United States
Environmental Protection
Agency

Health Effects Research
Laboratory
Research Triangle Park NC 27711

Research and Development

EPA-600/S1-81-045 July 1981

EPA

Project Summary

Potency Ranking of Chemicals Based on Enhancement of Viral Transformation

Bruce C. Casto

Treating primary hamster embryo cells with various classes of chemical carcinogens and mutagens leads to enhancement of transformation by simian adenovirus SA7. It appears that carcinogenic chemicals render the individual cells more sensitive to viral transformation, thus increasing the total number of cells integrating SA7 DNA. Enhancement of viral transformation appears to be a sensitive indicator for chemical agents with the potential to damage cell DNA by either direct or indirect means and thus may be useful as a screening tool to detect these chemicals in the environment.

The Project Report summarizes and compares the results for 136 chemicals (both carcinogenic and noncarcinogenic) assayed for enhancement of SA7 transformation, chemical transformation, and induction of DNA strand breaks and DNA repair synthesis. In addition, these chemicals are ranked by lowest effective concentration in the assay for enhancement of viral transformation.

This report was submitted in fulfillment of Contract No 68-02-2566 by Northrop Services, Inc.-Environmental Sciences, under the sponsorship of the U.S. Environmental Protection Agency. This report covers the period from June 1980 to August 1980, and work was completed as of August 1980.

This Project Summary was developed by EPA's Health Effects Research Laboratory, Research Triangle Park, NC, to announce key findings of the research project that is fully documented in a separate report of the same title (see Project Report ordering information at back).

Introduction

The U.S. Environmental Protection Agency (EPA) has been given the responsibility for regulating the release of toxic chemicals into the environment under the Toxic Substances Control Act, Resource Conservation and Recovery Act, and the Clean Air and Clean Water Acts.

Assessment of the chronic effects of chemical exposure is a complex task, since the consequences of exposure may appear long after the initial contact. To determine these effects, lifetime studies in animals must be conducted at considerable expense using specialized facilities and personnel. Single tests for carcinogens may cost more than $300,000 and last for 2 to 3 years. Some 50,000 chemicals are commercially produced in the United States and 700 to 1,000 new chemicals are introduced each year, yet the present capacity to conduct long-term animal studies is limited to approximately 500 compounds per year. Initial reliance on short-term tests for the identification of toxic chemicals, carcinogens, and mutagens is mandatory if the introduction of new chemicals continues at its present rate and estimates of 70-80% for environmentally-induced cancer are valid.

Several methods for *in vitro* testing have been described and many new test systems are being developed. It is anticipated that a battery of tests will eventually be available with a high degree of reproducibility and correlation with *in vivo* activity. These tests should: generate no false-negatives and only a low percentage of false-positives, be responsive to the various classes of chemical and physical agents, and lend themselves to quantitation. All of these attributes must be met if effective judgments are to be made concerning the relative risk of the test agent for humans. In addition, other parameters such as exposure, production volume, lability of the chemical, dose-effect at low concentrations, and the type of population at risk must be considered.

Presently, the *in vitro* systems receiving the most attention for prediction of a chemical's potential to cause chronic effects are: mammalian cell cytotoxicity and transformation; mutagenesis assays in microbial and mammalian cells; and analysis of DNA damage and repair. For a number of chemicals of known activity, the detection of carcinogens has been stated to be as high as 90 percent with systems such as microbial mutagenesis, mammalian cell transformation, or mammalian cell mutagenesis. However, none of the above *in vitro* tests can be used alone to predict the chronic toxicity potential of suspect environmental agents.

It has been proposed that testing of environmental agents proceed through a phased or tier approach. Such an approach is presently being employed by governmental and industrial laboratories, progressing from routine detection systems, involving microbial cells, to more complex assays utilizing mammalian cell cultures, and subsequently to whole-animal testing. Decisions concerning the degree of testing to be done are made at different levels of testing (and cost) depending upon the nature and use of the chemical being evaluated. As a final assessment of risk to man, the suspect agent is tested in animals using genetic and pharmacological evaluations based on dose, route of administration, and length of exposure.

Because routine microbial assays may be insensitive for detection of certain toxic chemicals such as hydrazine derivatives, inorganic metals, steroid hormones, asbestos, and chlorinated hydrocarbons that are detected by selected mammalian cell assays, some type of rapid mammalian cell bioassay must be included in the screening of suspect toxic environmental chemicals.

A different approach to the *in vitro* assay of carcinogens and mutagens has been described in which the ability of various chemicals to enhance adenoviral transformation is evaluated in hamster embryo cells. Cells are either pretreated for 2 or 18 hours with a series of chemical dilutions prior to viral inoculation or post-treated 5 hours after viral infection. The cells are transferred for survival (cloning) and focus assays (virus transformation) and maintained for 8 days and 25-30 days, respectively. To inhibit growth of normal cells and promote growth of virus-transformed cells, the cells for transformation assays (employing adenovirus) are cultured in a low calcium medium under 0.3% agar. Foci do not develop in cells treated only with chemicals since the conditions favorable for virus transformation inhibit the development of chemically-transformed HEC.

The classes of chemicals assayed in the enhancement assay include: alcohols and phenols, aliphatic amines, alkyl sulfates and sulfones, aromatic amines, aryl halides, carbohydrates and derivatives, hydrazines, hydroxylamines, metals and derivatives, mycotoxins, and the polycyclic hydrocarbons. The majority of agents examined have been: (1) those chemicals more commonly used in other short-term *in vitro* assays, (2) those used in various industrial applications, (3) chemicals annually produced in large volumes and (4) inorganic metal salts. Data from approximately 105 test performances in 29 different chemical classes have been published from the enhancement of viral transformation assay. Unpublished or preliminary data exist for approximately 100 other chemicals.

The enhancement assay is a reflection of the capacity of a chemical to damage cell DNA by either direct or indirect means. Mutation assays in microbial and mammalian cells or carcinogen assays in mammalian cells with a variety of short-term tests (inhibition of DNA synthesis, induction of DNA repair, breakage of cell DNA) also presume that mutagenic/carcinogenic agents damage or alter cell DNA.

The enhancement of viral transformation appears to be a sensitive indicator for chemical agents with the potential for damaging cell DNA either by direct or indirect methods and therefore may be useful as a screening tool to detect these chemicals in the environment.

Results and Discussion

Four assays for determination of carcinogenic or mutagenic potential have been conducted in Syrian hamster embryo cells with a large number of compounds. Chemicals were tested for viral enhancement, induction of DNA fragmentation or DNA repair, and morphological transformation. With 136 negative or positive carcinogens tested, 94% agreed with their current classification. Fifty chemicals were tested in all four assays: stimulation of DNA repair synthesis correctly classified 50%, DNA fragmentation 72%, and chemical transformation 92%.

Data for the 136 chemicals, carcinogens and noncarcinogens,obtained from replicate experiments using the enhancement of viral transformation assay, have been ranked based upon the least effective concentration (μg/ml). The upper limit for testing was usually 1 mg/ml unless solubility or toxicity dictated using a lower dose.

For those compounds testing positive the range of effective concentrations varied by a factor of 2.5×10^5 with 7,12-dimethylbenz(a)anthracene being the most potent (0.004 μg/ml) and hydrazine sulfate or nickel sulfide being the least potent (1000 μg/ml).

Chemicals have been placed into 3 groups depending upon the concentration necessary to produce a positive enhancement response. Those showing the highest enhancement activity (at 10 μg/ml or less) were classified as Group I; Group II consisted of those compounds active at 10-100 μg/ml; Group III was composed of those chemicals only active at more than 100 μg/ml. Approximately 46 chemicals could be classified as highly active (Group I), 22 as intermediate (Group II) and the remaining as weak (Group III); the only known false-positive (not carcinogenic or mutagenic) was caffeine. Three compounds were unclassified (±) due to the failure to be consistently positive in several experiments (e.g. zinc sulfate was positive in 3/7 trials).

Known carcinogens testing negative for enhancement (e.g. N-2-acetylaminofluorene, N-nitrosodimethylamine, N-nitrosodiethylamine) were those apparently not metabolized *in vitro* by hamster fibroblasts since other tests (transformation, DNA breakage, and DNA repair) were also consistently negative. Incorporation of an exogenous activating

ystem (a liver S9 mix) or exposure to hemical *in utero* converts many of the egative compounds to positive in enancement assays or transformation ssays, respectively. Other chemicals egative for enhancement that are uspect carcinogens in humans or nimals include: 1,2 diethyl-2-thiourea; richloroethylene; red dye #2; and 1,4 ioxane.

Of 35 chemicals tested in both *Salmonella* and the enhancement of viral ransformation assays, there was agreenent between the two tests with 26 of he compounds. Four carcinogens were etected with the viral enhancement ssay, but not with *Salmonella*. The emaining three required activation not rovided by hamster embryo cells and vere positive in *Salmonella* in combinaion with a liver S9 mix.

Forty-two of the top 50 volumeroduced compounds have been tested. Vith the exception of a few agents, nost were tested at least twice in the nhancement assay. Among these ompounds, ethylene dichloride, proylene oxide and vinyl acetate have aused enhancement. Butadiene (1,3-) he 27th ranked compound) has not een tested, but 2-chloro-1,3-butadiene nhances transformation. The temporal eriod between viral and chemical reatment with these compounds is imilar to that found previously with affeine, $MnCl_2$, and Ara-C. In experinents with ethylene dichloride, enancement was only observed at the ighest dose used (1 mg/ml). Routinely, oncentrations higher than 1 mg/ml re not tested, but with two other hemicals, phthalazinone and ethylene ibromide, enhancement continued to ncrease when 2 mg/ml were used. thylene dichloride was also positive at oses higher than 1 mg/ml. None of the emaining chemicals from the top 50 list ave shown any indication that they ay cause enhancement when added ither before or after virus.

The mutagenic and carcinogenic ctivity of many of these chemicals has een under investigation in recent ears. Chlorobutadiene (CBD) was eported to be negative in the *Salmonella* ssay in some laboratories and positive n others; additionally CBD has been eported to cause chromosome aberraions in lymphocytes of exposed workers nd to be related to the appearance of kin and lung tumors among rubber orkers.

Propylene oxide (PO) used in the anufacture of propylene glycols, polyglycols and propylene glycol esters, as a fumigant herbicide, as a solvent for cellulose nitrate or acetate and vinyl chloride or acetate, and used on foods to control spoilage, was produced (1974 data) at the rate of 1.78 billion lbs/yr and has been shown to be mutagenic in *Drosophilia* and carcinogenic. In addition to the enhancement of viral transformation, PO was shown to transform HEC in the focus assay.

Studies in *Salmonella* by both have been negative for vinyl acetate, and have not found any carcinogenic activity when VA was used in long-term bioassays as a control for vinyl chloride. Nevertheless, VA was positive in repeat experiments for viral enhancement when added to HEC after SA7 and was positive for focus formation when tested alone.

Styrene was negative in two experiments (-18 hr and + 5 hr treatment periods). Produced in the US at the rate of 6 billion lbs/yr and used in the production of plastics or resins and in styrene-butadiene rubber, styrene may be metabolically converted to the mutagenic form styrene oxide. In a series of experiments with styrene and styrene oxide, using the yeasts *S. cerevisiae* or *S. pombe* and Chinese hamster cells, styrene oxide was uniformly mutagenic whereas styrene was negative even in the presence of a liver microsome activating system. In a host-mediated assay, styrene was weakly mutagenic for *S. pombe* when Swiss mice were treated with 1 gm/Kg. Styrene and styrene oxide have been tested in 5 strains of *Salmonella* (TA98, TA100, TA1535, TA1537, TA1538) and again styrene was negative; styrene oxide induced mutations in TA100 (Milvy and Garro, 1976).

The findings of certain chemicals such as vinyl acetate, positive in the enhancement assay but negative in the *Salmonella* assay is not uncommon. For example, several recognized mutagens or carcinogens have not been mutagenic for *Salmonella*, even when incubated with a S-9 activating system, but were positive for viral enhancement including: thioacetamide, IUdR, 1,2-dimethylhydrazine, hydroxylamine phthalazinone, and the metal carcinogens or mutagens.

A total of 46 metal salts have been tested in the viral enhancement assay. Positive enhancement was found with salts of antimony, arsenic, beryllium, cadmium, cobalt, copper, iron, lead, manganese, molybdenum, nickel, selenium, platinum, thallium, vanadium and zinc. Negative metals include the acetate, chloride or sulfate salts of aluminum, barium, calcium, lithium, magnesium, potassium, stronthium, titanium and zirconium. Both the positives and negatives from above are in excellent agreement with the *in vitro* infidelity of DNA synthesis assay; the only exception being $FeCl_2$ that was positive in the viral enhancement assay and negative for introducing copy error in the infidelity of DNA synthesis assay. Although metallic iron has not been shown to be carcinogenic iron dextran will induce tumors in rats, mice, and hamsters. In contrast to the above, ferrous chloride or ferric chloride and sulfate were earlier found to induce point mutation in *E. coli*.

The enhancement data with metals also agree with data obtained using rec-assays with *B. subtilis*. In these studies of 56 metal salts, arsenic, cadmium, chromium, mercury, manganese and molybdenum were considered positive. Three of the strongly positive metals, arsenic, chromium and molybdenum, were also mutagenic in *E. coli*. The rec-assays, however, failed to detect beryllium, copper, iron, lead, nickel, antimony or zinc. There are several other reports on the carcinogenic and mutagenic activity of the metals shown to be positive in the viral enhancement assay.

Because of the use of metals in various industrial applications and the increasing evidence that many are involved in human carcinogenesis, sensitive and reliable assays for potential mutagenic or carcinogenic activity are necessary. The good agreement between the viral enhancement assay in HEC and the mutagenic or carcinogenic activity of the metals in other systems justifies the use of the SA7 transformation assay as one of the tests to be included in assays for potential environmental mutagenic or oncogenic metal complexes.

☆ U.S. GOVERNMENT PRINTING OFFICE: 1981 -757-012/7212

Bruce C. Casto is with Northrop Services, Inc.—Environmental Sciences, Research Triangle Park, NC 27709.

***Stephen Nesnow** and **Michael D. Waters** are the EPA Project Officers (see below).*

The complete report, entitled "Potency Ranking of Chemicals Based on Enhancement of Viral Transformation," (Order No. PB 81-210 080; Cost: $6.50, subject to change) will be available only from:

National Technical Information Service
5285 Port Royal Road
Springfield, VA 22161
Telephone: 703-487-4650

The EPA Project Officers can be contacted at:

Health Effects Research Laboratory
U.S. Environmental Protection Agency
Research Triangle Park, NC 27711

United States
Environmental Protection
Agency

Center for Environmental Research
Information
Cincinnati OH 45268

Postage and
Fees Paid
Environmental
Protection
Agency
EPA 335

Official Business
Penalty for Private Use $300