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Consequences of Synergy between Environmental Carcinogens

M. C. BERENBAUM

Department of Experimental Pathology, St. Mary's Hospital Medical School, London, United Kingdom

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As it is generally impossible to determine close–response relationships for carcinogens at the low concentrations in which they occur in the environment, risk–benefit considerations are by consensus based on the linear, no-threshold model. on the assumption that this represents the worst case. I-lowever, this assumption does not take into account the possibility of synergistic interactions between carcinogens. It is shown here that, as a result of such interactions, the dose–response curve for added risk due to any individual carcinogen will generally be steeper at lower doses than at higher doses, and consequently the risk at low environmental levels will be higher than would be expected from a linear response. Moreover. this excess risk at low doses is shown to increase as the general level of environmental carcinogens rises and, independently of this effect, it may also increase with the number of carcinogens present.

INTRODUCTION

The form of the dose–response curve of a carcinogen at low doses (i.e., in the region of interest for most environmental carcinogens) is of considerable public health importance. There has been much argument as to whether such curves have thresholds or not, for the existence of a threshold suggests there may he safe levels of exposure. However, for most carcinogens, it is almost impossible to determine the shape of the dose–response curve at the low levels which prevail in the environment. There is therefore a consensus that the safest assumption is that curves are generally linear, without thresholds. It is widely believed that this model probably overestimates the effects of carcinogens in the low-dose, low-rate region, that it thus defines an upper limit **of** risk, and, therefore, that risk–benefit calculations based on this model will err, if at all, on the side of safety (International Commission on Radiological Protection, 1966; Hoel ***et al.***, **1975;** Upton, 1977; Brown, 1977; Schneiderman and Brown, 1978; Pochin, 1978; Committee on Biological Effects of Ionizing Radiations, 1980; Rodricks, 1981).

Crump ***et al.*** (1976) and Peto (1978) pointed out that, in any case, for carcinogens of environmental concern, the shape of the dose–response curve for an individual carcinogen in isolation is more of academic than **of** practical importance. They showed that, for a carcinogen added in low levels to an environmenl already containing substantial amounts of other carcinogens, the incidence rate of additional cancers would be very nearly proportional to the amount **of** added carcinogen, irrespective of the form of its dose–response curve. Further, Guess and Crump (1978) showed that, cvcn when ***a*** carcinogen has a highly nonlinear

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dose–response curve and data are obtained in a perfectly conducted, large-scale experiment with no background effect, statistical tests would probably not enable the linearity hypothesis to be rejected at reasonable significance levels. Guess *et al.*, (1977) also showed that, at low doses, the upper statistical confidence limits on added risk would almost certainly be linear.

These statistical arguments therefore assert that, whatever the true nature of carcinogen dose–response curves at low levels, in practice the extra effect due to adding a new one to the environment will be very nearly linear with dose. Thus, the risk–benefit considerations which should decide whether or not a new carcinogen is introduced into the environment and which should determine its acceptable level should be based on the assumption of a linear dose–response curve.

The purpose of this communication is to show that linear dose–response curves do not in Fact generally indicate a safe upper limit of risk for carcinogens added to the environment. There is substantial evidence suggesting that environmental carcinogens interact synergistically in causing cancers in humans (Selikoff *et al.*, 1968, 1980; Lundin *et al.*, 1969; Kothman and Keller, 1972; Hammond and Selikoff, **1973;** Selikoff and Hammond, 1975; Doll, 1977; Saracci, 1977). Speculation about possible mechanisms of carcinogen interactions is unlikely to be profitable when so much is still obscure about the mechanisms of action of individual carcinogens. However, it is shown here that such interactions affect the shapes of dose–response curves and that, in consequence, risks in the low-dose region may be considerably greater than indicated by the linear model.

EFFECTS OF INTERACTIONS

Interaction means that the effect of exposure to two or more different carcinogens is not what is expected from their individual dose–response curves. Various criteria have, from time to time, been suggested for determining what to expect from combinations of agents that do not interact. The most widespread assumptions are that the effect of a zero-interactive combination should be either the product or the sum of the effects of its constituents. It is shown in detail elsewhere (Berenbaum, 1981, 1985) that the former assumption is correct only for agents with simple exponential dose–response curves and the latter only for agents with linear curves. Levels of environmental carcinogens are generally in the low-effect region, where it would be difficult or impossible to distinguish between linear and nonlinear curves, so the assumption of linearity is reasonable here, and in this case the effect of a combination of noninteracting carcinogens would closely approximate to the sum of their individual effects. However, an approach that is independent of the shapes of dose–effect curves is afforded by the construction of isoboles (isoeffect curves or surfaces) (Loewe and Muischnek, 1926; Loewe, 1953; Berenbaum, 1981, 1985). When agents do not interact, their isoboles are straight lines (for combinations of two **agents),** flat surfaces (for combinations of three), and, in general, $(n - 1)$-dimensional hyperplanes (for combinations of n agents).

Figures 1A, **2A, 3A,** and **4A** show various types **of** isoboles for combinations of E and X where E is a set of existing environmental carcinogens (considered in the first place as if it were a single agent) and X is a new carcinogen added to

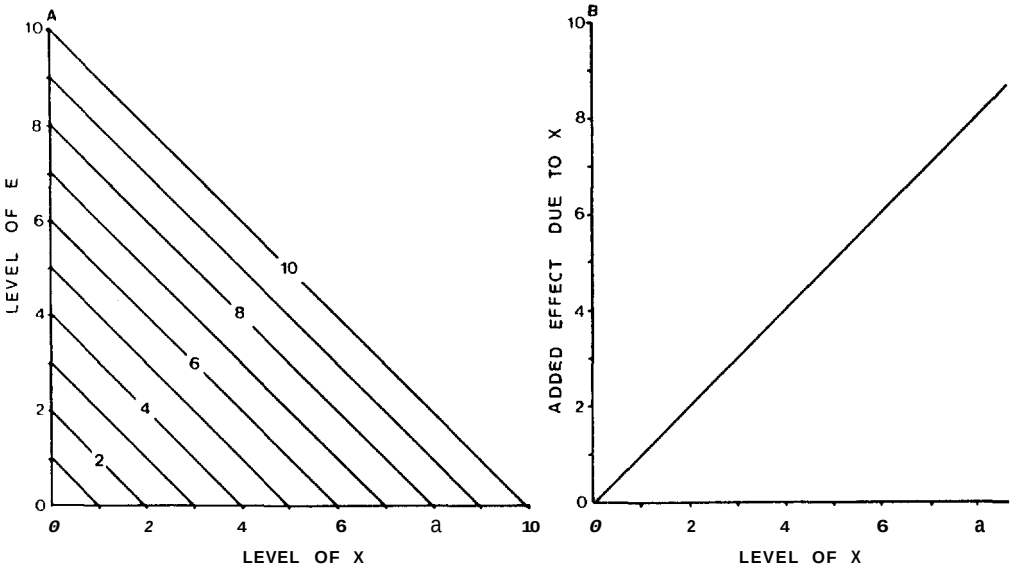

FIG. 1. **(A) Isoboles showing zero interaction between existing environmental carcinogen *E* and an added carcinogen *X* (levels in pg/day). Any particular combination of levels of *X* and *E* is represented by a point with these levels as coordinates, and the incidence of cancers per 10^6 exposed individuals is indicated by the isobole passing through that point. (B) The excess rate of cancers caused by adding *X* to a fixed level of *E*. In the case of zero interaction, the excess rate is independent of the level of *E* and is equal to the rate caused by *X* alone.**

the environment. It is assumed that *E* and *X* have linear dose–response curves, as discussed above, and that the risk of cancers per 10^6 exposed individuals is equal to the dose rate in picograms/day (for simplicity *E* and *X* are here assumed to have the same dose–response curves, but this is not essential).

Now consider the situation in which the level of environmental carcinogens is fixed, say at 2 pg/day, resulting in two cancers/10^6 individuals, and that we add to this different levels of the new carcinogen *X*. In Fig. 1A, *E* and *X* do not interact, as shown by the straight isoboles. The effect of any combination of *E* and *X* is simply the sum of the effects of *E* and *X* (as expected from a zero-interacting combination of agents with linear dose–response curves). The resulting total incidence due to 2 pg/day of *E* and any particular level of *X* is indicated by the isobole intersecting the horizontal line representing 2 pg/day of *E* at the appropriate level of *X*. Subtracting the two cancers/10^6 due to *E* gives the excess rate caused by adding *X* to the environment. Thus, the added effect due to different levels of *X* may be depicted as in Fig. 1B. Clearly, the excess due to *X* is proportional to the added amount of *X*, as expected from the statistical analyses mentioned above. If this exercise is repeated at different fixed levels of *E*, we find that the added effect due to *X* is unchanged, so the straight line function in Fig. 1B is independent of the level of *E* and, in fact, it is identical to the curve for *X* alone.

Let us now suppose that *E* and *X* do interact. This is shown by nonlinear isoboles. When *E* and *X* are synergistic the isoboles are concave up, reflecting the fact that a given cancer incidence is produced by lower levels of the carcinogens together than would be expected from their individual dose–response

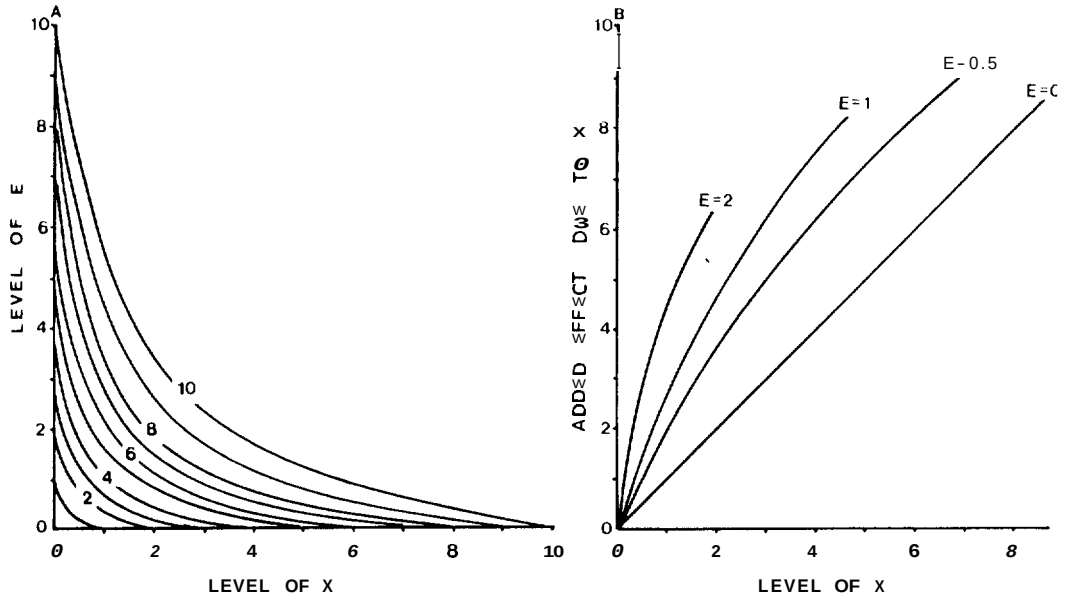

FIG. 2. (A) Isoboles showing synergy between *E* and *X*. The production of any particular incidence of cancers requires lower levels of *E* and *X* in combination than in the case of zero interaction (compare Fig. 1A). (B) The excess rate of cancers due to *X* increases with increasing levels of *E* and exceeds that in the case of zero interaction (compare Fig. 1B).

curves (compare Figs. 1A and 2A). If we now calculate the effects of different levels of *X* added to a fixed level of 2 pg/day of E, it is evident that the curve for extra cancers due to *X* is no longer linear but is steeper at low levels than the dose–response curve for *X* alone (Fig. 2B) and that, over the whole dose range, the yield of additional cancers due to *X* exceeds that which would be produced by *X* in the absence of environmental carcinogens.

Further, in contrast to the case of zero interaction, when there is synergy between *E* and *X* the slope of the curve for added risk increases as *E* increases, for the following reason. This slope depends on the horizontal separation of the isoboles along the line corresponding to the level of *E*. When the dose–response curves for *E* and *X* are linear and the isoboles are concave up, their horizontal separation will tend to decrease as *E* increases; therefore the slope of the curve for added risk due to *X* tends to increase with increasing levels of environmental carcinogens (Fig. 2B).

The effects of antagonism between *E* and *X* are shown in Figs. 3 and 4. The isoboles are concave down, reflecting the fact that a given incidence of cancer requires more of *E* and *X* together than would be expected from their individual dose–response curves. In Fig. 3A, antagonism is moderate and in Fig. 4A it is marked (the difference being that combinations of *E* and *X* in Fig. 4A are actually less effective in producing cancer than each of their constituents alone). The effect of moderate antagonism is to make the dose–response curve for added cancers due to *X* shallower at low doses than the curve for *X* alone (Fig. 3B). The effect of marked antagonism is to produce a threshold in the curve (Fig. 4B).

Although it has been assumed above that *E* and *X* have linear dose–response curves, the argument applies also to nonlinear curves. For example, Brown (1977)

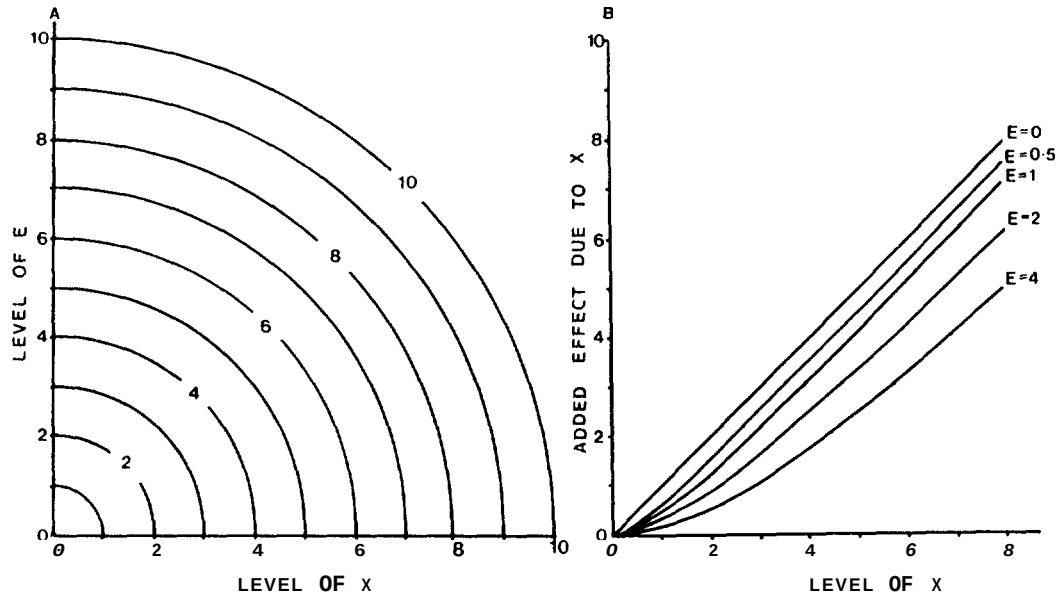

FIG. 3. **(A) Isoboles showing moderate antagonism between *E* and *X*. The production of any particular incidence of cancers requires higher levels of *E* and *X* in combination than in the case of zero interaction. (B) The excess rate of cancers due to *X* decreases with increasing levels of *E* and is less than that in the case of zero interaction.**

and Upton (1977) suggest that dose–response curves for radiation-induced cancer may be linear-quadratic rather than linear, and Hoel *et al.* (1983) have shown how kinetic processes involved in the metabolism and cellular actions of carcinogens may produce "hockey stick" rather than linear curves. In both cases, the dose–response curve is shallower at low doses than at intermediate doses. The effect of synergy on dose–response curves in a linear-quadratic case is illustrated in Fig. 5. The curves for extra risk due to X are again nonlinear, but this in itself is not of particular significance here as the curve for X on its own is also nonlinear. Of more importance is the fact that the yield of cancers due to X is again increased over the whole dose range in the presence of environmental carcinogen and that this effect increases with the level of E. (If this exercise is performed with curves that decrease in slope with increasing dose, the reverse is found—the yield of cancers is reduced in the presence of E and the reduction increases with E. The biological counterpart would be experiments in the high-dose region in which carcinogenic mechanisms are becoming saturated. Such situations, fortunately, would rarely be relevant to human exposure.)

In all the foregoing cases, the curve for extra risk due to X in the presence of nonzero levels of E is nonlinear. However, models can be devised in which interactions between X and E give rise to linear curves for extra risk. Suppose, for example, that risks due to X and E, respectively, are given by $\alpha_1 X + \alpha_2$ and $\beta_1 E + \beta_2$ ($\alpha_1, \beta_1 > 0$), and that the combined risk is $(\alpha_1 X + \alpha_2)(\beta_1 E + \beta_2)$. This function yields concave-up (synergistic) isoboles. The extra risk due to X is then $(\alpha_1 X + \alpha_2 - 1)(\beta_1 E + \beta_2)$, which is strictly linear in X when $\alpha_2 = 1$ (otherwise it is affine in X). The extra risk is linear in E when $\beta_2 = 1$. Such artificial models are of dubious biological relevance. Nevertheless, in these cases also, the extra

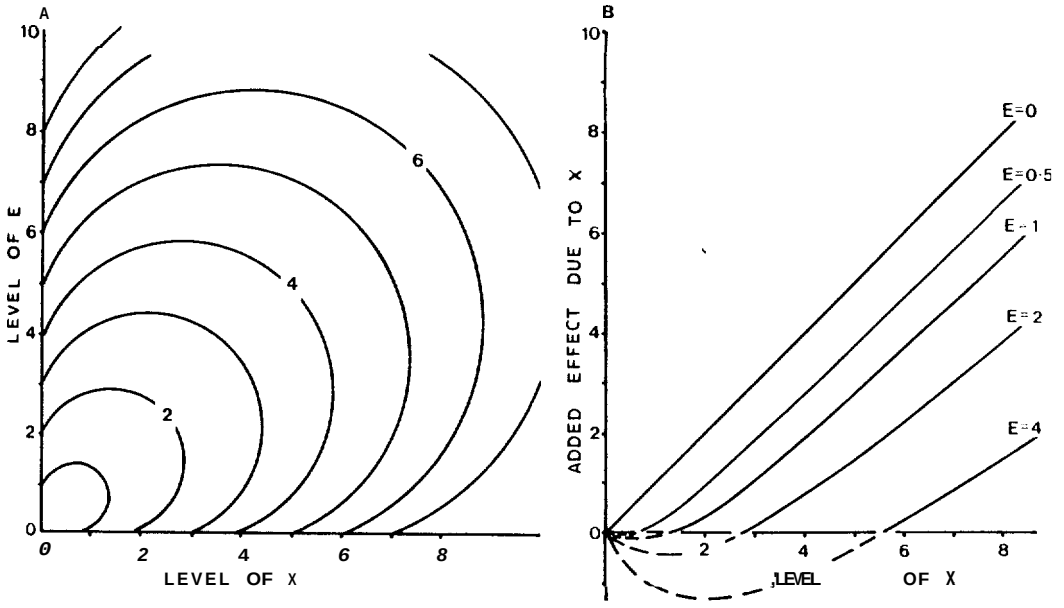

FIG. 4. **(A) Isoboles showing marked antagonism between *E* and *X*. Combinations of *E* and *X* generally produce fewer cancers than the constituent levels of *E* or *X* alone. (B) The excess rate of cancers due to *X* decreases with increasing levels of *E* and the reduction is large enough to produce a threshold in the dose–response curves of *X*.**

risk due to X in the presence of ***E*** exceeds that due to X alone, and this extra risk increases with the level of ***E***.

EFFECTS OF AGENT MULTIPLICITY

A further consequence of these interactions should be pointed out. ***E*** has been treated here as if it were a single agent. In fact, the environment contains several carcinogens which may interact, so that the situation that we must consider is not simply that due to possible interactions between two carcinogens, X and a supposedly single ***E***, but that due to adding X to an already interacting set of carcinogens. We must therefore consider how the expression of synergy might be affected by sheer multiplicity of agents.

Even for combinations of only two agents, the literature shows evidence of considerable confusion as to the criteria by which interactions between agents may be examined (Berenbaum, 1981, 1985). It is not surprising, therefore, that there is very little useful information on the behaviour of combinations of more than two agents. In fact, adequate information on interactions of this complexity appears to be available only for antimicrobial agents. Here, it has been found that combinations of three antibiotics show higher degrees of synergy than combinations of any pair of the three (Berenbaum *et al.*, 1983) and that combinations of four antifungal drugs show greater synergy on average than combinations of two or three (Odds, 1982). Further, whereas combinations of two antibiotics commonly varied in the type of interactions shown, being sometimes synergistic and sometimes antagonistic, depending on the ratio of the antibiotics, combinations of three were almost invariably synergistic, irrespective of ratios. Moreover, com-

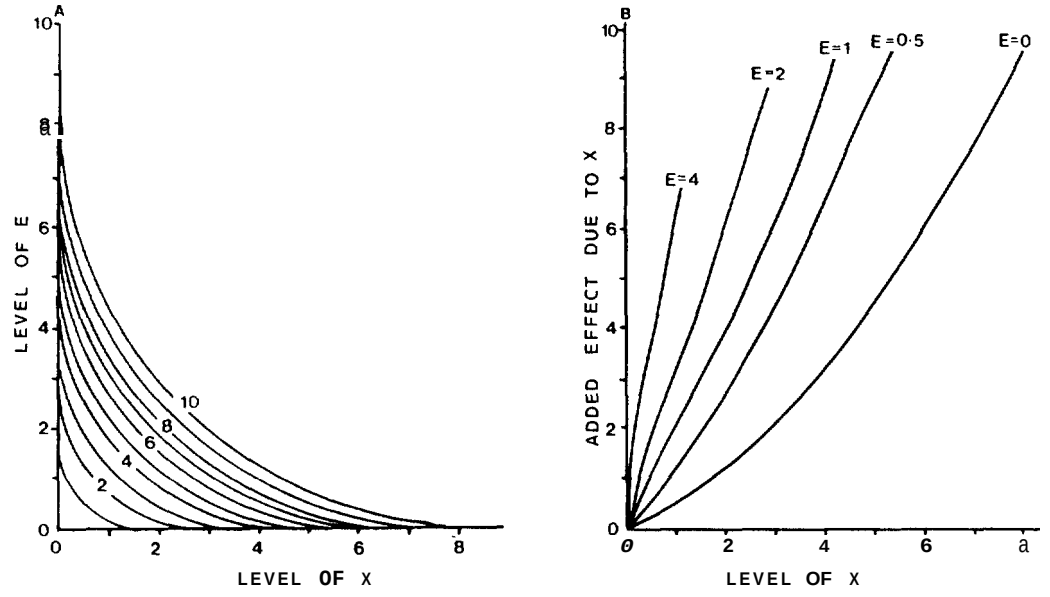

FIG. 5. (A) Isoboles showing synergy between E and ***X***. (B) The dose–response curve for ***X*** (indicated by ***E*** = 0) is given by $y = 0.4x + 0.1x^2$, where y is the number of cancers per 10^6 exposed individuals and x the dose of X or ***E*** in pg/day. It is assumed that E and x have the same dose–response curve. The excess rate of cancers caused by adding X to fixed levels of E increases markedly with increasing levels **of** E.

binations of three usually showed synergy even in cases where combinations of any two of the three were almost always antagonistic (Berenbaum ***et*** *al.*, 1983). It appears, therefore, in the case of antimicrobial agents, that increasing the number of agents interacting increases the likelihood that the interaction will be synergistic and increases the degree of synergy shown. It cannot be assumed, without further investigation, that other classes of agents will behave similarly, but studies with combinations of two agents show that interactions are very common phenomena, irrespective of the class of agent (these include antibiotics, cancer chemotherapeutic agents, ionising radiations, immunosuppressive agents, and enzyme inhibitors, to name but a few) (Berenbaum, 1981, 1985) and there are no evident differences between these in the sorts of behaviours observed. It is therefore not unreasonable to assume that carcinogens will prove to behave similarly when they are properly investigated, and it appears sensible to assume this until proved otherwise.

CONCLUSION

The main conclusions to be drawn from this analysis are that, even when the dose–response curve of a carcinogen is truly linear (or indistinguishable from linear over the relevant range), synergistic interactions between it and carcinogens already present in the environment will generally lead to its dose–response curve for extra risk being nonlinear and steeper at lower than at higher doses, so that extrapolation from a high-dose experiment would underestimate the effect of low doses. These features would be more pronounced at higher levels of en-

vironmental carcinogens (as long as these are not at saturating levels). **Risk** is also likely to increase with the number of carcinogens present.

These conclusions are clearly relevant to public policy on environmental carcinogens. They also point to the need for a systematic examination of interactions between carcinogens and of the mechanisms underlying their interactions. Neither topic has hitherto received adequate attention.

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