

A summary of the results obtained is shown in the Table. Phosphoramidate (II) and HMPA (I) gave positive results on each occasion and the trianilide (V) and the thioamide (VI) gave negative results. The results shown in Fig. II for dimethylnitrosamine and diphenylnitrosamine were obtained under similar conditions and are recorded here for comparison. Full details of these experiments will appear elsewhere (Styles, in preparation).

DISCUSSION

The object of the present study was to define an appropriate short-term test with which to evaluate the potential carcinogenicity of chemical relatives of HMPA, and further, to attempt to find a chemical explanation for its carcinogenicity. The results shown in Table I and Table II clearly establish that only the cell transformation test meets the test selection criteria described earlier and it is, therefore, the preferred assay for this class of compounds. Further, the data obtained provides a basis for understanding the in vivo and in vitro effects so far observed.

Consideration will first be given to the possible ways by which the carcinogenicity of the HMPA could be mediated. First, an intermediate alkylating species might arise from the dimethylamino group which is present in this compound. This functional group has been directly implicated in the carcinogenic activation of several other carcinogens such as dimethylnitrosamine, dimethylcarbamoyl chloride and 4-dimethylaminoazobenzene. Alternatively, the in vivo effect may derive from purely physical interactions associated with the unusual lipid-aqueous solvent properties of HMPA (Lloyd 1975) which is a liquid. In this connection, it may be significant that the primary tumours observed in the rat inhalation study occurred in the immediate nasal region. Both of these possible methods of action are prevented in phosphoric trianilide (V) which is a solid (mp 212 - 215°) and which is also devoid of potential alkylating groups.

The positive effects given by HMPA in these two short-term tests together with the fact that it produces mutagenic effects in Drosophila (Benes and Sram 1969) supports the view that this compound, or an active metabolite derived from it, reacts chemically with genetic material. Further, the negative response observed in the present study for the phenyl analogue (V), which is devoid of methyl groups, implicates the methyl groups

in this process. These results equally make the non-specific physical hypothesis less plausible as the functionally related dipolar solvents dimethylformamide (DMF) and dimethylsulphoxide (DMSO) are without effect in both tests (Purchase et al 1976, 1977 in preparation).

The difference in test response observed when comparing HMPA with the phenyl analogue (V) (Figs. Ia and Ic) is similar to that found when comparing the test response for the carcinogenic biological alkylating agent, dimethylnitrosamine (VII) with its non-carcinogenic, phenylated analogue, diphenylnitrosamine (VIII) (Fig. II). The analogy between these two classes of carcinogen is further strengthened by the fact that the erratic response given by the Ames test for HMPA has also been observed for dimethylnitrosamine (Bartsch et al 1976, Purchase et al 1977, in preparation) when using the same plate incorporation assay technique. It may be that both of these erratic responses are due to variations in the rate of formation or effective half life of a common alkylating species. Such variations could result from changes in the enzyme profile or balance between different batches of microsomes or from slight changes in the chemical environment in the test medium.

There is, therefore, sufficient evidence to initially assume that the broad structural requirements for carcinogenicity observed for the nitrosamine carcinogens apply equally to phosphoric amides.

The structure-activity relationships observed for nitrosamines have been reviewed (Druckrey 1975) and the major requirement for activity is that the amine nitrogen atom should carry at least one alkyl group having a free α -position potentially capable of undergoing metabolic α -hydroxylation. On this basis, it is possible to predict that hexaethylphosphoramidate (IV) and phosphoric trimorpholide (X), for example, would both have carcinogenic potential by analogy with the carcinogens diethylnitrosamine and nitrosomorpholine (X) respectively. Clearly, the first step in the evaluation of such compounds would be to submit them to the cell transformation test with HMPA (I) as the positive control.

The validity of the above chemical class analogy is partially confirmed by the observation that HMPA (I), and two of its alkylated analogues, undergo in vivo and in vitro α -hydroxylation leading to formaldehyde formation. (Jones and Jackson 1968). We have confirmed this observation for HMPA (I) using the S-9 liver fraction described above, a dose-dependent relationship between the concentration of HMPA (I) and formaldehyde formation being observed. These experiments, which mirror those described for dimethylnitrosamine (VII) (McLean and Day 1974), together with others aimed at trapping the postulated intermediate alkylating species formed from HMPA (I) will be described in a subsequent publication (Ashby, Lefevre and Penman in preparation).

The usefulness of a reliable test is illustrated by the problem posed when attempting to evaluate a compound such as the thioamide (VI). This compound is superficially related to HMPA yet it has no counterpart in nitrosamine chemistry. The negative result obtained (Fig. 1d) clearly indicates that this compound can be dissociated from the HMPA type in vivo carcinogenic activity.

Finally, it must be mentioned that the in vitro test response and the in vivo leukaemogenicity observed for phosphoramidate (II) require an explanation not involving alkyl groups (since none is present). Any separate hypothesis might, of course, be additionally involved in explaining the carcinogenic activity of HMPA itself. Curiously, a similar situation is encountered when considering the carcinogenicity of derivatives of hydrazine (NH_2NH_2) (Toth 1975). In this case the activity of a variety of alkylated hydrazines can be explained in terms of derived alkyl carbonium ions yet this leaves the activity observed for hydrazine itself unexplained (Biancifiori and Ribacchi 1962).

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Inhalation toxicity of hexamethylphosphoramidate.

TABLE I

Legend

Results given by the Ames test and the cell transformation test for hexamethylphosphoramide (I), phosphoramidate (II), phosphoric trianilide (V) and N,N',N''-trimethylphosphorothioic triamide (VI).

EXPERIMENT \ COMPOUND	(I) (+ve control)	(II)	(VI)	(V) (-ve control)
Ames test				
1	+		-	-
2	-	-		-
Cell transformation				
1	+		-	-
2	+	+		-

Fig I Survival and transformation dose response curves of
(Legend) BHK cells treated with compound (I)(Fig Ia), (II)
(Fig Ib), (V)(Fig Ic), (VI)(Fig Id), benzidine
(Fig Ie) (—●—●—●) and DMSO (Fig Ie)(—▲—▲—▲—)

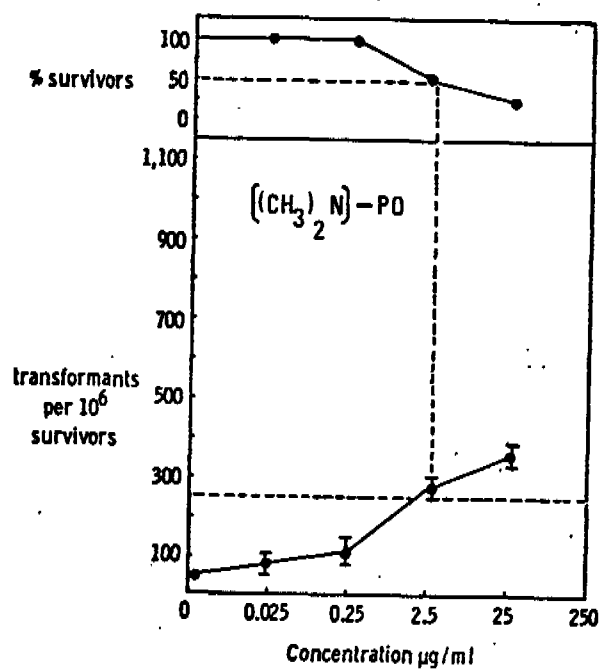


Fig Ia.

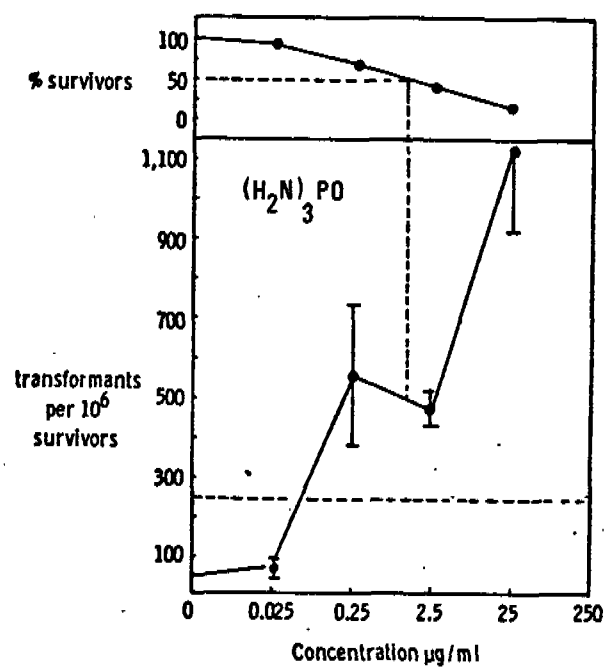


Fig Ib.

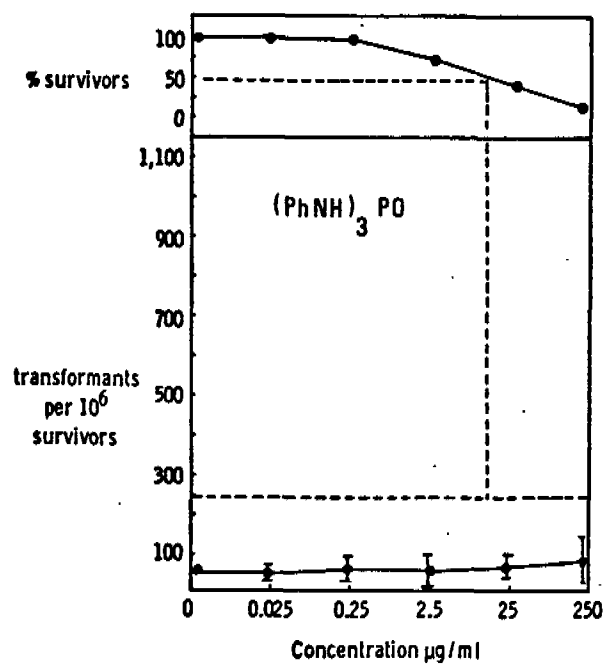


Fig 1c.

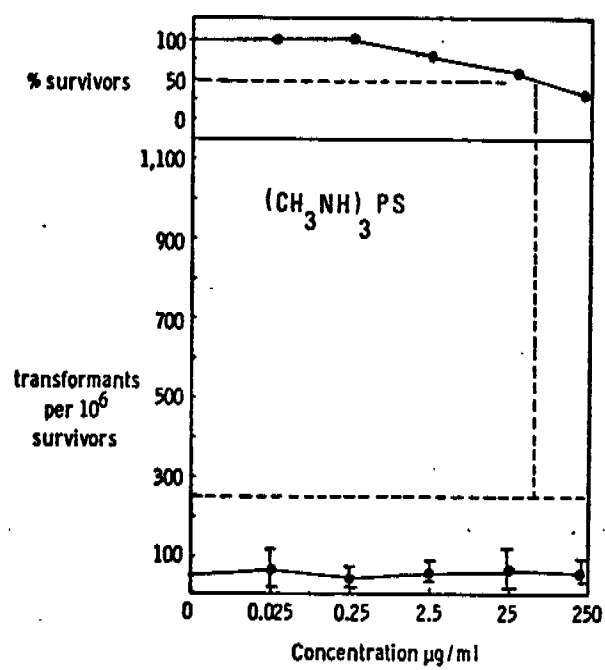


Fig Id.

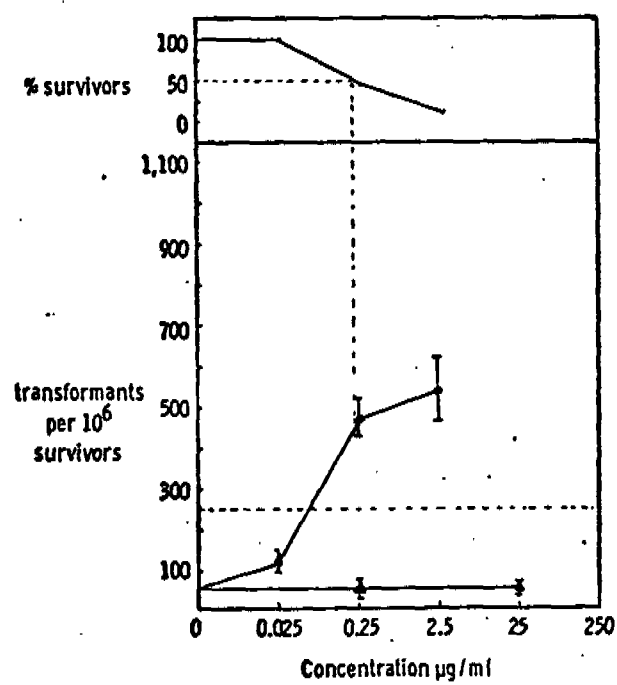
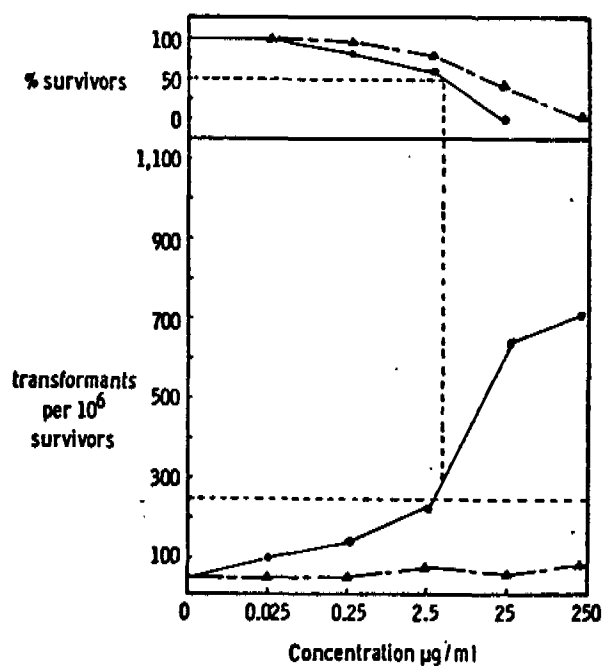
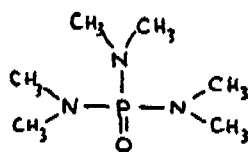


Fig 1e.

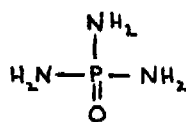
Benzidine —●—●—
DMSO —▲—▲—

Fig II . Survival and transformation dose response curves of
(Legend) BHK cells treated with diphenylnitrosamine (—▲—▲—)
and dimethylnitrosamine (—●—●—)

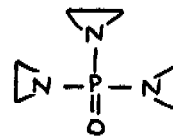




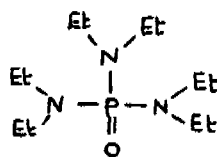
(I)



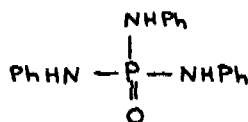
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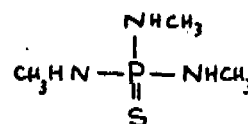
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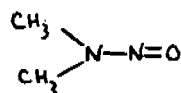
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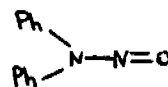
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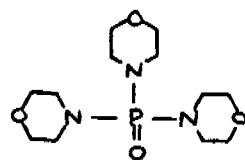
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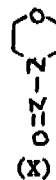
(VII)



(VIII)



(IX)



(X)