Carcinogenicity and Genetic Toxicity
Results on Single Chemicals: Implications for Testing Mixtures

Raymond W. Tennant, David M. DeMarini and Judson W. Spalding

ABSTRACT
The problem of defining the effects of chemical mixtures requires further testing and research efforts at several levels. To know the predictive value of the various end-points of genetic toxicity for the carcinogenicity of single chemicals, it is necessary to develop a much more systematic data base that includes many non-carcinogens. Few complex chemical mixtures have been tested for carcinogenicity or for most categories of genetic toxicity. The ability to predict the carcinogenicity of complex mixtures in rodents based upon the genetic toxicity of such mixtures is more limited than the ability to predict the carcinogenicity of single chemicals. Until the predictive value of genetic toxicity tests is better defined for single chemicals, it will be difficult to effectively use such results to predict the genetic toxicity of chemical mixtures. Genetic toxicity assays, however, are valuable in the monitoring, separation and identification of toxic chemicals and mixtures so that mutagens can be eliminated where the technology permits. However, the limited studies to date suggest that a variety of interactions between chemicals are possible and that the genetic toxicity of complex mixtures cannot be predicted from the genetic toxicity of individual chemicals. Therefore, the genetic toxicity of mixtures must be determined by empirical tests.

1 INTRODUCTION
There are several problems that complicate the topic of this manuscript. The first concerns the relationship between genetic toxicity and carcinogenicity: for the results of short-term tests which measure the genetic effects of chemicals to be used prospectively, a thorough retrospective analysis must be possible. It is only by
retrospective evaluations that the limits of the predictive value of short-term tests can be established. Second, there are many potential sources of exposure to complex mixtures, including ambient air, cigarette smoke, and effluents from industrial processes or chemical waste sites, to name a few. Each mixture poses different problems in terms of sampling and testing in each test system. A third problem is that the interaction (synergism, additivity, or antagonism) of compounds in a mixture is not predictable from the properties of a few of the compounds in the mixture.

There have been numerous reports on the use of genetic toxicity data in relation to chemical mixtures (e.g. Epler, 1980; Waters et al., 1982), and the reader is directed to these for specific applications of genetic toxicity assays. The use of genetic toxicity assays to predict the potential carcinogenicity of single chemicals is, perhaps, the key issue in the application of these assays. Therefore, we shall first focus on this aspect of the problem.

We recently completed an evaluation of 74 assays of single chemicals for carcinogenicity that were conducted in mice and rats by the National Cancer Institute and National Toxicology Program (Tennant et al., 1986). The purpose of the evaluation was to compare patterns of tumorigenicity and genetic toxicity to determine if any relationship might exist between subsets of tumour types and the various end-points for genetic toxicity. This report will summarize the results of that evaluation, and discuss the implications of these and other results in relation to chemical mixtures.

2 SUMMARY OF PREVIOUS EVALUATION

Although approximately 240 reports are available on tests for carcinogenicity in rodents conducted by the National Cancer Institute and the National Toxicology Program, this evaluation included only 74 of the most recent reports. The analysis was confined to those reports, chosen on a chronological basis, because they were conducted under a standardized protocol. Because the animals were exposed at relatively high concentrations (defined as the 'maximum tolerated dose') of test chemical for a significant portion of their lifespan (103–107 weeks), these studies represent one of the few sources of identified non-carcinogens where the exposures were adequate to reduce the probability of missing weak carcinogens.

The conclusions used were the same as those stated in the original reports. Other evaluations of rodent carcinogens (Chu et al., 1981; Griesemer and Cueto, 1980; Weisburger, 1983) identified the type and frequency of tumours in each sex/species and the malignancy of the tumours. In our evaluation we also took into account the relationship between the induced tumours and the spontaneous or background frequency (incidence) of tumours at that site or sites. The background tumour frequencies for B6C3F1 mice and F344 rats were historical
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control values derived from at least 2000 animals per group (Haseman, 1983).

Genetic toxicity test results for the same chemicals were derived from the published literature or from the National Toxicology Program, Cellular and Genetic Toxicology testing contracts. Literature results were obtained from sources that provided data that could be evaluated by criteria established by the EPA Gene-Tox Evaluation Committees (for example, see Brusick et al., 1980). Data obtained from the National Toxicology Program were derived from tests conducted with coded chemicals under a defined protocol (e.g. Haworth et al. 1983). To be considered in the evaluation, results from at least two of four broad categories of genetic toxicity tests were required: mutagenicity; chromosomal effects—chromosome aberrations or sister chromatid exchanges (SCEs); DNA damage; or mammalian cell neoplastic transformation. Of the 74 chemicals considered, sufficient genetic toxicity results were available for only 33 chemicals. Of these, 25 were carcinogens, five were non-carcinogens, and three were suspect.

Of the 41 carcinogens identified, many caused tumours of more than one type at more than one site, and 19 of the 41 induced tumours in at least one sex of both species. Common tumours (those that are the same type as the spontaneous tumours for that sex/species) and uncommon tumours (those that rarely occur spontaneously in that sex/species) together were induced by 20 of the 41 carcinogens; four chemicals (diglycidyl resorcinol ether, allyl isothiocyanate, melamine, and p-quinone dioxime) induced only uncommon tumours, three of which were transitional cell carcinomas or papillomas in the bladder of rats; 21 carcinogens induced only common tumours. The liver was the principal site of induction for common tumours; 27 of the 41 carcinogens induced liver tumours. Although the male B6C3F1 mouse has been considered to be the principal sex/species of liver tumour induction, it was never the only sex/species in which liver (or any other) tumours were induced. More chemically induced tumours occurred in the liver than at any other site, and the occurrence of these liver tumours was independent of the spontaneous incidence of liver tumours (i.e. in male mice with a spontaneous incidence of 32%, 16 chemicals were liver carcinogens, while in male rats with a spontaneous incidence of 3.5%, 11 chemicals induced liver tumours). However, it is predictable that the liver should be a predominant site for high-dose two-year studies that involve a per os route of administration because the liver is the principal site of chemical metabolism and carcinogen activation.

Comparison of the rodent carcinogenicity results with genetic toxicity results showed a high degree of association between the two parameters, and 23 of 25 rodent carcinogens showed evidence of genetic toxicity. However, the predictive capacity of the genetic toxicity assays cannot be determined because only a small number (5) of non-carcinogens has been adequately tested. It is clear, however, that in vitro mutagenicity assays by themselves are inadequate to predict the potential rodent carcinogenicity of some chemicals.
3 EVALUATION OF SELECTED CHEMICALS THAT INDUCE COMMON OR UNCOMMON TUMOURS

As stated previously, liver tumours were induced in at least one sex/species by 27 of the 41 carcinogens, but the liver was the only site of induction in a single species for just seven chemicals (Table 1). One chemical (C.I. solvent yellow 14) was positive only in rats; six chemicals (trichloroethylene, 1,1,1-trichloroethane, 1,1,1,2-tetrachloroethane, pentachloroethane, di(2-ethylhexyl)adipate, and 2,6-dichloro-p-phenylenediamine) were positive only in mice. Relatively few genetic toxicity results were available for these chemicals, but four chemicals showed evidence of genetic toxicity in at least one system. However, the volatility of the halogenated aliphatics creates some problems of interpretation. Unless tests are conducted under conditions suitable for volatile chemicals, it is not possible to determine if the chemicals are actually negative or if the tests are inadequate.

Twenty of 41 carcinogens induced uncommon tumours in at least one sex/species, and, in addition, most of these 20 carcinogens also induced common tumours at other sites. For example, three chemicals that induced nasal epithelium papillomas and/or carcinomas or rhabdomyosarcomas (uncommon tumours) also induced common tumours in the same or other sex/species (Table 2). These carcinogens also showed evidence of genetic toxicity in three of the four categories of in vitro assays. Although the nasal tumours induced by 1,2-dibromo-3-chloropropane and 1,2-dibromomethane are probably related to inhalation exposure, 2,6-xylidine was administered in the feed, and all three compounds induced tumours at other sites that were not directly related to the exposure route (e.g. adrenal cortical adenomas, haemangiosarcomas, mammary and subcutaneous fibroadenomas and fibromas). Likewise, three chemicals that induced thyroid follicular cell tumours, which are uncommon in the female rat, also induced common tumours at other sites (Table 3). Also, they induced common tumours in at least one sex of mice.

All of the chemicals listed in Tables 2 and 3 induced common and uncommon tumours, and were positive for genetic toxicity in more than one of the four general categories. Because not all of the chemicals have been tested adequately in all four categories, it is possible that they could be positive in additional genetic toxicity end-points. Individual genetic toxicity assays differ in sensitivity, endogenous metabolic capacity, etc.; therefore, it is unlikely that most chemicals will be positive in all tests. Table 4 shows the relationship between genetic toxicity results that were available in at least three of the four categories for the chemicals that induced tumours in at least one sex of both species. Nine of these 11 chemicals induced uncommon tumours, eight of the 11 were positive in at least two genetic toxicity categories, and three were positive in one category. Again, testing is not complete, and results are not available in many categories.

The results described above are in contrast to the results of some chemicals that induced only uncommon tumours in a single sex/species (Table 5).
Table 1 Genetic toxicity of chemicals which induced liver tumours in only one rodent species

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Carcinogenicity</th>
<th>Genetic toxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F344 rat</td>
<td>B6C3F&lt;sub&gt;1&lt;/sub&gt; mouse</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>F</td>
</tr>
<tr>
<td>C.I. solvent yellow 14 (feed)</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Trichloroethylene (gavage)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1,1,1-Trichloroethane (gavage)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1,1,1,2-Tetrachloroethane (gavage)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Di(2-ethylhexyl)adipate (feed)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Pentachloroethane (gavage)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2,6-Dichloro-p-phenylenediamine (feed)</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

+ = positive response.
- = negative response.
Blank = untested.
? = uncertain response.
+w = positive only with addition of exogenous activation system (89).
Table 2 Genetic toxicity of chemicals which induced tumours of nasal epithelium

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Carcinogenicity</th>
<th>Genetic toxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F344 rat</td>
<td>B6C3F₁ mouse</td>
</tr>
<tr>
<td>1,2-Dibromo-3-chloropropane (inhalation)</td>
<td>U</td>
<td>U,C</td>
</tr>
<tr>
<td>1,2-Dibromoethane (inhalation)</td>
<td>U,C</td>
<td>U,C</td>
</tr>
<tr>
<td>2,6-Xyldine (feed)</td>
<td>U,C</td>
<td>U,C</td>
</tr>
</tbody>
</table>

U = uncommon tumour.
C = common tumour.
+ = positive response.
− = negative response.
Blank = not tested.
Table 3 Genetic toxicity of chemicals that induced thyroid and other tumours

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Carcinogenicity</th>
<th></th>
<th>Genetic toxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F344 rat M F</td>
<td>B6C3F₁ mouse M F</td>
<td>Mutagenesis Sal LYM Other</td>
</tr>
<tr>
<td>4,4'-Methylenedianiline·2HCl (water)</td>
<td>C, L U</td>
<td>C, L C, L</td>
<td>+</td>
</tr>
<tr>
<td>4,4'-Methylene-bis(N,N-dimethyl)aniline (feed)</td>
<td>C U</td>
<td>L</td>
<td>+ +</td>
</tr>
<tr>
<td>4,4'-Oxydianiline (feed)</td>
<td>L, C L, U C, L,C</td>
<td>+ +</td>
<td>+</td>
</tr>
</tbody>
</table>

- C = common tumour.
- L = liver (common) tumour.
- U = uncommon tumour.
- + = positive response.
- - = negative response.
- Blank = not tested.
- + w = positive only with addition of exogenous activation system (S9).
Table 4  Genetic toxicity of chemicals that induced tumours in at least one sex of both species

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Carcinogenicity</th>
<th>Genetic toxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F344 rat</td>
<td>B6C3F1 mouse</td>
</tr>
<tr>
<td>1,2-Dibromo-3-chloropropane</td>
<td>+ +</td>
<td>+ +</td>
</tr>
<tr>
<td>1,2-Dibromoethane</td>
<td>+ +</td>
<td>+ +</td>
</tr>
<tr>
<td>Dichloromethane</td>
<td>+ +</td>
<td>+ +</td>
</tr>
<tr>
<td>Di(2-ethylhexyl)phthalate</td>
<td>+ +</td>
<td>+ +</td>
</tr>
<tr>
<td>4,4'-Methyleneedianiline·2HCl</td>
<td>+ +</td>
<td>+ +</td>
</tr>
<tr>
<td>Michler's ketone</td>
<td>+ +</td>
<td>+ +</td>
</tr>
<tr>
<td>4,4'-Oxydianiline</td>
<td>+ +</td>
<td>+ +</td>
</tr>
<tr>
<td>Benzyl acetate</td>
<td>+ +</td>
<td>+ +</td>
</tr>
<tr>
<td>Cinnamyl anthranilate</td>
<td>+ +</td>
<td>+ +</td>
</tr>
<tr>
<td>4,4'-Methylene-bis(N,N'-dimethyl)aniline</td>
<td>+ +</td>
<td>+</td>
</tr>
</tbody>
</table>

+ = positive response.
- = negative response.
Blank = untested.
? = uncertain response.
+w = positive only with addition of exogenous activation system.
Table 5  Genetic toxicity of chemicals that induced bladder tumours in rats

| Chemical                        | Carcinogenicity | Genet
|                               | F344 rat | B6C3F1 mouse | Mutagenosis | Chromosomal effects | Transformation | DNA damage |
|--------------------------------|-----------|-------------|----------------|---------------|------------------|---------------|-----------|
|                                | M | F | M | F | Sal | LYM | Other | CA | SCE | Cell | Viral | UDS | Rec |
| Allyl isothiocyanate (gavage)  | + | - | - | - | -   | -   | -    | +  | +   | -    | -    | -   |     |
| 11-Aminoundecanoic acid (feed) | + | - | - | - | -   | -   | -    | -  | +   | -    | -    | -   |     |
| Melamine (feed)                | + | - | - | - | -   | -   | -    | -  | -   | -    | -    | -   |     |

+ = positive response.
- = negative response.
Blank = not tested.
Allyl isothiocyanate, 11-aminoundecanoic acid, and melamine induced transitional cell bladder tumours only in male rats. Neoplastic nodules of the liver were also associated with exposure to 11-aminoundecanoic acid. Two of these chemicals show evidence of only chromosomal effects, and one is negative in all four categories. Chemicals that induce uncommon tumours in a single sex/species may reflect high tissue/species specificity, probably involving cell-specific metabolism or a highly specific target site.

4 SOME IMPLICATIONS OF SINGLE CHEMICAL TEST RESULTS

The range of chemical classes and the carcinogenicity and genetic toxicity results available for this analysis limit the conclusions that can be drawn. However, the results do allow for some hypothetical inferences which may be tested by future results. A general pattern that is suggested by the results is that some carcinogens that induce common and uncommon tumours in at least one sex of both species tend to be positive in several categories of genetic toxicity tests. It is possible that such carcinogens are more significant biologically because they may be more likely to be positive in other species than those carcinogens that induce highly specific or uncommon tumours in a single sex/species. These latter carcinogens demonstrated less potential genetic toxicity. Similarly, the carcinogens that induce only common (usually liver) tumours in one sex/species also tended to show less genetic toxicity and may have relatively less trans-species carcinogenic potential than those carcinogens that induced both common and uncommon tumours.

However, we are not proposing that the chemicals be classified on the basis of potential genetic toxicity alone. Such a classification is inappropriate at this time because sufficient results do not exist for most chemicals to distinguish between those that are not genotoxic and those that are inadequately tested. This is particularly true in the absence of results from short-term in vivo assays because tissue- or species-specific metabolism, target cells, or indirect effects (i.e. induction of endogenous sources of potential genetic toxicity; e.g. Totter, 1980) can be missed in in vitro systems. It has been proposed that carcinogens that are not genotoxic induce tumours by indirect mechanisms such as tumour promotion (Clayson, 1981; Pitot et al., 1982; Schulte-Hermann et al., 1983). This may be plausible for some carcinogens that induce common tumours where other toxic properties of the chemical may select for preneoplastic cells, cause compensatory proliferation, decrease time to tumour, etc. However, the hypothesis is less plausible for chemicals that induce uncommon tumours. If conventional conceptions of tumour promotion apply, then some ubiquitous or ambient inducer must be proposed. If the inducer is ubiquitous, then one must explain why the tumour is uncommon. At this time, therefore, we think that the systematic application of tests for the major categories of genetic toxicity end-points may be useful in distinguishing those chemicals with the greatest...
trans-species potential. No one end-point alone, such as gene mutation, is sufficient, and results should be obtained in a variety of end-points. Finally, too few bona fide non-carcinogens have been tested adequately to know the predictive capacity of any genetic toxicity assay.

5 GENETIC TOXICITY STUDIES ON SELECTED CHEMICAL MIXTURES

The mutagenicity of complex mixtures has been investigated by two general approaches. In one approach, a complex mixture is fractionated into a variety of chemical classes and then the fractions are tested for mutagenic activity in the Salmonella/microsome assay. The other approach involves combining two or more pure chemicals and comparing the mutagenicity of the mixture to that of the single compounds. We will discuss some of the details and findings from both approaches.

The fractionation of complex mixtures often involves analytical chemistry techniques such as gas chromatography/mass spectrometry and high-performance liquid chromatography to further characterize the mutagenic compounds that may account for some of the observed mutagenicity of the original complex mixture. Although mutagenicity test systems other than the Salmonella assay have been used, the vast majority of mutagenicity studies of complex mixtures used mutagenicity in Salmonella as the sole genetic end-point.

The types of complex mixtures that have been investigated for mutagenic activity include drinking water (Loper, 1980), airborne particles (Chrisp and Fisher, 1980), oils and fuels made from coal (Epler, 1980), cigarette smoke (DeMarini, 1983), food (Felton et al., 1986), automobile emissions (Lewtas, 1983), roofing tar and coke oven fumes (Nesnow and Lewtas, 1981), various industrial effluents (Nestmann et al., 1984), and commercial products (Pham et al., 1979).

Although many complex mixtures have been studied for mutagenic activity, very few have been adequately tested in rodents for carcinogenic activity. In general, the majority of such studies have applied the mixture to mouse skin and used skin tumours as the carcinogenic end-point. Such studies have been performed primarily with combustion products such as coal oils, cigarette smoke condensate, automobile emissions, and broiled meat pyrolysates.

The interactions that may (and undoubtedly do) occur among the components of complex mixtures include synergisms, antagonisms, inhibitions, etc. Sorting out these complexities is difficult enough; the effects of these interactions on the final end-point (mutagenesis or carcinogenesis) are complicated even further by the other biological activities (such as cytotoxicity) which may not be measured but may affect the measured end-point.

The following observations have been made for two complex mixtures: coal oils and cigarette smoke condensate (CSC). For CSC, the neutral polyaromatic
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hydrocarbon (PAH) fraction is responsible for the mouse skin tumor activity, but not for the Salmonella mutagenicity. Most of the mutagenicity seems to be due to the basic (aromatic amines, etc.) fraction. For coal oils, most of the mouse skin tumor activity is due to PAHs and neutral nitrogen heterocyclics, but the Salmonella mutagenicity is due to the neutral polar fractions.

Thus far there has been little work to allow comparison of chemical classes or to explore sites of tumorigenesis other than skin. Likewise, little is known about which chemical classes are important for mutagenesis in organisms other than Salmonella and at endpoint other than gene mutation (e.g. cytogenetic effects, etc.). Therefore, a comprehensive understanding of the mutagenicity and carcinogenicity of complex mixtures is not likely to be accomplished until such an understanding is available for a large number and variety of single chemicals.

This brings us to the second approach to the study of the mutagenicity of complex mixtures. We will describe some of the types of interactions that have been identified between single chemicals and provide a few examples of each. One type of interaction that has been described is exemplified by compounds called desmutagens, which are agents that directly inactivate mutagens. A desmutagen interacts with the mutagen rather than the cell or organism. For example, L-cysteine is desmutagenic towards MNNG, 4-NQO, and captan in the Bacillus subtilis Rec assay (Onitsuka et al., 1978). Thus, these three compounds, which are positive when tested alone in the B. subtilis Rec assay, are negative when L-cysteine is added.

A variation of this observation is demonstrated by chemicals that inhibit exogenous metabolic activation systems. Haugen and Peak (1983) showed that a neutral fraction from coal-derived oil suppressed the mutagenicity of the indirect mutagens benzo[a]pyrene, 7,12-dimethylbenz[a]anthracene, 2-aminofluorene, and 2-acetylaminofluorene; however, the neutral fraction did not suppress the mutagenicity of the direct-acting mutagens 2-nitrofluorene and benzo[a]pyrene diol-epoxide. Thus, interactions of chemicals with each other, with S9, and/or with the cell/organism may be an important determinant in the results of mutagenicity studies with mixtures.

Another type of interaction is exemplified by antimutagens that reduce the mutant yield of cells that have already been exposed to a mutagen (Kada et al., 1982). When Escherichia coli cells were exposed to either MNNG (Kada and Kanematsu, 1978) or gamma rays (Kada et al., 1979), the induced mutation frequencies were reduced when cobaltous chloride was present in the post-treatment medium.

Other types of antimutagens exhibit more complex interactions; there are chemicals that inhibit the mutagenicity of some chemicals but not of others. For example, either sodium ascorbate or butylated hydroxytoluene (BHT) reduced the DNA damage and repair synthesis induced by dimethylnitrosamine (DMN) in human skin fibroblasts; however, only BHT reduced the repair synthesis induced by aflatoxin B1 (Wei et al., 1981). In Salmonella, however, BHT had no
effect on the mutagenicity of MNNG (Rosin, 1981). This demonstrates that the effects seen by mixing two chemicals and treating *Salmonella* may not be indicative of the effects produced by the mixture in another test system and/or at another genetic end-point.

Another complication is that a particular chemical may enhance the mutagenicity of some chemicals but suppress, at an equimolar dose, the mutagenicity of other chemicals. For example, propyl gallate enhanced the mutagenicity of *N*-hydroxy-2-acetylaminofluorene in *Salmonella*, but it reduced the mutagenicity of *N*-acetoxy-2-acetylaminofluorene, which is the ultimate mutagenic form of the former compound (Rosin, 1981). Thus, one chemical (propyl gallate) may have the opposite effect on two closely related compounds.

Synergistic effects between mutagens also have been observed. For example, 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) and streptozocin were mutagenic in *Salmonella* and in mammalian cells (V79), with streptozocin being more mutagenic than BCNU in both systems. However, when cells were treated by a mixture of the two compounds, the resultant mutation frequencies were greater than those of either compound alone (Harbach *et al.*, 1982). Thus, synergistic effects have been noted in both *Salmonella* and mammalian cells. Synergism has also been found for end-points other than gene mutation. Schwartz *et al.* (1984) found that 3-aminobenzamide, MMS, and ENU each induced SCEs in CHO cells. However, 3-aminobenzamide acted on MMS- or ENU-treated cells to increase the SCE frequency beyond that seen if the effects were simply additive. In addition, this synergism did not seem to be mediated by an inhibition of the removal of DNA adducts. Synergistic effects also have been found with complex mixtures. For example, cigarette smoke condensate combined with 2-acetylaminofluorene gave a greater mutant yield in *Salmonella* than did either alone (Gairola and Hsu, 1983).

Other chemicals exhibit a type of interaction that is called comutagenesis. A comutagen is an agent that is not mutagenic itself, but which will enhance the mutagenicity of a mutagen. For example, catechol is not a mutagen in *Salmonella*; however, it enhances the mutagenicity of benzo[a]pyrene (Yoshida and Fukhara, 1983). This demonstration that catechol is a comutagen for benzo[a]pyrene in *Salmonella* is interesting because catechol also has been shown to be a cocarcinogen for benzo[a]pyrene on mouse skin (Van Duuren and Goldschmidt, 1976). However, the results of comutagenesis experiments in one test system cannot be used to predict results in another test system. For example, harman and norharman are comutagens for a variety of compounds in *Salmonella*, but are not comutagens in mammalian cells when combined with the same mutagens as used in the *Salmonella* experiments (Schwartz *et al.*, 1983).

Another form of interaction is revealed by 3-aminobenzamide, which induces SCEs synergistically with MMS or ENU. However, it is not mutagenic in the *CHO/HGPRT* assay. Nonetheless, it enhances EMS- or MNNG-induced
mutagenesis at the HGPRT locus (Schwartz et al., 1983). Therefore, 3-amino-
benzamide is an example of a chemical that acts synergistically with chemicals at
one end-point (SCEs), but which acts as a comutagen with the same chemicals at
another end-point (gene mutation). Thus, even simple extrapolations are not
justified between cell systems or genetic end-points.

An additional variation on the theme of comutagenesis is exemplified by
3-aminopyridine and norharman, neither of which is mutagenic in Salmonella
when tested separately. However, when these two compounds are combined, the
mixture is mutagenic (Wakabayashi et al., 1982). Once again, extrapolation from
results with single chemicals to potential results with mixtures of those chemicals
is not possible. Other complex interactions have been noted. For example,
neither methylguanidine nor nitrite alone induced chromosomal aberrations in
human skin fibroblasts; when the two compounds were combined they induced a
high frequency of chromosomal aberrations which were reduced when ascorbic
acid was present (Lo and Stich, 1978). The inhibition was due mainly to a
competitive reaction between ascorbic acid and methylguanidine for nitrite,
leading to decreased N-nitrosation of methylguanidine.

Thus, although many chemical mixtures have been studied for mutagenicity,
much more work must be done in order to develop an understanding of the
myriad of interactions that occur in such mutagenesis studies. Because of the
limited number of studies and the nature of the experiments, no unifying or
fundamental principles have emerged from these investigations. Therefore, there
are as yet no guides for predicting the mutagenic effect in a particular organism
under specific conditions when the organism is exposed to two chemicals
simultaneously—even if the mutagenicities of the two individual compounds are
known. Because of the complexities associated with the mutagenicity of complex
mixtures, simple mixing studies with two or three chemicals in just a few systems
may not lead to any fundamental principles that would be applicable to a broad
range of very complex mixtures.

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