Effects of Metals in *in Vitro* Bioassays

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The capacity of *in vitro* bioassays to detect the potential carcinogenicity of metal compounds is reviewed. The *in vitro* bioassays discussed include: bacterial reversion analysis to determine the capacity of metal salts to revert *Salmonella typhimurium* histidine auxotrophs or to revert *Escherichia coli* WP 2 tryp*+* to tryptophan prototrophy; examination of the ability of metal salts to preferentially inhibit cell growth in *Bacillus subtilis* cells deficient in DNA repair pathways; determination of the ability of metal salts to induce resistance to base analogs in mammalian cells; the capacity of metal salts to enhance viral transformation of mammalian cells or to transform cells in the absence of virus; and the ability of metal salts to induce chromosomal aberrations in mammalian cells. Using each of these *in vitro* bioassays, diverse metal compounds have been identified as potential carcinogens. Furthermore, the use of different compounds of a specific metal may allow a determination of the valence which may be required for carcinogenesis.

*Introduction*

Repeated observations have suggested that environmental agents are causative factors in the development of human neoplasia (1-5). As epidemiological studies as well as animal experimentation can be performed on only a few compounds at great expense and over extended intervals, recent attention has focused on the development of short-term *in vitro* bioassays through which suspected carcinogens may be detected (6-10). The most widely used bioassay is that developed by Ames which uses reversion to histidine prototrophy in *S. typhimurium* as a means to detect potential human mutagens and carcinogens (11, 12). This bioassay, as well as most others which have been developed, ultimately quantitate the capacity of environmental agents to induce an alteration in the nucleotide sequences which comprise the genetic information in the cells DNA.

Metals have been shown to be carcinogenic in laboratory animals (13-15) and have been implicated as human carcinogens (16, 17). In this review the main *in vitro* bioassays which have been used to screen a large number of metal compounds for their mutagenicity and for their potential carcinogenicity will be discussed. In particular, this review will examine the capacity of metal compounds to induce mutations in procaryotes and in eucaryotes; to enhance the viral transformation of mammalian cells in culture; to induce transformation of hamster embryo cells *in vitro*; and to induce sister-chromatid exchanges and other chromosomal aberrations in eucaryotic and in human cells. The other main bioassay used to detect potential metal mutagens and carcinogens, the infidelity of DNA synthesis *in vitro* (18-20), is discussed in a companion paper.

In this review I shall also consider the hypothesis that short-term *in vitro* bioassays may be used to determine which compounds of specific metal carcinogens pose the greatest risk with regard to the induction of human neoplasia.

*Bacterial Mutagenesis as an in Vitro Bioassay for Metal Compounds*

The observation that many chemical carcinogens are also mutagens has led to the development of several bacterial systems as *in vitro* bioassays. The basis of these assays in the quantitation of the capacity of environmental agents to revert previously induced mutations or to inhibit their capacity for cell growth. A brief description of these assays will be provided for the purpose of this

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review. A more extensive review of each assay can be consulted for more detailed analysis of the systems per se or for a further consideration of metal mutagenesis (12, 21, 22).

Foremost among in vitro bioassays is that developed by Ames in which the reverse mutation of histidine requiring strains of *S. typhimurium* to histidine prototrophy is quantitated. The tester strains used contain additional defects to facilitate mutagenesis. In the initial experiments defining the Salmonella assay approximately 250 chemicals were examined (23, 24). Of the 175 chemical carcinogens tested, 157 or 90% were mutagenic. In contrast, of the 108 non-carcinogenic chemicals tested, 94 or 87% did not produce detectable his*+ revertants above background levels. Subsequent analysis using this assay has been extended to a wide variety of individual chemicals as well as to complex mixtures of environmental agents (11).

The second bacterial system which has been used to screen metal compounds measures reverse mutations in *E. coli* strain WP 2 tryp– (22). Analogous to the Salmonella assay this system quantitates the capacity of exogenous agents to revert the bacteria to tryptophan prototrophy. As DNA is considered the critical target for chemical carcinogens, the *E. coli* WP 2 strains employed are usually deficient in specific DNA repair pathways. This deficiency enhances the potential for detecting environmental mutagens as DNA lesions will tend to persist for greater intervals due to the cells inability to excise the DNA adducts.

The third bacterial system utilized as an in vitro bioassay for metal compounds measures the capacity of exogenous agents to inhibit cell growth (25). Recombination proficient (rec+) and recombination deficient (rec−) strains are used. The hypothesis which forms the basis of this bioassay is that the agent tested will inhibit cell growth to a greater degree in the rec− strains which is deficient in DNA repair. One presumes that the persistent lesions in DNA will prevent the expression of specific genes whose products are essential to cell growth or that the DNA lesions will prevent DNA replication and thus inhibit cell growth. Thus, in parallel cultures, one can measure an inhibition zone of cell growth for rec+ cells as well as for rec− cells. The expectation is that the greater the difference in inhibition zones, the greater the potential mutagenicity of the compound tested.

### Effects of Metals in Bacterial Bioassays

Chromium has been implicated as a human carcinogen inducing the formation of lung tumors after industrial exposure (15). Venitt and Levy (26) in 1974 reported that chromates were mutagenic in the *E. coli* B WP 2 system. Using hexavalent chromium compounds they observed a 3- to 4-fold increase in the number of revertants per plate as compared to untreated controls. Similarly, Nishioka (27) using the rec assay demonstrated that hexavalent chromium compounds strongly inhibited cell growth and thus had a demonstrative rec effect. In parallel studies, he also showed that potassium dichromate was mutagenic in *E. coli* B WP 2 tryp− uvr A− which is deficient in nucleotide excision repair (28).

In spite of the usefulness of the Ames assay in analyzing diverse types of chemicals, initial studies using this in vitro assay failed to detect the mutagenicity of several metal carcinogens (23, 24). This might have reflected technical problems which related to the chelation of free metal cations by components of the bacterial medium. However, later studies by several groups, including that of Ames, have been successful in determining metal mutagenesis using this in vitro assay. Lofroth and Ames (29) reported that chromate and dichromate induced frameshift mutations in three different strains of *S. typhimurium*. Further studies by Petrilli and De Flora (30) confirmed that hexavalent chromium compounds (sodium dichromate, chromic acid and calcium chromate) produced significant increases in histidine reversion using four tester strains. The nature of the reverse mutations required to regain histidine prototrophy suggested that the chromium compounds produced both frameshift and base pair mutations. Furthermore, the mutagenic activity of hexavalent chromium was independent of microsomal metabolism. Using lead chromate, Nestman et al. (31) reported increased mutagenicity in both the Ames Salmonella assay and the *E. coli* WP 2 tryp-fluctuation test. As both chromium and lead are potential human carcinogens, it was of interest to observe that the substitution of lead chloride for lead chromate resulted in an elimination of the mutagenic effect of the metal compound.

In contrast to the mutagenic capacity of hexavalent chromium, parallel studies demonstrated that trivalent chromium was inactivated as a mutagen (29, 30). With the use of two trivalent chromium compounds (chromium potassium sulfate and chromic chloride), no increase in the reversion frequency was observed in the presence or absence of a microsomal fraction from rat liver or from human erythrocytes. Although hexavalent chromium could be deactivated by enzymatic metabolism (32), trivalent chromium could not be converted to the hexavalent form. Of interest was the observation that microsomes from rat lung had no effect on the...
mutagenicity of hexavalent chromium. These results may be of importance as chromium may localize and accumulate in human lung during occupational or environmental exposure. Thus the inability of human lung to deactivate hexavalent chromium compounds may play a role in chromium carcinogenesis in humans. However, trivalent chromium could be chemically oxidized in vitro by using potassium permanganate (29). Similarly, Nishioka had reported that the rec effect of potassium dichromate was lost after treatment with Na$_2$SO$_3$, which is the reducing agent (27). Thus, it would appear that although chromium per se is a metal carcinogen, the valence state of the metal within a specific compound may be of prime importance (Table 1). Further study is required to determine whether trivalent chromium compounds can be considered relatively innocuous with regard to carcinogenicity and whether the focus should remain on hexavalent compounds. Arsenic compounds have been examined in each of these three bacterial in vitro assays (Table 1). Trivalent arsenic (arsenite) and pentavalent arsenic (arsenate) were inactive in the Ames assay (29). In contrast, Nishioka reported that rec effects were observed with both trivalent (AsCl$_3$ or NaAsO$_2$) and pentavalent (Na$_2$HAsO$_4$) arsenic (27). The result with AsCl$_3$ in the rec assay is the initial report that a free metal cation (As$^{3+}$) was a potential mutagen. Furthermore, trivalent arsenic as sodium arsenite induced tryp$^+$ reversions in *E. coli* WP2. However, recent results suggest that arsenite may not induce mutations in *E. coli* WP2 cells (T. Rossman, M. Molina, D. Stone, and W. Troll, Mutat. Res. Submitted). It was suggested that the results observed by Nishioka might have been due to the intrinsic variability of background mutation frequencies. As arsenic is implicated as a human carcinogen, the absence of arsenic induced mutation would predicate another mechanism. One intriguing possibility is that arsenic may inhibit the enzymes which comprise the excision repair pathways (28, 34).

Selenium has been investigated in both the rec assay and in the Ames Salmonella test. Using the rec assay, Nakamuro et al. (23) tested five selenium compounds (35). They reported that selenate failed to produce any difference in inhibition zones. However, selenite did show a small but significant rec effect. In contrast, Lofroth and Ames reported that using the Salmonella test system, selenite was not a mutagen (<< 0.01 revertants/mole) but that selenate produced a small number of mutations (0.03 revertants/mole) (29). Recently, Noda et al. reexamined this question (36). They reported that selenate and selenite were weak mutagens in both the rec assay and in the Salmonella test. In their experiments which did not utilize a microsomal activating system, selenite induced 0.2 revertants/ mole while selenate induced 0.05 revertants/mol in the Ames assay. The basis for these conflicting results is unknown but may spring from the marginal effects of selenium as a bacterial mutagen.

The role of platinum compounds as cancer chemotherapeutic agents has resulted in several investigations on the capacity of these compounds to act as bacterial mutagens (37-40). In particular, the stereospecificity implicit in the usefulness of cis-platinum (II) diamminedichloride (cis-PDD) as compared to its trans isomer in chemotherapy has also been examined in *in vitro* bioassays. Beck and Brubaker initially reported that cis-PDD was

Table 1. Metal mutagenesis in bacterial in vitro bioassays.$^a$

| Metal     | Valence or charge | Reversion of *S. typhimurium* his$^-$, revertants/n mole | Reversion of *E. coli* WP 2 tryp$^-$, colonies/plate | Rec effect in *B. subtilis* (difference in inhibition zone) | Reference |
|-----------|------------------|--------------------------------------------------------|-----------------------------------------------------|--------------------------------------------------------|-----------|
| Arsenic   | 3                | 0.01                                                   | N.D.                                                | +                                                     | (27, 29)  |
|           | 5                | 0.01                                                   | 37.7 (8.0)                                          | +                                                     | (27, 32, 33) |
| Chromium  | 3                | 172 (194)$^b$                                         | N.D.                                                | -                                                     | (27, 32, 33) |
|           | 6                | 925 (238)$^b$                                         | 41.7 (8.0)                                          | +                                                     |           |
| Platinum  | -2               | 2                                                      | N.D.                                                | N.D.                                                  | (44)      |
|           | 0                | 100                                                   | N.D.                                                | N.D.                                                  |           |
|           | +2               | 0.2                                                   | N.D.                                                | N.D.                                                  |           |
| Selenium  | 4                | 0.20                                                  | N.D.                                                | ±                                                     | (29, 35, 46) |
|           | 6                | 0.05                                                  | N.D.                                                | ±                                                     |           |

$^a$Determination of the capacity of metal compounds to induce mutations or to preferentially inhibit cell growth are described in the respective references. The numbers in parentheses refer to the number of mutations observed in simultaneous untreated controls. Charge refers to the net charge on the metal compound while valence defines the state of a metal within a compound. The convention of Nishioka has been used as a determination of the rec effect (27).

$^b$In revertants per plate.
cytotoxic to several *E. coli* strains deficient in DNA repair (41). They concluded that this cytotoxicity was due to the production of intrastrand crosslinks in DNA.

Mutagenicity studies in the Salmonella assay demonstrated the capacity of cis-PDD to induce histidine reversions. In a study of 12 platinum compounds, LeCointe et al. investigated the stereospecificity of platinum-induced mutagenesis (42). They observed that cis-PDD was the most potent mutagen tested. In the Ames assay cis-PDD produced 100 reversions/moles as compared to a reversion frequency of between 0.2-2.0 reversions/mole observed for the other eleven platinum compounds. LeCointe et al. also reported that PDD produces base-pair mutations. Of interest was the observation that the charge of the platinum compound appeared to directly affect the reversion frequency (Table 1).

Although chromium, arsenic, selenium, and platinum have been examined in some detail, rigorous analysis of other metals using all three *in vitro* bioassays remains undetermined. Manganese induced mutations in bacteriophage T4 (43) and in yeast (44). Copper caused mutations in *E. coli* (45) and in *B. subtilis* (46). Nishioka has screened a large number of metal salts in the rec assay (27). In addition to the metals previously discussed, rec effects were observed for cadmium, mercury, manganese, and for molybdenum. However, silver, beryllium, cobalt, copper, iron, nickel, lead, and zinc were negative in this assay. However, a systematic analysis of metal mutagenesis has not yet been performed using either the Ames Salmonella assay or the *E. coli* WP 2 tryp− *in vitro* bioassays.

### Metal Mutagenesis in Mammalian Cells

The decreased sensitivity of mammalian cells to base analogues has been used to detect metal compounds as mutagens in mammalian cells (47, 48). In particular, mutants in the HGPRT locus can be identified by the acquired capacity to grow in media containing thioguanine or 8-azaguanine. The HGPRT locus specifies a purine salvage enzyme, the hypoxanthine-guanine phosphoribosyl transferase. A deficiency in this enzyme renders the cell insensitive to the base analogues. Thus, in contrast to bacterial systems which quantitate the reversion of a previously induced mutation, this system measures the induction of the initial mutation. In general, hamster cells have been used as model systems to approximate the effects of metals on the HGPRT locus in mammalian cells.

O’Neill et al. reported that platinum as cis-dichlorodiammine platinum (II) induced thioguanine resistance in Chinese hamster ovary cells (49). Using 3 μM cis-PDD they observed an increase of approximately 30-fold in induced mutation as compared to spontaneous mutations in parallel cultures. Similar results were reported by Turnbull et al. using V79 Chinese hamster cells (50). A comparison of six platinum compounds by Taylor et al. (51) demonstrated that a variety of platinum compounds induced 8-azaguanine resistance in CHO cells. In this study, both cis-PDD and Pt(SO₄)₂ were equally effective in inducing mutations. In contrast, similar treatment with varying concentrations of K₂PtCl₄ or K₂PtCl₆ did not induce any alteration in sensitivity to 8-azaguanine. However, repeated subculture of CHO cells in 10 μM K₂PtCl₆ resulted in a 2- to 3-fold increase in the induction of 8-azaguanine-resistant colonies. This induction required exposure of the cells for 10 population doublings and was maintained after 20 population doublings. Further analysis by Zwelling et al. in V79 Chinese hamster lung cells demonstrated that cis-PDD was more mutagenic than trans-PDD at equitoxic doses (52). This confirms the previous results observed using bacterial *in vitro* assays. Furthermore, Zwelling et al. reported that the number of interstrand cross-links produced by both compounds were identical. These results would indicate that other lesion(s) were the causative DNA lesions for mutagenesis.

Chromium has also been examined in the Chinese hamster V79 system for the induction of 8-azaguanine resistance. In agreement with those results obtained in bacterial assays, Newbold et al. observed that hexavalent chromium compounds induced mutations while trivalent chromium compounds were inactive (53). Three hexavalent compounds were used, each of differing solubility in water; potassium dichromate which is highly water-soluble, zinc chromate which is slightly water-soluble, and lead chromate which has a very low solubility. Both potassium dichromate and zinc chromate induced significant numbers of colonies resistant to 8-azaguanine. In contrast, lead chromate was inactive yielding results comparable to those observed with trivalent chromium compounds. These results are of interest in that lead chromate was mutagenic in the Ames assay. The reason for this discrepancy remains unknown. Other metals have been analyzed in mammalian mutagenesis assays (54). These results should be available in the near future (J. P. O’Neill, personal communication).
Enhancement of Viral Induced Transformation by Metal Compounds

Exposure of hamster embryo cells to the simian adenovirus SA7 results in viral transformation of the cells (55). Subsequent analysis after transformation demonstrated that the viral genome was integrated into the hamster DNA as defined by nucleic acid hybridization studies. In a series of publications, Casto and DiPaolo reported that treatment of the cells with diverse chemical carcinogens resulted in an enhancement of the frequency of viral induced transformation (56, 57). In particular, treatment with polycyclic aromatic hydrocarbons or alkylating agents significantly enhanced viral transformation as compared to that observed with the virus alone. They hypothesized that this increase resulted from additional sites in DNA which were now accessible to the virus for integration. The production of these extra sites were due presumably to DNA alterations induced by carcinogen-DNA interactions.

Using this system as an in vitro bioassay, they then examined whether specific metal compounds could similarly enhance viral transformation (58). In these experiments they investigated 25 metals in a total of 38 metal salts. Of the 38 metal salts which were examined, 24 significantly enhanced viral transformation. These metal compounds were divided into three categories according to the metal concentration required for enhancement. The first group which showed highest activity at the lowest concentrations included antimony, arsenic, cadmium, chromium and platinum. The second group contained beryllium, cobalt, copper, lead, manganese, mercury, nickel, silver, thallium, and zinc. The third group contained only iron. There was a direct relationship between the capacity of metal salts to enhance viral transformation and their reported mutagenicity or carcinogenicity. Of the metals tested, only the results with zinc were inconclusive. A partial summary of the data is presented in Table 2.

It is interesting to note that treatment of the cells with soluble metal salts resulted in increases in viral transformation. This is in contrast to the results observed using bacterial assays in which most metal cations did not induce mutations. Of interest also is the agreement of the results in this bioassay as compared to the results obtained using the fidelity of DNA synthesis in vitro as a test system (18-20). Those metals which enhanced viral transformation altered the accuracy of DNA synthesis; metals which did not increase transformation did not affect fidelity. The only exception was iron which enhanced transformation but did not alter fidelity.

Transformation of Syrian Hamster Cells by Metal Compounds

Recent studies by DiPaolo and Casto have demonstrated that morphological transformation of Syrian hamster cells could itself by used as an in vitro bioassay to detect potentially carcinogenic metal compounds (59). In these experiments, cells were exposed to varying concentrations of metal salts for one day. After 7-8 days of culture, the cells were analyzed for transformation. The cells were classified

| Metal     | Valence | Enhancement of viral transformation (enhancement ratio; mM) | Transformation of Chinese hamster cells (transformed colonies/total colonies), %<sup>a</sup> |
|-----------|---------|----------------------------------------------------------|--------------------------------------------------------------------------------------------------|
| Arsenic   | 3       | 2.4 (0.1)                                                | 4.13 (5)                                                                                         |
| Beryllium | 2       | 2.6 (0.56)                                               | 6.40 (5)                                                                                         |
| Cadmium   | 2       | 2.2 (0.001)                                              | 4.02 (1)                                                                                         |
| Chromium  | 6       | 2.4 (0.01)                                               | 3.48 (1)                                                                                         |
| Nickel    | 2       | 3.4 (0.38)                                               | 5.45 (10)                                                                                        |
| Barium    | 2       | 0.9 (4.8)                                                | —                                                                                                 |
| Calcium   | 2       | 0.8 (6.8)                                                | —                                                                                                 |
| Lithium   | 1       | 0.5 (23.6)                                               | —                                                                                                 |
| Strontium | 2       | 0.9 (3.8)                                                | —                                                                                                 |
| Titanium  | 4       | 0.8 (12.5)                                               | —                                                                                                 |

<sup>a</sup>The capacity of metals to enhance viral transformation or to transform mammalian cells in the absence of exogenous virus was determined as described (59, 60). The enhancement ratio was determined by calculating the ratio of transformed foci observed in the presence of metals to that observed in untreated cells.

<sup>b</sup>The numbers in parentheses refer to the lowest concentration required to observe enhancement or the highest concentration used for compounds scored as negative.

<sup>c</sup>The numbers in parentheses refer to the concentration (μg/ml) required to observe that percentage of transformation.

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as transformed if the colony formation was altered due to uncontrolled cell growth and due to irregular cell arrangement.

In this system 12 metals were examined (Table 2). Six metal compounds induced morphological transformation. Positive results were achieved with nickel sulfate, cadmium acetate, sodium chromate, nickel subsulfide, beryllium sulfate, and sodium arsenate. In each instance increasing concentrations of the metal salt resulted in an increase in the number of transformed colonies observed. No transformation was observed in untreated cultures. Furthermore, each metal in the compounds used has been implicated as a carcinogen in animals or in humans.

In contrast to the positive results observed with those metal salts, no transformation was observed using ferric oxide, titanium dioxide, sodium tungstate, zinc chloride, aluminum chloride, or with amorphous nickel sulfide. Of these metals tested in vivo, the determination of the carcinogenicity of iron and of zinc have been inconclusive. Iron in the form of iron-dextran and intratesticular injection of zinc have produced tumors (60, 61). However, administration of the metals by all other routes tested or in other forms has not resulted in tumor formation. Thus the negative results obtained in this bioassay as well as other results obtained in other in vitro tests could serve potentially as supportive data for a determination of the hazards of iron and of zinc compounds.

### Induction of Chromosomal Aberrations by Metal Compounds

The capacity of exogeneous agents to perturb normal chromosome structure has been used quite successfully as an in vitro bioassay (62-65). Cytogenic analysis provides a rapid and reproducible test to determine whether exposure to specific chemicals results in the production of diverse chromosomal lesions. This type of analysis also permits the simultaneous determination of various types of chromosomal perturbations. The facilitation by exogeneous agents of genetic disturbances at the chromosomal level would presumably be due to DNA damage. In particular, strand breaks or the induction of DNA crosslinks would provide attractive sites for the transfer of nucleotide sequences to sites on other chromosomes.

The in vitro determination of chromosomal aberrations produced in vivo in cells from individuals exposed to specific chemicals may provide the most relevant bioassay to monitor environmental hazards. In particular, human lymphocytes provide a readily accessible source with which to assay large populations of exposed individuals. Using populations of industrial workers, recent studies on the capacity of metals to produce chromosomal aberrations have been equivocal. Bauchinger et al. determined the extent of chromosomal perturbations in lymphocytes from workers in a zinc smelter plant (66). These individuals had increased blood levels of lead and of cadmium. These workers had a 2- to 3-fold increase in the number of aberrations/cell with respect to comparable controls. Furthermore, the increase in aberrations was not selective but instead reflected an overall increase for each type of perturbation. However, other studies by Evans and O'Riordan (67) and by O'Riordan et al. (68) failed to observe any increase in chromosomal aberrations in industrial workers exposed to lead or to cadmium.

Analysis of the capacity of chromium compounds to induce chromosomal aberrations substantiates the importance of hexavalent chromium in metal carcinogenesis. Using hamster embryo cells, Tsuda and Kato reported that increasing concentrations of potassium chromate induced increasing numbers of chromosome gaps, chromatid or chromosome breaks, or chromosome exchanges (69). However, prior treatment of potassium dichromate with Na2SO3 eliminated the induction of chromosomal aberrations. This is in agreement with previous results in bacterial systems demonstrating that metabolism of hexavalent chromium eliminated chromium induced mutagenesis.

Similar studies in other mammalian cells demonstrated the capacity of hexavalent chromium to induce chromosomal perturbations. In human lymphocytes, Nakamuro et al. examined the chromosome breaking capacity of hexavalent chromium (70). At low levels of exposure, the yield of aberrations was linear with dose. In Chinese hamster ovary cells, hexavalent chromium induced a 10 fold increase in the number of chromosomal perturbations as compared to the untreated control (71). With sodium dichromate, the number of chromatid breaks or gaps was dose-dependent. In mouse cells, both K2Cr2O7 and CrO3 induced large numbers of aberrations (72). Sister chromatid exchanges were observed in human fibroblasts exposed to either K2CrO4 or to K2Cr2O7 (73).

In contrast to the results observed with hexavalent chromium, exposure of mammalian cells to trivalent chromium did not result in the production of similar numbers of chromosomal perturbation (70). Although these results support those observed in bacterial assays, trivalent chromium did affect chromosomal structure in some studies. In human
leukocytes, hexavalent chromium compounds were significantly more effective in inducing chromosomal rearrangements than were trivalent chromium compounds. However, all of the compounds tested were comparably effective inducing chromatid gaps. The significant differences were observed in the number of chromosome breaks or exchanges. These results would suggest that the latter type of perturbation is of more importance with regard to potential chromium carcinogenesis.

Exposure to cadmium salts has resulted in the induction of chromosomal aberrations. Using Chinese hamster cells, Rohr and Bauchinger reported that exposure to $10^{-4} M \text{CdSO}_4$ resulted in a significant production of chromosomal perturbations (74). Determination of the mitotic index suggested a pronounced effect of cadmium. As unsynchronized cells were used in this experiment, it was not possible to ascertain whether a specific part of the cell cycle was affected or whether cadmium exhibited a nonspecific inhibitory effect. Using a system comparable to that of Casto and DiPaolo, Zasukhina et al. (75) examined the ability of CdCl$_2$ to affect the induction of chromosomal aberrations caused by Kilham virus. Using primary rat embryo cells, they reported that CdCl$_2$ by itself failed to produce any detectable number of aberrations above background levels. In contrast, administration of CdCl$_2$ to virus-containing cultures increased by 2-fold the number of perturbations which were observed. These two studies are of interest in that they demonstrated the effect of free metal cations on the induction of chromosomal aberrations. In contrast to many bacterial studies reporting the lack of activity of free metal cations, this study suggests that the interaction of free metal cations with chromosomal structures may result in specific perturbations.

Several systems have been used to determine that platinum as cis-dichlorodiammine platinum (II) induced chromosomal aberrations. In human lymphocytes, Wiencke et al. (76) demonstrated that exposure to increasing concentrations of cis-PDD resulted in a proportional increase in the number of sister chromatid exchanges (SCE). At a cis-PDD concentration of 1.0 $\mu g/ml$, they reported an approximately 5-fold increase in the number of SCE. As a function of sequential cell division, there was an inverse relationship between the number of SCE and the number of cell divisions after exposure. Further analysis in Chinese hamster cells demonstrated that caffeine produced a synergistic effect on the induction of chromosomal aberrations by cis-PDD (77).

**Conclusion**

In this review I have attempted to briefly summarize recent studies using the major in vitro bioassays to evaluate the potential carcinogenicity of metal compounds. In particular, most investigations have focused on those metals for which carcinogenicity data are available. As shown in Table 3, some of these metals have been analyzed in several of the bioassays. However, as is evident from this compilation, wide gaps remain with

| Metal    | Carcinogenicity in vivo | Bacterial mutagenesis | Mammalian mutagenesis | Enhancement of viral transformation | Transformation of mammalian cells | Chromosomal aberrations |
|----------|-------------------------|-----------------------|-----------------------|------------------------------------|---------------------------------|-------------------------|
| Arsenic  | +                       | -?                    | N.D.                  | +                                  | +                               | N.D.                    |
| Beryllium| +                       | N.D.                  | N.D.                  | +                                  | +                               | N.D.                    |
| Cadmium  | +                       | -?                    | N.D.                  | +                                  | +                               | +                       |
| Chromium | +                       | - (3)                 | - (3)                 | N.D. (3)                           | N.D. (3)                        | -? (3)                  |
| Cobalt   | ?                       | N.D.                  | N.D.                  | +                                  | N.D.                           | N.D.                    |
| Copper   | ?                       | + *                   | N.D.                  | +                                  | N.D.                           | N.D.                    |
| Iron     | ?                       | ?                     | N.D.                  | +                                  | N.D.                           | N.D.                    |
| Lead     | +                       | -?                    | N.D.                  | +                                  | N.D.                           | N.D.                    |
| Manganese| ?                       | + *                   | N.D.                  | +                                  | N.D.                           | N.D.                    |
| Mercury  | ?                       | - (1)                 | N.D. (1)              | N.D. (1)                           | N.D.                           | N.D.                    |
| nickel   | +                       | - (2)                 | N.D. (2)              | + (2)                              | N.D.                           | N.D.                    |
| Platinum | ?                       | + (2)                 | + (2)                 | + (2)                              | N.D.                           | N.D.                    |
| Selenium | ?                       | +                     | N.D.                  | N.D.                              | N.D.                           | N.D.                    |
| Zinc     | ?                       | -                     | N.D.                  | +                                  | -                               | N.D.                    |

*aThe determinations of the interactions of metal compounds with in vitro bioassays are taken from the text and from the appropriate references. Determinations of carcinogenicity are taken from the references (13-17). The designation of -? refers to compounds tested in only one of the available assays. The number in parenthesis refers to the valence or charge of the metal. The asterisk (*) refers to results obtained in systems other than the major in vitro bioassays.
respect to our knowledge of the effects of many of these metals.

The greatest use of in vitro bioassays with respect to metals may be to determine which forms of a specific metal may pose the greatest risk. It may be argued that in vivo data have indicated which metals are potential human carcinogens. However, it may also be a reasonable expectation that not all forms of a given metal pose this hazard. The valence of the particular metal within the compound, the net charge on the metal compound, the orientation of the metal within the compound as well as the solubility of the metal salt may be determining factors. Thus, several investigations performed with chromium compounds suggest that, although in vivo data demonstrate the carcinogenicity of chromium in animals or in man, in vitro experiments would indicate that exposure to hexavalent chromium which crosses cell membranes is biologically more active than comparable exposure to trivalent chromium which does not cross cell membranes. Unfortunately, similar studies have not been performed with most of the other metals listed. Furthermore, most metals have not been systematically analyzed with respect to their capacity to act as bacterial mutagens or for their capacity to induce chromosomal aberrations in mammalian cells.

Although many metals have been tested for in vivo carcinogenicity, other metals have not been adequately tested. Thus, in vitro bioassays may also serve to identify other metals which are potential human carcinogens. In vitro bioassays may thus function to screen a large number of metal compounds as a first step to focus attention on specific metal salts. These salts may then be examined in vivo. For example cis-dichlorodiammine platinum (II) induces bacterial mutations as well as inducing chromosomal aberrations. It is of interest that this and related compounds produced sarcomas after s.c. injections in rats, and that it was an initiator for mouse skin after IP injections (78). In this respect, it behaves like most cancer chemotherapeutic agents which are carcinogenic. However, if in vitro bioassays are used as a “first tier” test for metal compounds, the unique solubility problems of metal salts and their potential interactions with media components have to be considered with regard to negative results.

Recent evidence suggests that diverse metals may act synergistically to potentiate the effects of other mutagens or carcinogens. Stich et al. reported that the mutagenicity of ascorbic acid in the Ames Salmonella assay was increased 80-fold by the addition of copper (79). Similarly, copper, cobalt, manganese, iron and zinc increased the mutagenic effect of cis-5,6-dihydro-6-hydroperoxy-5-hydroxy-thymine on transforming DNA of H. influenzae (80). Further studies demonstrated that copper could increase the induction of sister chromatid exchanges in Chinese hamster cells caused by exposure to ascorbic acid or to sodium bisulfite (81). Furthermore, manganese, copper, or iron enhanced unscheduled DNA synthesis induced by isoniazid or other hydrazines (82). Thus, the synergistic effects of transition metals suggest the possibility that in vitro bioassays may serve as useful tools to further investigate the capacity of metals to enhance the mutagenicity or carcinogenicity of other agents.

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REFERENCES

1. Epstein, S. S. Environmental determinants of human cancer. Cancer Res. 34: 2425 (1974).
2. Miller, E. C. and Miller, J. A. Biochemical mechanisms of chemical carcinogenesis. In: The Biology of Cancer, H. Busch, Ed., Academic Press, New York, 1974, pp. 377-402.
3. Cairns, J. Mutation selection and the natural history of cancer. Nature 255: 197 (1975).
4. Heidelberger, C. Chemical carcinogenesis. Ann. Rev. Biochem. 44: 79 (1975).
5. Doll, R. Strategy for detection of cancer hazards to man. Nature 265: 588 (1977).
6. Committee 17 of the Council of the Environmental Mutagen Society. Environmental mutagenic hazards. Science 187: 593 (1975).
7. Bridges, B. A. Short-term screening tests for carcinogens. Nature 261: 195 (1976).
8. Purchase, I. F. M., Longstaff, E., Ashby, J., Styles, J. A. Anderson, D., Lefevre, P. A., and Westwood, F. R. Evaluation of six short—term tests for detecting organic chemical carcinogens and recommendations for their use. Nature 264: 624 (1976).
9. Bartch, H. Predictive value of mutagenicity tests in chemical carcinogenesis. Mutat. Res. 38: 177 (1976).
10. de Serres, F. J., and Shelby, M. D. Recommendations on data production and analysis using the Salmonella/microsome mutagenicity assay. Mutat. Res. 64: 159 (1979).
11. Ames, B. N. Identifying environmental chemicals causing mutations and cancer. Science 204: 587 (1979).
12. Hollstein, M., McCann, J., Angelosanto, F. A., and Nichols, W. W. Short term tests for carcinogens and mutagens. Mutat. Res. 65: 133 (1979).
13. IARC. Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Man. Vol. 2, Some Inorganic and Organometallic Compounds. IARC, Lyon, 1973.
14. Sunderman, Jr., F. W. Metal carcinogenesis. Adv. Mod. Toxicol. 1: 287 (1977).
15. Furst, A. Inorganic agents as carcinogens. Adv. Mod. Toxicol. 3: 209 (1977).
16. Lee, A. M. and Fraumeni, Jr., J. F. Arsenic and respiratory cancer in man: an occupational study. J. Natl. Cancer Inst. 170
17. IARC Working Group. An evaluation of chemicals and industrial processes associated with cancer in humans based on human and animal data: IARC Monographs 1-20, 40: 1-12 (1980).

18. Sirover, M. A. and Loeb, L. A. Metal-induced infidelity during DNA synthesis. Proc. Natl. Acad. Sci. (U.S.) 73: 2331 (1976).

19. Sirover, M. A. and Loeb, L. A. Infidelity of DNA synthesis in vitro: Screening for potential metal mutagens or carcinogens. Science 194: 1434 (1976).

20. Sirover, M. A. and Loeb, L. A. A molecular analysis of mutagenesis by oncogenic agents. In: Recent Advances in Cancer Research: Cell Biology, Molecular Biology and Tumor Virology, R. C. Gallo, Ed., CRC Press, Cleveland, Ohio, 1977, pp. 49-65.

21. Flessel, C. F. Metals as mutagenic initiators of cancer. In: Trace Elements in Health and Disease, N. Karash, Ed., Raven Press, in press.

22. Greene, M. H. L. and Muriel, W. J. Mutagen testing using TRP reversion in Escherichia coli. Mutat. Res. 38: 8 (1976).

23. McCann, J., Choi, E., Yamasaki, E., and Ames, B. N. Detection of carcinogens as mutagens in the Salmonella/microsome test: Assay of 300 chemicals. Proc. Natl. Acad. Sci. (U.S.) 72: 5135 (1975).

24. McCann, J., and Ames, B. N. Detection of carcinogens as mutagens in the Salmonella/microsome test: Assay of 300 chemicals: Discussion. Proc. Natl. Acad. Sci. (U.S.) 75: 950 (1976).

25. Kada, T., Sadea, Y., and Tutikawa, T. In vitro and host-mediated "rec assay" procedure for screening chemical mutagens and phloxine, a mutagenic red dye detected. Mutat. Res. 16: 165 (1972).

26. Venitt, S. and Levy, L. S. Mutagenicity of chromates in bacteria and its relevance to chromosome carcinogenesis. Nature 250: 495 (1977).

27. Nishioka, H. Mutagenic activities of metal compounds in bacteria. Mutat. Res., 31: 185 (1975).

28. Grossman, L., Braun, A., Feldberg, R., and Mahler, I. Enzymatic repair of DNA. Ann. Rev. Biochem. 44: 19 (1975).

29. Lofroth, G., and Ames, B. N. Mutagenicity of inorganic compounds in Salmonella typhimurium: arsenic, chromium and selenium. Mutat. Res. 53: 65 (1967).

30. Petrilll, F. L. and De Flora, S. Toxicity and mutagenicity of hexavalent chromium on Salmonella typhimurium. Appl. Environ. Microbiol. 32: 805 (1975).

31. Nestman, E. R., Matula, T. L., Douglas, G. R., Bora, K. C., and Kowbell, D. J. Detection of the mutagenic activity of lead chromate using a battery of microbial tests. Mutat. Res. 66: 357 (1979).

32. Petrilll, F. L. and De Flora, S. Metabolic deactivation of hexavalent chromium mutagenicity. Mutat. Res. 54: 139 (1978).

33. Petrilll, F. L. and De Flora, S. Oxidation of inactive trivalent chromium to the mutagenic hexavalent form. Mutat. Res. 58: 167 (1978).

34. Lindahl, T. DNA glycosylases, endonucleases for apurinic/apyrimidinic sites and base excision repair. Prog. Nucleic Acid Res. Mol. Biol. 22: 135 (1979).

35. Nakamura, K., Yoshikawa, K., Sayato, Y., Kurata, H., Tonomura, M., and Tonomura, A. Studies on selenium-related compounds. V. Cytogenetic effect and reactivity with DNA. Mutat. Res. 40: 177-184 (1976).

36. Noda, M., Takano, T., and Sakurai, H. Mutagenic activity of selenium compounds. Mutat. Res. 66: 175 (1979).

37. Beck, D. J. and Brubaker, R. R. Mutagenic properties of cis-platinum (II) diaminedichloride in Escherichia coli. Mutat. Res. 27: 181 (1975).

38. Monti-Bragadin, C., Tamaro, M., and Banfi, E. Mutagenic activity of platinum and ruthenium complexes. Chem.-Biol. Interact. 11: 469 (1975).

39. Benedict, W. F., Baker, M. S., Haroun, L., Choi, E., and Ames, B. N. Mutagenicity of cancer chemotherapeutic agents in the Salmonella/microsome test. Cancer Res. 37: 2209 (1977).

40. Anderson, K. S. Platinum (II) complexes generate frameshift mutations in test strains of Salmonella typhimurium. Mutat. Res. 67: 209 (1979).

41. Beck, D. J., and Brubaker, R. R. Effect of cis-platinum (II) diaminedichloride on wild type and deoxyribonucleic acid repair-deficient mutants of Escherichia coli. J. Bacteriol. 116: 1247 (1973).

42. Leconte, P., Macquit, J.-P., Butour, J.-L., and Paollete, C. Relative efficiencies of a series of square-planar platinum (II) compounds on Salmonella mutagenesis. Mutat. Res. 48: 139 (1977).

43. Orgel, A., and Orgel, L. E. Induction of mutations in bacteriophage T4 with divalent manganese. J. Mol. Biol. 14: 453 (1966).

44. Putrament, A., Baranowska, H., Ejchart, A., and Prazmo, W. Manganese mutagenesis in yeast. Methods in Cell Biol. 2: 95-94 (1978).

45. Demerec, M., Bertani, G., and Flint, J. A survey of chemicals for mutagenic action on E. coli. Am. Naturalist 85: 119 (1951).

46. Weed, L. L. Effects on copper on Bacillus subtilis. J. Bacteriol. 56: 1003 (1953).

47. Chu, E. H. Y., and Malling, H. V. Mammalian cell genetics. II. Chemical induction of specific locus mutations in Chinese hamster cells in vitro. Proc. Natl. Acad. Sci. (U.S.) 61: 1306 (1968).

48. Huberman, E., and Sachs, L. Cell-mediated mutagenesis of mammalian cells with chemical carcinogens. Int. J. Cancer 13: 326 (1974).

49. O'Neill, J. P., Couch, D. B., Machanoff, R., San Sebastian, J. R., Brimer, P. A. and Hsie, A. W. A quantitative assay of mutation induction at the hypoxanthine-guanine phosphoribosyltransferase locus in Chinese hamster ovary cells (CHO/HGPRT/SYSTEM): Utilization with a variety of mutagenic agents. Mutat. Res. 45: 103 (1977).

50. Turnbull, D., Popescu, N. C., DiPaolo, J. A., and Myhr, B. C. cis-Platinum (II) diaminedichloride causes mutation, transformation, and sister chromatid exchanges in cultured mammalian cells. Mutat. Res. 66: 267 (1979).

51. Taylor, R. T., Carver, J. H., Hanna, M. L., and Wanders, D. L. Platinum-induced mutations to 8-azaguanine resistance in Chinese hamster ovary cells. Mutat. Res. 67: 65 (1979).

52. Zwelling, L. A., Bradley, M. O., Sharkey, N. A., Anderson, R., and Kohn, K. W. Mutagenicity, cytotoxicity and DNA crosslinking in V79 Chinese hamster cells treated with cis- and transPt(II) diaminedichloride. Mutat. Res. 67: 271 (1979).

53. Newbold, R. F., Amos, J., and Connell, J. R. The cytotoxic, mutagenic and clastogenic effects of chromium-containing compounds on mammalian cells in culture. Mutat. Res. 67: 55 (1979).

54. Haie, A. B., Couch, D. B., San Sebastian, J. R., Sun, W. N. C., Riddle, J. C., Brimer, P. A., Machanoff, R., Forbes, N. L., Kenny, F. T., and Hsie, M. H. Mutagenicity of carcinogens as determined in a quantitative mammalian cell mutation system, CHO/HGPRT: Study of 90 agents. Proc. Amer. Assoc. Cancer Res. 19: 93 (1978).

55. Casto, B. C. Enhancement of viral oncogenesis by chemical carcinogens. In: Chemical Carcinogenesis, Part B, P.O.P.
56. DiPaolo, J. A., Donovan, P. J., and Casto, B. C. Enhancement by alkylating agents of chemical carcino-
gen transformation of hamster cells in culture. Chem. Biol. Interact. 9: 351 (1974).

57. Casto, B. C., Miyagi, M., Myers, J., and DiPaolo, J. A. Increased integration of viral genome following chemical and viral treatment of hamster embryo cells. Chem. Biol. Interact. 25: 255 (1979).

58. Casto, B. C., Myers, J., and DiPaolo, J. A. Enhancement of viral transformation for evaluation of the carcinogenic or mutagenic potential of inorganic metal salts. Cancer Res. 39: 193 (1979).

59. DiPaolo, J. A., and Casto, B. C. Quantitative studies in in vitro morphological transformation of Syrian hamster cells by inorganic metal salts. Cancer Res. 39: 1008 (1979).

60. Roe, F. J. C., and Carter, R. L. Iron-dextran carcinogenesis in rats: Influence of dose on the number and types of neoplasms induced. Int. J. Cancer. 2: 370 (1967).

61. Guthrie, J., and Guthrie, O. A. Embryonal carcinomas in Syrian hamsters after intratesticular inoculation of zinc chloride during seasonal testicular growth. Cancer Res. 34: 2612 (1974).

62. Setka, D. G. and Wolff, S. Sister chromatid exchange as an assay for genetic damage induced by mutagen-carcinogens. II. in vitro test for compounds requiring metabolic activation. Mutat. Res. 41: 343 (1976).

63. Popescu, C., Turnbull, D., and DiPaolo, J. A. Sister chromatid exchange and chromosomal aberration analysis with the use of several carcinogens and non-carcinogens. J. Nati. Cancer Inst. 59: 289 (1977).

64. Wolff, S. Sister chromatid exchange. Ann. Rev. Genet., 11: 183 (1977).

65. Carrano, A. V., Thompson, L. H., Lindl, P. A., and Miniker, J. L. Sister chromatid exchange as an indicator of mutagenesis. Nature 271: 551 (1978).

66. Bauchinger, M., Schmid, E., Einbrodt, H. J., and Dresp, J. Chromosome aberrations in lymphocytes after occupational exposure to lead and cadmium. Mutat. Res. 40: 57 (1976).

67. Evans, H. J., and O'Riordan, M. L. Human peripheral blood lymphocytes for the analysis of chromosome aberrations in mutagen test. Mutat. Res. 31: 135 (1975).

68. O'Riordan, M. L., Hughes, E. G., and Evans, H. J. Chromosome studies on blood lymphocytes of men occupationally exposed to cadmium. Mutat. Res. 58: 305 (1978).

69. Tsuda, H., and Kato, K. Chromosomal aberrations and morphological transformation in hamster embryonic cells treated with potassium dichromate in vitro. Mutat. Res. 46: 87 (1977).

70. Nakamura, K., Yoshikawa, K., Sayato, Y., and Kurata, H. Comparative studies of chromosomal aberration and mutagenicity of trivalent and hexavalent chromium. Mutat. Res. 58: 175 (1978).

71. Majone, F., and Levis, A. G. Chromosomal aberrations and sister chromatid exchanges in Chinese hamster cells treated in vitro with hexavalent chromium compounds. Mutat. Res. 67: 231 (1979).

72. Umeda, M., and Nishimura, M. Inducibility of chromosomal aberrations by metal compounds in cultured mammalian cells. Mutat. Res. 67: 221 (1979).

73. Macrae, W. D., Whiting, R. F., and Stich, H. F. Sister chromatid exchanges induced in cultured mammalian cells by chromate. Chem. Biol. Interact. 26: 281 (1979).

74. Rohr, G., and Bauchinger, M. Chromosome analyses in cell cultures of the Chinese hamster after application of cadmium sulphate. Mutat. Res. 40: 125 (1976).

75. Zasukhina, G. D., Sinelschikova, T. A., Lvova, G. N. and Kirkova. Molecular-genetic effects of cadmium chloride. Mutat. Res. 45: 169 (1977).

76. Wiencke, J. K., Cervenka, J., and Paulus, H. Mutagenic activity of anticancer agent cis-dichlorodiammineplatinum-II. Mutat. Res. 68: 69 (1979).

77. Van Den Berg, H. W. and Roberts, J. J. Post-replication repair of DNA in Chinese hamster cells treated with cis-platinum (II) diammine dichloride. Enhancement of toxicity and chromosome damage. Mutat. Res. 33: 279 (1975).

78. Leopold, W. R., Miller, E. C., and Miller, J. A. Carcinogenicity of antitumor cis-platinum (II) coordination complexes in the mouse and rat. Cancer Res. 39: 913 (1979).

79. Stich, H. F., Karim, J., Koropatnick, J. and Lo, L. Mutagenic action of ascorbic acid. Nature 260: 722 (1976).

80. Thomas, H. F., Herriott, R. M., Hahn, B. S., and Wang, S. Y. Thymine hydroperoxide as a mediator in ionizing radiation mutagenesis. Nature 259: 341 (1976).

81. Macrae, W. D. and Stich, H. F. Induction of sister chromatid exchanges in Chinese hamster cells by the reducing agents bisulphite and ascorbic acid. Toxicol. 13: 167 (1978).

82. Whiting, R. F., Wei, L., and Stich, H. F. Enhancement by transition metals of unscheduled DNA synthesis induced by isoniazid and related hydrazines in cultured normal and xeroderma pigmentosum in human cells. Mutat. Res. 62: 506 (1979).