MUTAGENICITY TESTING OF SELECTED INDUSTRIAL CHEMICALS

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SUMMARY

The mutagenicity of 147 industrial chemicals and structurally related compounds have been studied by the Utah Biomedical Test Laboratory at Salt Lake City, Utah, under the contract with the National Institute for Occupational Safety and Health. The Salmonella typhimurium-microsome plate incorporation test developed by Ames and Co-workers was used as the assay system. The assays were conducted with the tester strains TA 1535, TA 1537, TA 98 and TA 100 in the presence and absence of S-9 prepared from the liver of Aroclor 1254 pretreated Sprague Dawley rats.

The results of these studies indicate that 120 of 147 compounds were not mutagenic to any of the testers tested with or without metabolic activation. Twenty-three compounds were directly mutagenic to one or more tester strains, and the remaining four compounds required metabolic activation for their mutagenic activities.

INTRODUCTION

It is well documented that many synthetic and naturally occurring compounds can interact with the genetic material and cause mutations in somatic and/or germ cells. Induction of mutations in these cells may lead to one or more of the following deleterious effects: Genetic disease, malformation, spontaneous abortion, and cancer. Recent studies have estimated that more than 1000 known genetic disorders and diseases can be related to gene mutations. It has been estimated that approximately 50% of all spontaneous abortions involve chromosomal defects and more than 0.5% of total live births in the United States carry clinically serious chromosomal aberrations[4]. The large number of chemicals created by modern industrial technology may account for this prevalence of genetic diseases and disorders and for the high incidence of cancer noted in humans in recent years.

During the past decade many short-term tests for mutagenesis have been developed. Among these tests, the histidine reverse mutation system of Salmonella typhimurium developed by Ames and co-workers[1] is probably the most sensitive and useful system for mutagenesis testing. In this test system, the use of in vitro metabolic activation has helped detect the mutagenic activity of promutagens[2]. By using different tester strains, mutagenic specificity (base-pair substitution vs. frameshift mutation) of chemicals can be determined. With this test system, McCann et al.[5], have shown that there is an excellent correlation between mutagenicity and carcinogenicity among the 300 compounds studied.

Many industrial workers are routinely exposed to occupation-related chemicals. To protect these workers from any potential mutagenic and

carcinogenic hazards, it is necessary to detect and identify the mutagenic activity of industrial and related chemicals. A contract was, therefore, initiated by NIOSH in 1977 for Utah Biomedical Testing Laboratory to study the mutagenic activity of 147 industrial chemicals and related compounds in the Salmonella/microsomal assay system.

MATERIALS AND METHODS

Bacterial Strains

The bacteria used in this study were Salmonella typhimurium strains TA 98, TA 100, TA 1535 and TA 1537. They were provided by Dr. Bruce N. Ames, University of California at Berkeley. Upon receipt, each strain was subjected to the appropriate procedures to confirm its genotype. Strains were reisolated, genotypes tested, and new frozen stocks prepared at bimonthly intervals. Stocks were stored at -80° C, and inocula were prepared fresh for each experiment by subculture in nutrient broth.

Mutagenesis Assay

Most chemicals were tested by the plate incorporation method. Some chemicals were tested by the pre-incubation technique. Details of the methodology of Salmonella/microsome mutagenesis assay have been described by Ames and co-workers[3]. A brief description of both assay systems is as follows:

1. Plate Incorporation Assay

   Agar plates containing histidine-deficient Vogel Bonner Medium E[6] and fortified with 2% glucose were prepared and incubated overnight at 37° C prior to use (VBME plates). Aliquots of top agar (0.6% Bacto-agar in 0.5% NaCl) were melted on the day they

were to be used and maintained at 45° C. Each 100 ml aliquot of top agar was supplemented with a final concentration of 0.05 mM histidine and 0.05 mM biotin immediately before use. Five ml of a 16 hour nutrient broth culture of the appropriate Ames' tester strain of Salmonella typhimurium was added to the 100 ml aliquot of top agar, and the mixture transferred to sterile, disposable screw capped tubes in 2.0 ml aliquots and held at 45° C. Test materials were added to the tubes, mixed, and the contents of the tube were poured onto the surface of VBME plates. All plates were incubated at 37° C for three days, and the number of colonies was determined using a manual Quebec colony counter.

S-9 was prepared from livers of male Sprague-Dawley rats (150-200 g) injected with Aroclor 1254 at a dose of 1000/mg/kg five days prior to sacrifice. For tests in the presence of S-9, each tube containing top agar, inoculum and test material received 0.5 ml of S-9 mix containing 0.45 ml of S-9 base[3] and 0.05 ml of S-9 fraction. Tube contents were poured onto the surface of VBME plates immediately after addition of the S-9.

2. Pre-incubation Assay (as above with the following exceptions)

Test materials were added to sterile, disposable screw capped tubes and inoculated with 0.1 ml of a 16 hour nutrient broth culture of the appropriate Ames' tester strain of Salmonella typhimurium (TA 1535, TA 1537, TA 98, or TA 100). The tubes were incubated at 37° C for 30 minutes with shaking. Two ml aliquots of top agar at 45° C were then added to the

tubes, the tubes were mixed, and the contents were poured onto the surface of VBME plates. For tests in the presence of S-9, each tube containing sample plus inoculum received 0.5 ml of S-9 mix prior to the 30 minute incubation at 37° C.

Five different doses of each chemical were tested with and without microsomal activation. The highest concentration used was limited by solubility and toxicity. Five different concentrations of S-9 (10, 20, 30, 40 and 50 µl per plate) were used for each dose in the plate incorporation test while only one concentration of S-9 (50 µl/plate) was used for each dose in the preincubation test. All tests were performed in duplicate and each compound was tested along with positive and negative controls. The positive control compounds used are: Propylene oxide for TA 1535 and TA 100, 9-aminoacridine for TA 1537, and 2-nitrofluorene for TA 98. Ethidium bromide was used as a positive control compound for metabolic activation. All gases and volatile compounds were tested in a sealed jar.

Chemicals

Chemicals used in this study, with the exception of platinum related compounds, were obtained from commercial chemical companies. Platinum related compounds were provided by Dr. Dave Groth, NIOSH, Cincinnati, Ohio. Chemicals were dissolved in sterile distilled water (whenever feasible) or in dimethyl sulfoxide immediately before used.

RESULTS

The results are shown in Tables 1 and 2. Among the 147 compounds tested, 120 were not mutagenic to any of the testers either with or without metabolic activation. Twenty-three compounds were directly mutagenic to one or more than one tester. The remaining four compounds (1,2,3-trichloropropane, dimethoxyethylphthalate, 2,4-dimethylaniline and 2,6-dinitrotoluene) required metabolic activation for their mutagenic activities. A compound is classified as mutagenic if it causes a dose related increase in the number of revertants and the increase is more than two times the background level. The ranges of background revertants were 10-50 for TA 1535, 5-25 for TA 1537, 16-90 for TA 98 and 100-350 for TA 100.

Two (nitromethane and monochloroethane) of the 27 mutagenic compounds were mutagenic only for TA 1535 and two other compounds (dimethoxyethylphthalate and n-nitrosoaniline) were mutagenic only for TA 1537. Pt (bipyridyl) Cl_2 and 2,4,-dimethylaniline were mutagenic only for TA 98 and TA 100, respectively. Several compounds were mutagenic only for one or two testers if tested without *in vitro* microsomal activation. However, they were mutagenic for more than two testers if tested with S-9 from liver of Aroclor 1254 pretreated rats. Some compounds showed higher mutagenic activity when a low concentration (20 µl/plate) of S-9 was used, whereas other compounds required a higher concentration (50 µl/plate) of S-9 for mutagenic activity.

DISCUSSION

In this study, we found that industrial chemicals such as 1,2,3-trichloropropane, nitromethane, 2-nitropropane, methyl bromide, methylene chloride, monochloroethane, 2,3-dinitrotoluene, 2,5-dinitrotoluene, 2,6-dinitrotoluene, 2,4,5-trinitrotoluene, p-dinitrobenzene, and m-dinitrobenzene are mutagenic for Salmonella typhimurium. More studies need to be conducted in mammalian and other submammalian mutagenesis test systems, and the potential mutagenic and carcinogenic hazards of these compounds for the exposed population need to be determined.

Several interesting phenomena were noted in this study. PtK_2Cl_4 was mutagenic for TA 98 and TA 100 if it was dissolved in water. If dimethyl sulfoxide (DMSO) was used as the solvent, however, negative results were obtained. It seems that DMSO could interact with PtK_2Cl_4 and inhibit or diminish the mutagenic activity of this compound. This result emphasizes the importance of making an effort to dissolve chemicals in aqueous solution for mutagenesis testing.

S-9 from the liver of mammals can activate promutagens to mutagenic metabolites and, in some instances, enhance the mutagenic activity of directly acting mutagens. A concentration of 50 µl S-9 per plate is used by most laboratories for mutagenesis testing. In this study, however, the best mutagenic response of several compounds was found when a lower concentration of S-9 (20 µl/plate) was used. Recently, we have also found that m-aminophenol and the dye, direct blue-15 are mutagenic only when these compounds were tested with S-9 from hamsters.

There appears to be a structure and function relationship among several structurally related chemicals studied. For instance, nitrophenol is mutagenic if hydroxy and nitro groups are in a meta arrangement, but not mutagenic if both groups are in an otho arrangement Similar results were found with dinitrobenzene. m-dinitrobenzene was mutagenic but o-dinitrobenzene was not.

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TABLE 1

Compounds That Did Not Exhibit Mutagenic Activity

for Salmonella Typhimurium

Alicyclic and Heterocyclic Compounds:

Adenine (1500 µg)[a]

Dipyridyl (5000 µg)

Furfuryl alcohol (15 µl)

Hexachlorocyclopentadiene (0.0002 µl)

2-mercaptobenzothiazole (500 µg)

N-methyldicyclohexylamine (10 µl)

Aliphatic Amines:

sec-butylamine (10 µl)

2-dibutylamino ethanol (50 µl)

2-diethylamino ethanol (30 µl)

Ethanolamine (30 µl)

Hexamethylenediamine (3 µg)

Hexamethylenetetramine (5 µg)

n-hexylamine (4.5 µl)

n-pentylamine (8 µl)

n-propylamine (10 µl)

Aliphatic Carboxylic Acids and Other Aliphatic Compounds:

Acetone cyanohydrin (0.05 µl)

Acetonitrile (300 µl)

Acrolein (0.2 µl)

Acrylamide (50 µg)

TABLE 1 (CONT'D)

Acrylonitrile (25 µl)

Adiponitrile (100 µl)

Allylchloride (4 µl)

Butyl isocyanate (0.01 µl)

N-butyronitrile (100 µl)

Diethylenetriamine pentaacetic acid (1 µl)

N,N-dimethylacetamide (1000 µl)

Ethylene (100%)

Ethyleneglycol-bis-(ß-aminoethylether)-N,N'-tetra
acetic acid (500 µg)

Glyconitrile (3500 µg)

Hexachlorobutadiene (500 µl)

Hexachloroethane (4000 µg)

Iso-butyronitrile (5 µl)

Malononitrile (0.5 µl)

Methyl ethyl ketone peroxide (0.4 µl)

Bis-(2-methoxyethyl) ether (500 µl)

Oxalic acid (2000 µg)

Pentachloroethane (5 µl)

Perchloroethylene (100 µl)

n-propyl isocyanate (0.01 µl)

Proprionitrile (200 µl)

Succinonitrile (4000 µg)

1,1,2,2-tetrachloroethane (2 µl)

Tetramethyl succinonitrile (4000 µg)

1,1,1-trichloroethane (500 µl)

Trichloroethylene (100 µl)

Aromatic Amines:

m-aminophenol (1000 g)

2,3-dimethylaniline (2 µl)

2,5-dimethylaniline (2 µl)

2,6-dimethylaniline (4 µl)

3,4-dimethylaniline (1250 µg)

3,5-dimethylaniline (100 µl)

O-methoxyaniline (30 µl)

N-methylaniline (60 µl)

p-nitrobenzyl-N,N-propylamine (5000 µg)

N-phenyl-N'-2-octylparaphenylenediamine (250 µl)

Triphenylamine (2000 µg)

Aromatic Hydrocarbons:

Benzoyl peroxide (100 µg)

Bisphenol A (200 µg)

m-dichlorobenzene (10 µl)

Ethyl benzene (0.2 µl)

Hexachlorobenzene (150 µg)

Naphthalene (500 µg)

2,4-toluene diisocyanate (3 µl)

1,3,5-trichlorobenzene (100 µg)

TABLE 1 (CONT'D)

1,2,4-trimethylbenzene (0.3 µl)

Triortho cresyl phosphate (500 µ)

m-vinyl toluene (0.1 µl)

O-vinyl toluene (0.1 µl)

p-vinyl toluene (0.1 µl)

Metals, Metal Salts, and Organometallics:

$BeCl_2$ (5000 µg)

Bipyridyl $BeCl_2$ (300 µg)

Bis (dimethylglyoxime) Pt(II) (300 µg)

Bis (2-pyridinaldoximinato) Pt(II) (300 µg)

Dichloro (2-formimidoyl pyridine) Pt(II) (100 µg)

H(Pt adenine Cl_3) (300 µg)

Nitro Aromatics:

o-dinitrobenzene (200 µg)

4,6-dinitro-o-cresol (150 µg)

2,4-dinitrophenol (250 µg)

2,6-dinitrophenol (250 µg)

2,3-dinitrotoluene (50 µg)

2,4-dinitrotoluene (400 µg)

3,4-dinitrotoluene (500 µg)

Metaoxon (2000 µg)

Mononitrobenzene (4 µl)

o-nitrophenol (500 µg)

p-nitrophenol (500 µg)

2,4,6-trinitrophenol (1500 µg)

TABLE 1 (CONT'D)

Solvents:

Acetic acid (500 µl)

Acetone (500 µl)

Benzene (500 µl)

Cyclohexanone (50 µl)

N, N-dimethylacetamide (500 µl)

Dimethylformamide (500 µl)

Dimethylsulfoxide (500 µl)

Ethanol (500 µl)

2-ethoxyethanol (20 µl)

Glycerol (500 µl)

2-methoxyethanol (500 µl)

Phosphoric acid, in H_2O, pH4 (500 µl)

Potassium acid phthalate (2%) (500 µl)

Sodium hydroxide, in H_2O, pH 9.5 (500 µl)

Sodium phosphate, dibasic (2%) (500 µl)

Miscellaneous:

Antioxidant 2246 (200 µg)

Butylene oxide (100 µl)

Carbon disulphide (100 µl)

Cyanogen (0.01%)

Geltrol (20 µl)

α-(histamine $AlBr_3)^+$ (2000 µg)

Hydrogen sulphide (0.1%)

TABLE 1 (CONT'D)

Isopropyl isocyanate (0.01 µl)
Methyl parathion (300 µg)
Nitrous oxide (90.6%)
Phenyl isocyanate (0.01 µl)
Phosgene (0.0001%)
O-terphenyl (5000 µg)
p-terphenyl (1500 µg)
Tert-butyl isocyanate (0.1 µl)
(Tetrazene $AlBr_2$)$^+$ (500 µg)
p-toluene sulfonyl isocyanate (2 µl)

[a]The number in parentheses represents the highest concentration of chemical tested in terms of quantity per plate.

TABLE 2

Compounds with Mutagenic Activity in Salmonella Typhimurium

Compound	S-9[a]	Dose/Plate[b]	No. of His$^+$ Revertants/Plate[c] TA 1535	TA 1537	TA 98	TA 100
		Alicyclic and Heterocyclic Compounds				
Vinyl cylohexene dioxide	+	15 µl	669 (9)	28 (12)	283 (78)	<2000 (226)
	-	15 µl	647 (11)	-	-	<2000 (285)
		Aliphatic Carboxylic Acids and Other Aliphatic Compounds				
Diethyl carbamoyl chloride	+[e]	200 µl	82 (16)	21 (9)	157 (59)	896 (183)
	-	200 µl	107 (43)	-	-	690 (261)
Ethylene oxide	+	0.1 %[f]	>2000 (12)	-	-	>2000 (195)
	-	0.1 %[f]	592 (12)	-	-	1640 (215)
2-nitropropane	+	50 µl	+	-	+	>4000 (NT)
	-	50 µl	NT	-	NT	>4000 (141)
Methyl bromide	+	0.53 %[f]	124 (8)	-	-	710 (159)
	-	0.53 %[f]	28 (13)	-	-	700 (151)
Methylene chloride	+	500 µl	70 (22)	-	632 (58)	>2000 (142)
	-	500 µl	88 (33)	-	190 (26)	1836 (170)

TABLE 2 (CONT'D)

Compound	S-9[a]	Dose/Plate[b]	No. of His+ Revertants/Plate[c] TA 1535	TA 1537	TA 98	TA 100
Monochloroethane	+	5000 µl	594 (14)	-	-	-
	-	5000 µl	159 (18)	-	-	-
Nitromethane	+	50 µl	146 (19)	-	-	-
	-	50 µl	105 (36)	-	-	-
1,2,3-Trichloropropane	+	0.5 µl	385 (21)	100 (9)	-	>2000 (219)
	-	0.5 µl	-	-	-	-
Dimethoxyethylphthalate	+	100 µl	-	34 (8)	-	-
	-	100 µl	-	-	-	-
2,4-dimethylaniline[d]	+	2 µl	-	-	-	446 (194)
	-	2 µl	-	-	-	-
N-nitrosoaniline	+	100 µg	-	75 (14)	-	-
	-	100 µg	-	143 (13)	-	-
		Metals, their Salts and Organometallics				
[Pt(bipyridine) (adenine)]Cl_2	+		NT	NT	NT	NT
	-	40 µg	-	-	511 (40)	543 (221)

TABLE 2 (CONT'D)

Compound	S-9[a]	Dose/Plate[b]	No. of His+ Revertants/Plate[c] TA 1535	TA 1537	TA 98	TA 100
$Pt(bipyridyl)Cl_2$	+		NT	NT	NT	NT
	-	10 µg	-	-	709 (50)	-
$Pt\ K_2\ Cl_4$	+		NT	NT	NT	NT
	-	100 µg	-	-	267 (40)	689 (221)
		Nitroaromatics				
m-dinitrobenzene	+[e]	200 µg	-	-	219 (41)	362 (180)
	-	200 µg	-	-	160 (21)	569 (255)
p-dinitrobenzene	-[e]	50 µg	-	-	90 (48)	407 (172)
	-	50 µg	-	-	505 (44)	565 (209)
2,5-dinitrophenol	+[e]	20 µg	-	-	142 (64)	324 (193)
	-	20 µg	-	-	351 (57)	-
2,5-dinitrotoluene	+[e]	200 µg	61 (21)	-	112 (49)	-
	-	100 µg	-	-	233 (35)	-
2,6-dinitrotoluene	+	2000 µg	-	57 (19)	337 (88)	485 (243)
	-	2000 µg	-	-	-	-

TABLE 2 (CONT'D)

Compound	S-9[a]	Dose/Plate[b]	No. of His$^+$ Revertants/Plate[c]			
			TA 1535	TA 1537	TA 98	TA 100
1-nitronaphthalene	+[e]	100 µg	-	-	82 (38)	1000 (332)
	-	100 µg	-	-	76 (22)	1080 (300)
m-nitrophenol	+[e]	500 µg	36 (18)	-	127 (33)	505 (196)
	-	500 µg	-	-	205 (20)	-
Paraoxon	+	4 µl	54 (16)	-	-	536 (198)
	-	4 µl	71 (35)	-	-	565 (231)
2,4,5-trinitrotoluene	+[e]	10 µg	-	-	96 (52)	486 (178)
	-	10 µg	-	-	279 (44)	384 (209)
		Miscellaneous				
Propylene oxide	+[e]	100 µl	168 (16)	-	-	2500 (332)
	-	100 µl	120 (20)	-	60 (22)	2300 (300)
Styrene oxide	+	5 µl	NT	NT	NT	>4000 (199)
	-	5 µl	NT	NT	NT	>4000 (153)

TABLE 2 (CONT'D)

Compound	S-9[a]	Dose/Plate[b]	No. of His+ Revertants/Plate[c]			
			TA 1535	TA 1537	TA 98	TA 100
Tetramethyl thiuram disulphide	+	25 µg	43 (12)	-	-	510 (171)
	-	100 µg	-	-	-	453 (191)

[a] + = Tested with metabolic activation; - = Tested without metabolic activation.

[b] Dose which gave the highest mutagenic response.

[c] Number of revertants is an average of 2 plates; Number of spontaneous revertants is shown in parentheses; NT = not tested; - = Number of revertants is less than 2 times of the background.

[d] Tested by the pre-incubation assay system.

[e] The concentration of S-9 is 20 µl/plate rather than 50 µl/plate. Number of revertants decreased when 50 µl S-9/plate was used.

[f] Percent in air.

TABLE 3

Summary of the Test Results

Types of Compounds Tested	No. of Compounds Tested	No. of Compounds Found to be Mutagenic		
		Without Activation	Activation Required	Total
Alicyclic and Heterocyclic	7	1	0	1
Aliphatic Amines	9	0	0	0
Aliphatic Carboxylic Acids and other Aliphatics	38	7	1	8
Aromatic Amines and Aromatic Hydrocarbons	27	1	2	3
Metals, their Salts and Organometallics	9	3	0	3
Nitroaromatics	21	8	1	9
Solvents and Reagents	15	0	0	0
Miscellaneous	20	3	0	3
Total	147	23	4	27

Dr. O'Conor, NCI: I could not read the slide of the list, and you say you will have the list, but could you just tell us were there some metals that were positive?

Dr. Elliott, NIOSH: As you know, if you test the metals like cobalt, nickel and cadmium, they are not positive in the standard Ames' assay. The compounds that were positive in this study were organic platinum compounds. Dr. Groth is interested in beryllium compounds and in the way that they cause cancer. He has synthesized the platinum compounds and then he is going to repeat the same thing with beryllium. We do not have the data on those compounds, but beryllium metal and its salts, beryllium chloride, beryllium nitrate, and beryllium oxide, were negative.

Dr. Lee, EPA: On the same line, I have a question. I noticed that on your list there you did some of the toluene group, and you have about three or four different isomers. Did you check on most of those?

Dr. Elliott, NIOSH: Yes.

Dr. Lee, EPA: The reason is when I was at a Middle West research institute we undertook an extensive mammalian toxicity study for the munitions compounds, the compounds important to the Army, and in one group is trinitrotoluene and dinitrotoluene. Most of the isomers, unfortunately I don't remember which is positive and which is negative and this data has not been in the literature, but it is in the extensive report to the Army.

Dr. Elliott, NIOSH: We have looked at several dinitrotoluenes. The 2,3, 2,5 and 2,6 were negative, and I cannot remember which ones I had up on the slide. It does not make any difference, but--

Dr. Lee, EPA: You had three of them.

Dr. Elliott, NIOSH: The 2,5 and 2,6 dinitrotoluenes were positive along with trinitrotoluene.

Dr. Lee, EPA: One of the derivatives is the nitroaminotoluene, and if I remember that is a positive. I understand that, also, is one of the metabolites, both by bacteria reaction and, also, biological changes in the high species. That is, also, positive.

FIGURE CAPTIONS

Figure 1. Mean daily percent of intervals in which the chop sprayer and gelcoat sprayers 1, 2, and 3 placed molds properly within the spray booths, during baseline and training.

Figure 2. Mean daily work-duration, breathing zone exposures (ppm) for the resin-chop sprayer, rollout person, gelcoat sprayers 1, 2, and 3 and mold repair person, during baseline and training. Means across baseline and training conditions are denoted by (----).

NO DISCUSSION FOLLOWING THIS PAPER

NIOSH

PROCEEDINGS OF THE
FIRST NCI/EPA/NIOSH COLLABORATIVE WORKSHOP:
PROGRESS ON JOINT ENVIRONMENTAL AND
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MAY 6-8, 1980

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The papers included in these Proceedings were printed as they were submitted to this office.

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