular level of a carcinogen (this may be termed societal risk) or the probability that an individual exposed to a carcinogen will develop cancer (the individual risk). The route and rate of exposure may help to determine the site and time of action of the carcinogen.

4.1.3 Risk Acceptability and Limitation

Once the risk is established in qualitative and quantitative terms, society may judge whether it is acceptable, taking into account the availability of alternatives and the cost of imposing controls.

The importance of these processes to the assessment of the potency of a chemical carcinogen is that an estimation of potency is required at the dose level to which the population at risk is exposed. This is particularly critical for carcinogenic action because for the majority of carcinogens large doses are required to produce a significant elevation in cancer incidence in the experiments normally carried out with rodents; and because in the majority of situations where exposure to carcinogens occurs the level of exposure is low, often by a factor of 10^2 – 10^6 lower than used in the rodent experiments.

4.2 Potency Estimation from Rodent Studies

The estimation of potency is an integral component of carcinogenic hazard assessment which is quantifying an inherent property of the chemical. The usual assumption made is that the expression of carcinogenic potency is a feature common to the carcinogenic process in all species; details of experimental design which alter the expressed potency are confounding factors in this concept.

Most current attempts at estimating potency seek to express the carcinogenic response produced with respect to dose. In many, time is an incidental parameter which is avoided by suitable correction of the dose and/or incidence data. In none does the third component of potency, namely severity, play a significant part. Thus it is worth considering the problems of expressing dose and incidence, which are used in estimating potency.

4.2.1 Dose

In the majority of experiments it is only possible to express dose in terms of that applied to the rodent. The problem is to provide an integrated dose, which is meaningful for comparative purposes, that correctly takes into account duration, frequency, route and magnitude of exposure. Current approaches remove duration and frequency from the problem by the selection of the studies to be analysed and using only those in which exposure has been continuous over the lifetime. Crude approximations of the dose administered by the various routes may be made and expressed as mg/kg body weight/day. From these analyses the dose required to reduce by one-half the probability of animals being tumour-free when administered over a standard lifetime may be calculated. This index and similar indices are the best methods available for expressing potency. However, it must be recognized that they suffer from severe deficiencies, the most notable of which are:

- Experiments with designs substantially different from a standard two year daily dose protocol cannot be used.
- It is recognized that cancer incidence varies with the second to fourth power of time and thus the dose administered early in the experiment has a much more critical influence than that administered late in the experiment. This fact is not encompassed in the linear expression of dose.
- Only the applied dose is considered. Very large differences occur in the ratio between the applied dose and the target tissue dose at differing dose rates. Only the experimental dose and/or response range is used.

These inadequacies in the expression of dose mean that estimates of dose will be relatively inaccurate. Comparisons between species will have a level of inaccuracy due to differences in such parameters as surface area/body weight ratios or respiratory volumes in relation to body weight. Comparisons between routes of exposure could have similar levels of inaccuracy. Taken together, estimates of dose may be inaccurate by a factor of between 10^1 and 10^2 .

4.2.2 Response

The number of tumour-bearing animals is the usual parameter of response. A major drawback is that time is corrected for rather than being incorporated as a

component of the response. Analysis of the ED₀₁ study results by the Society of Toxicology Task Force (1981) demonstrated that time-to-tumour contributes information valuable for the estimation of carcinogenic response; its omission is a serious limitation. Important information for the assessment of human risk, such as the number and type of neoplasms and their site, is also omitted.

The expression of a carcinogenic response is subject to a wide variety of confounding factors which include dietary composition, quantity of diet, environmental conditions (temperature, light, contaminants), viral infections and a host of unknown factors. Experimental protocols attempt to eliminate these by the use of controls and by randomization, but the response finally observed may be limited to those circumstances and thus of little validity in extrapolation.

Variations in the incidence of cancer in control animals housed under nearly identical conditions gives the lower limit on the inaccuracies of estimating response. For some types of cancer, a two- to three-fold variation is quite common (Chu *et al.*, 1981). Interactions between these variations in control incidence and the carcinogenic action of a chemical may magnify these differences, and it is not uncommon to find the highest incidence of cancer in the lowest dose group.

4.2.3 Conclusion

Current methods of expressing potency of carcinogens in rodents suffer from severe limitations. When the variance in the estimate of dose and response is compounded by differences in route of administration and species, quite a large variability is observed. For example, compounds 17 and 14 in Figure 1 have estimates of potency which differ by over 10², and diethylstilboestrol potency varies by 10⁴ in the work described by Clive *et al.* (1979) (Figure 7).

4.3 Potency Estimations from *in vitro* Studies

The number of variables which have to be considered in these studies is much smaller than for *in vivo* studies. Mutagenic potency may be expressed either as the number of revertants (or mutants) per unit dose or as its reciprocal. An assumption is made that such estimations of potency are reasonably reliable. This topic was the subject of several papers (Ames and Hooper, 1978; Ashby and Styles, 1978a,b; Purchase, 1980; Bartsch *et al.*, 1980) with respect to the *Salmonella*/microsome assay. Briefly, Ashby and Styles were of the view that the correlation between mutagenic potency and carcinogenic potency should not be considered to be a general rule on the basis of the work of Ames and co-workers. The reasons were that there was a variety of confounding factors which can have a dramatic effect on the expression of potency (e.g., tester strain, type of protocol, details of preparation of S-9 mix, time of incubation, aerobic or anaerobic

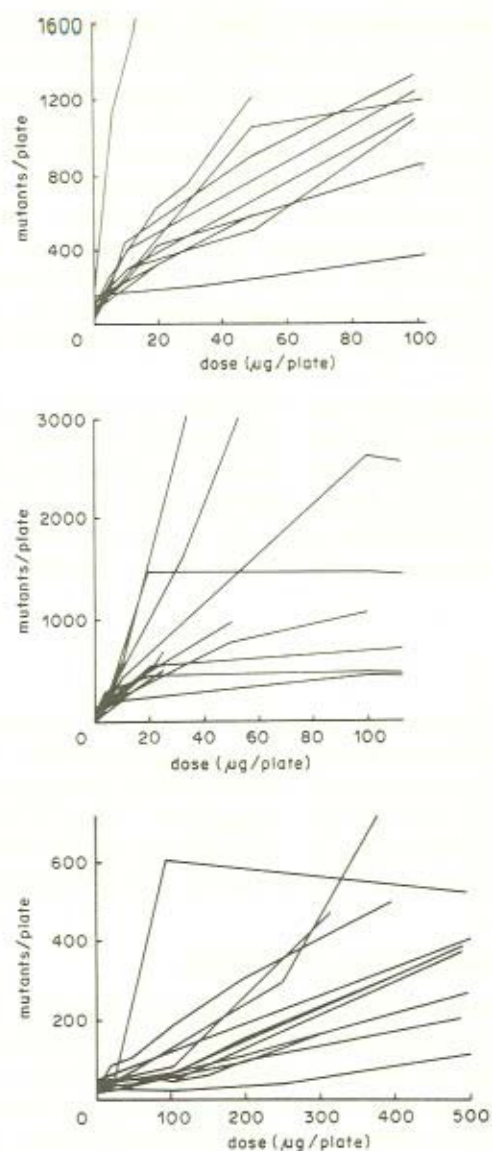


Figure 8 Dose-response curves for various chemicals in the Ames test (from Bridges, 1981). Top figure: dose-response curves for Prolac in the *Salmonella* test (TA100, no S9). Middle figure: dose-response curves for 2AAF in the *Salmonella* test (TA98, +S9). Bottom figure: dose-response curves for 4AAF in the *Salmonella* test (TA98, +S9) Reprinted by permission of the publisher from summary report on the performance of bacterial mutation assays by Bridges, in de Serres and Ashby, *Evaluation of Short-term Tests for Carcinogens*, pp. 49-60. Copyright 1981 by Elsevier Science Publishing Co

conditions, solvent, salt, buffer and nucleophilic content and other features). Ames, while criticizing technical aspects of their case, acknowledged that the potency estimates were not precise. Some recent data have given an idea of the quantitative implications of some of these points. In an international collaborative study (Bridges, 1981), 12 laboratories tested the same chemicals using the standard Ames' protocol (Ames *et al.*, 1975). Comparison of the results showed an inter-laboratory variation of up to 10^3 and 10^4 in the revertant colonies observed at a given dose (Figure 8). Changes in the quantity and source of S-9 mix can affect the counts of revertants by similar amounts (see, for example, McGregor, 1978). Responses may only be observed in one strain or may be greater in one strain, frequently TA100. These differences are frequently of the order of 10^3 . Similar differences may be observed in the mutagenic potency of chemicals among the data reviewed in section 3 of this paper (compounds 14 and 19 are methylnitrosourea and differ in potency by 10^1 , Figure 1).

Estimations of potency, even in the relatively simple *Salmonella*/microsome assay, are subject to substantial errors (probably on the order of up to 10^3) and this should be taken into account in any comparison with carcinogenic potency in rodents.

5 PROBLEMS PRESENTED BY COMPARISONS OF POTENCY

5.1 Different Nature of Mutagenesis and Carcinogenesis

Current knowledge of the molecular mechanism of mutagenesis allows a fairly detailed description of the events resulting from treatment of *Salmonella* or mammalian cells with a mutagen. In their simplest description, these events comprise damage to DNA from the reaction of the chemical with, usually, the nucleotides in DNA followed by the fixation of non-repaired or misrepaired DNA damage in daughter cells. A single dose of material followed by a period sufficient for a cell division is sufficient to fix the mutation; a variable period of, say, ten cell divisions is required for the phenotypic alteration to be expressed and become observable.

Carcinogenesis, on the other hand, requires repeated dosing, often over a major part of the lifetime of the animal, and cancer only appears after an extensive latent period, probably representing many cell divisions (there are some exceptions where single or few doses are sufficient to induce cancer). The events at the molecular and cellular level are not well understood. Somatic mutation may be involved in initiation but does not provide a complete explanation for these phenomena nor for subsequent promotion. The involvement of immune mechanisms and a wide variety of test and environmental factors which modulate carcinogenesis cannot be explained by somatic mutation. There is a group of chemical carcinogens without mutagenic activity which appear to induce cancer following production of prolonged and profound disturbances in the normal physiology of the host (e.g. hormonal changes).

Another area of major differences between the two phenomena is the frequency of events. Mutation is normally considered to be a relatively infrequent event (in the case of the Ames' test, 10^2 – 10^3 revertants per 10^8 cells). Carcinogenesis *in vivo* is much less frequent. Repeated dosing (daily for 2 years = 730 daily doses, or, if added to the diet, as a continuous dose) will produce a few cancer cells among the 10^{12} – 10^{14} cells in the mammalian body in a lifetime. Even if a single organ, e.g., the liver, is considered as the target, the frequency is much lower per unit dose than for mutations.

There are, therefore, several fundamental differences between mutagenesis and carcinogenesis. Mutagenesis may be induced after a single dose at a relatively high frequency and relatively rapidly. Carcinogenesis usually requires multiple dosing to produce a low level of response after a substantial latency.

5.2 Thresholds

Current attempts to compare potency involve an unstated assumption that there are no thresholds for mutagenesis or carcinogenesis; for if there were thresholds a more sensible approach would be to identify them as a means of defining a safe dose. The lack of thresholds is deduced for mutagenesis on the basis that it is theoretically possible for a single molecule to induce a mutagenic event in a cell. There are many that believe this to be improbable but few attempts have been made to demonstrate a threshold because of the technical problems involved. Jensen and Ramel (1980) have shown the presence of a clear threshold in the induction of 8-thioguanine-resistant mutants by alkylating agents in V79 cells *in vitro*. This observation is explained by the greater efficiency of repair at low dose levels.

For carcinogenesis, the technical problems of demonstrating a threshold are even greater. Based on the observation that cancer incidence is proportional to dose and a fixed power of time in the observable range, it has been assumed that no threshold can exist. This view is supported by extending the no-threshold concept in mutagenesis to the presumptive first step of somatic mutation in cancer. However, it is quite clear from experimental work and theoretical considerations that threshold doses of non-genotoxic carcinogens do in fact occur. The only convincing experimental evidence for genotoxic carcinogens is contradictory, in that acetylaminofluorene (AAF)-induced liver cancer showed no threshold, while AAF-induced bladder cancer did show a threshold in the ED₀₁ study (Society of Toxicology Task Force, 1981).

5.3 At Which Dose Should Potency be Expressed?

As an estimation of the dose required to produce a given level of effect (or its reciprocal), the commonly used index of potency may be quite misleading when

the quantitative estimation of risk is required at a much lower (or higher) dose (Figure 9). The only condition under which this technique is valid is when the two dose-response lines are parallel over the whole dose range of interest. In the absence of this specific condition, potency estimates are valid only at the given dose-response point or they should be expressed as the response at a given dose together with the slope and shape of the dose-response curve.

This argument severely limits the utility of comparisons of potency in two systems using a single dose-response point in each.

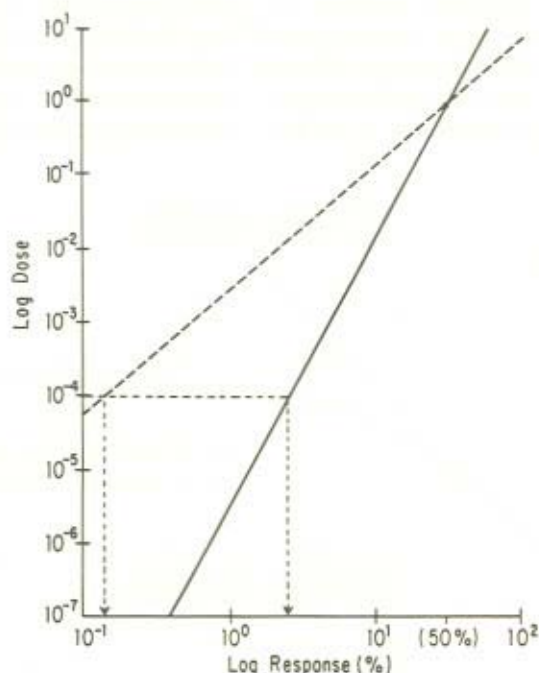
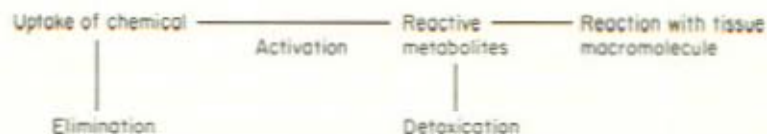


Figure 9 Idealized dose-response curves for a carcinogen in 2 assay systems. In both the ED_{50} is the same; at lower doses the responses are dramatically different

5.4 The Impact of Metabolic Kinetics

It has been recognized that the metabolic capability provided by S-9 mix in *in vitro* mutation assays differs quantitatively and qualitatively from *in vivo* metabolism. Krewski *et al.* (1982) have analysed the consequences of various types of kinetic models on the relationship between applied dose and concentration of the reactive metabolite. Their simple model is:



The relationship between applied dose and concentration of reactive metabolite may be convex or concave depending on the kinetic model used (Figure 10). *In vitro* tests differ significantly from *in vivo* systems in having no route for elimination, no enzymes for conjugation and readily available nucleophilic material in the incubation medium. An interesting example of the problems presented by metabolic processes is provided by Bartsch and co-workers (1979) who found a potency correlation between carcinogenicity of hydrocarbons and mutagenicity of their metabolic products. It would indeed be very surprising if the relationships were similar at all dose levels in any two assay systems.

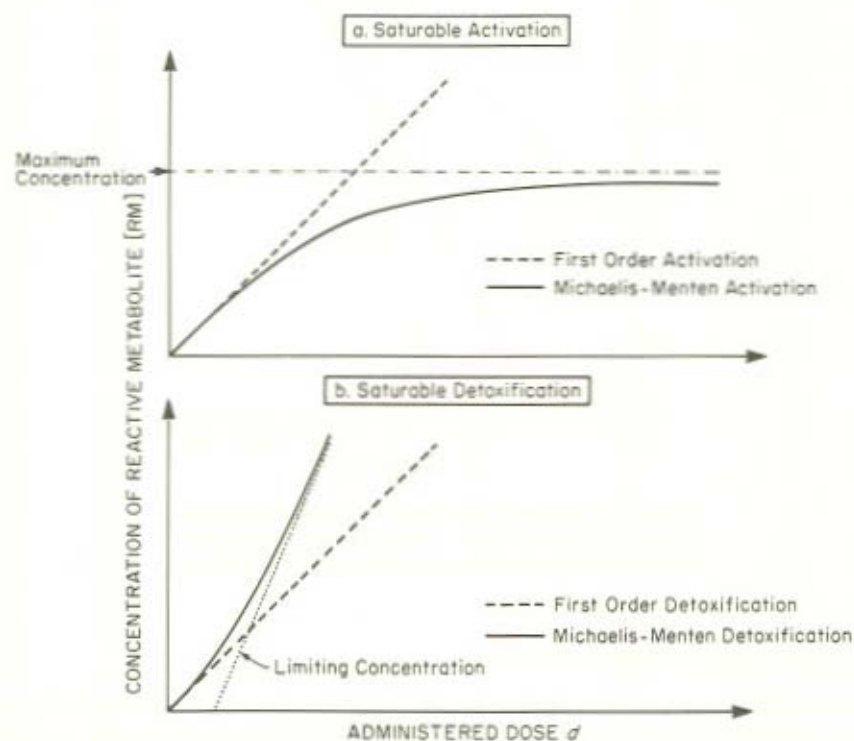


Figure 10 Formation of reactive metabolite under saturable metabolic activation or saturable detoxification. Reproduced by permission of Plenum Press from Krewski *et al.* (1982)

5.5 Conclusion

Comparisons of mutagenic and carcinogenic potency are extremely complex if they are to be accurate. The differences in biological processes, the need to assess potency over a wide dose range, and the differences in frequency and expression of response, present a formidable array of variables to correlate in a way which is sufficiently simple to be useful. Existing attempts to carry out this type of correlation have ignored most of the problems outlined in this section and it is therefore not surprising that no usable correlations have been substantiated.

6 FUTURE DEVELOPMENTS

The initial attempts to compare mutagenic and carcinogenic potency have not demonstrated a relationship which is universally accepted as useful for risk assessment using *in vitro* systems. The major limitations which will have to be overcome for these correlations to be useful are:

- An improved method of expressing dose which will allow comparisons between test systems. The use of radioactively labelled chemicals allows an estimation of residual radioactivity in target tissue over time; such estimates of dose circumvent metabolic differences.
- An improved method of expressing response for carcinogenesis which takes account of all available data, including time and severity.
- An understanding of dose-response relationships so that potency can be expressed in a way which avoids the problems of differing dose-response curves.

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