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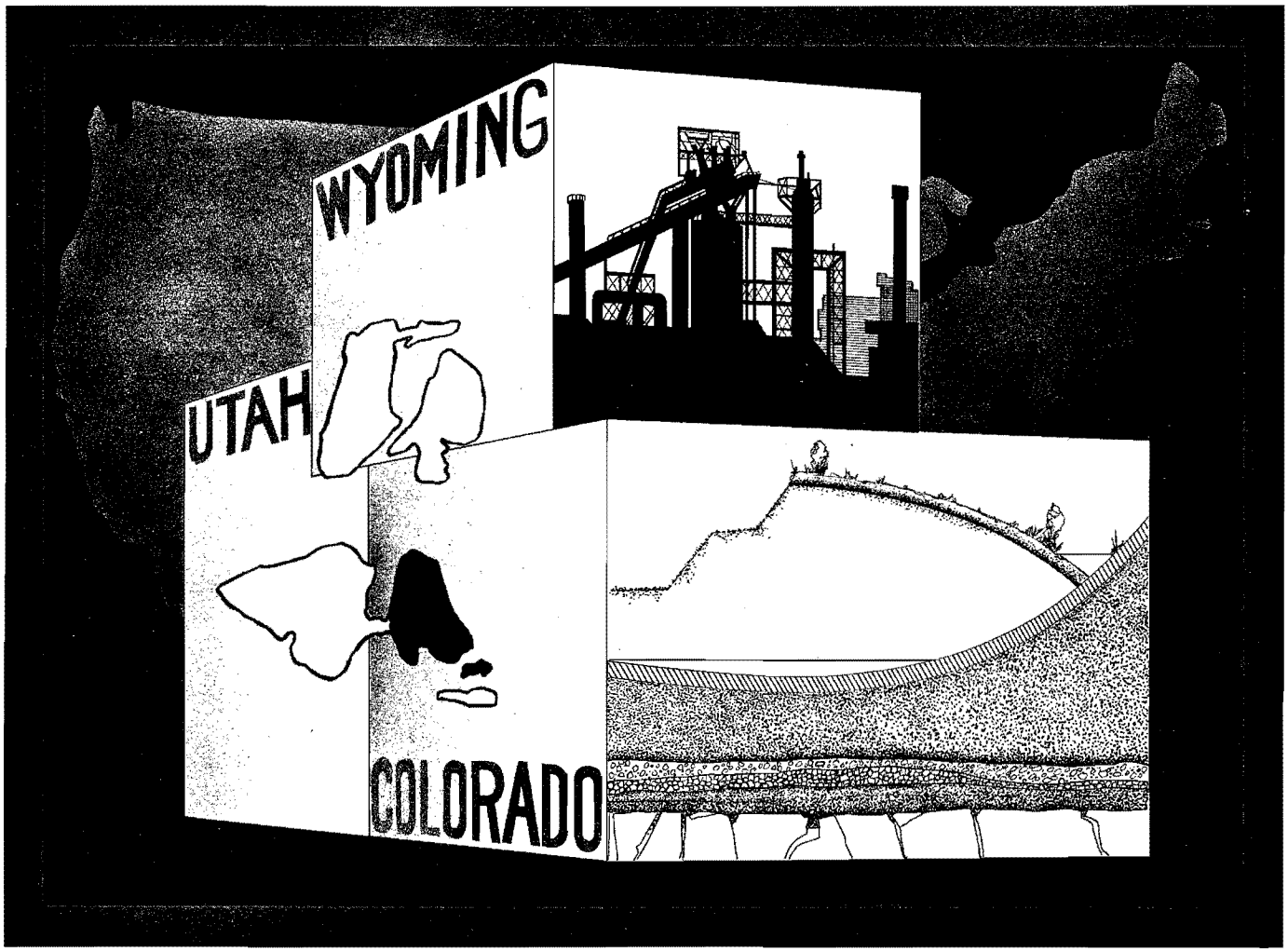

Utah Water Research Laboratory
Utah State University
Logan, Utah 84322

WATER QUALITY SERIES
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EVALUATION OF THE POTENTIAL FOR GROUNDWATER TRANSPORT OF MUTAGENIC COMPOUNDS RELEASED BY SPENT OIL SHALE

by

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ABSTRACT

The major focus of this study was on the potential mutagenicity of aqueous leachates from spent oil shale. Additional mutagenicity testing was also done on raw shale and coal.

The Ames salmonella microsomal bioassay was used to test for chemical mutagenicity. Spent oil shales from the Paraho and TOSCO II processes, a raw shale from Anvil Points, and a composite coal sample from the Wasatch plateau were extracted with water and organic solvents. Only organic solvent extraction of the TOSCO spent shale resulted in a mutagenic response. The lack of mutgenic response to organic extracts of Paraho spent shale was unexpected and was probably due to higher than typical temperatures at which it had been retorted.

Using TOSCO spent shale leachate and the organically extracted mutagen, a partition relationship between the spent shale and leachate water was developed. The mutagen was found to have a fairly high affinity for spent shale. Based on this it was estimated that mutagenicity of the TOSCO spent shale leachate will be low (in the range of chlorinated wastewater), however it will require many pore volumes to leach out of a pile potentially resulting in a chronic long-term problem.

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We would like to thank the Paraho Development Corporation for providing both a spent and a raw shale sample and the TOSCO corporation for providing and transporting to Logan a large quantity of spent shale. Valuable advice was obtained from both corporations.

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INTRODUCTION AND OBJECTIVE

Coal and oil shale mining have been increasing in the intermountain west and pose some new concerns for water quality control. Surface and groundwaters that are pumped during mine dewatering, seep into mines, or used in mining operations are collected, treated, and either evaporated or discharged into streams. Groundwater may become contaminated by leaching of either surface mine spoils, solid wastes of the industries (i.e., spent oil shale) or abandoned mines. Expanded mining generates new water quality concerns. Objective water quality management requires information on the effects of these waters on the aquatic environments and on potential uses of these waters. Potential carcinogenic and mutagenic effects are among the most important concerns.

It has been estimated (Wynder and Mabuchi 1972) that up to 90 percent of all cancer in the United States is induced by environment factors. Cairns (1975) estimated that limiting human exposure to the environmental factors contributing to cancer, would reduce the overall cancer rate in the United States 10 fold. The concern has multiplied efforts to identify carcinogenic compounds in drinking water and determine their sources and rates of accumulation in aquatic organisms.

Carcinogenic and mutagenic compounds have been found in many natural waters. Kool et al. (1981b) and Van Kreijl et al. (1980) found organic mutagens in the Rhine and Meuse Rivers. Parry et al. (1976) found mussels to accumulate mutagenic compounds in marine environments near areas of industrial development in England. Veldre et al. (1979) found benzo(a)pyrene (BaP), a known mutagen (Ames et al. 1975) and carcinogen (Kingsbury et al. 1979), in Estonian waters and accumulated in aquatic organisms in those waters. Oil shale wastes were considered the probable source. Numerous studies have found mutagens in waters receiving chlorinated wastewater effluents (Cumming et al. 1978; Grabow et al. 1980; Harrison et al. 1975; and Moore et al. 1980). Mutagenic and carcinogenic compounds have been identified in the drinking water of many major cities (Coleman et al. 1980; Grimm-Kibalo et al. 1981; Kool et al. 1981b; and Nestmann et al. 1979).

Energy development in the Upper Colorado River drainage basin will greatly increase the quantities of accrual water flowing from active and abandoned coal and oil shale mines. Israelsen et al. (1980) found the total organic carbon (TOC) concentration of water in contact with slurried coal to increase by as much as 30 mg/l. The Cathedral Bluffs Shale Oil Company (1981) has shown that leachate from raw oil shale may have a TOC as high as 512 mg/l. The nature of the organics coming from these natural materials is largely unknown. Characterization of the pollutants is further complicated by either in situ retorting or the return of the retorted spent shale residue to the oil shale mines. Both surface and groundwaters may become contaminated by leachate from coal, raw oil shale, and spent oil shale.

The oil shale industry has only begun to develop. There are no commercial scale retorts in the United States, and any of a variety of processes may be used to recover shale oil when and if commercial production begins. This

inability to characterize the waste products makes environmental assessment difficult; however, it also allows environmental concerns to be considered and incorporated into process design from the early stages of industrial development.

The overall objective of this study was to determine the extent to which mutagens are mobilized and leached from coal, oil shale, or spent shale and contaminate groundwater or accrual water. Important leaching mechanisms were evaluated and projected to a field situation to allow prediction of the potential for water contamination from these materials.

LITERATURE REVIEW

Oil Shale Processing Techniques

Oil shale is mined either by sub-surface techniques or by strip mining. The shale contains kerogen, a solid organic material which when heated decomposes to shale oil, a crude petroleum. The shale must be retorted to heat the kerogen and produce extractable shale oil. This can be done either above ground or in situ. There are two basic heating processes. The direct combustion process burns the organic residue in the spent shale while indirect processes use some external heat source to recover oil from the shale.

In Situ Retorting

Numerous in situ processes including bubble in situ (RISE), Oxy Modified in situ, horizontal modified in situ, multimineral modified in situ, true in situ, Geokinetics in situ, BX in situ, Talley energy in situ, and radio frequency retorting (Nowacki 1981) are currently being considered for use. The difference between a true in situ process and a modified in situ process (MIS) is that in a true in situ process all the retorting is done underground. An MIS process mines some of the oil shale for retorting ex situ.

The numerous direct combustion processes for in situ oil shale retorting differ in geometry and flow configuration but produce similar reactions. Steam injection and microwave indirect processes have been discussed but have not been tested on a large scale. The direct combustion processes may be characterized as a batch, fixed bed reactor, in which a combustion front is swept through the bed by a stream of injected gas. The sweep contains oxygen to sustain the combustion and steam or recycled product gas to control it (Nowacki 1981).

The MIS process (Figure 1) developed by Occidental Oil Shale, Inc., has been the most studied in situ process. The MIS process begins with mining 20 to 25 percent of the shale. After the mined shale is removed, the adjacent rock formation is explosively expanded into the room, producing an underground "retort."

After startup, the process reaches steady-state in which a combustion front moves down the retort. At the bottom, water condenses on the cool raw shale. The layer above this layer is the vaporization zone in which water, bound in the oil shale, vaporizes as oil condenses. Above this is the intra particle coking zone where kerogen is decomposed to oil and the oil vaporizes. Above this is the zone of combustion for residual organics. The warm spent shale is at the top. Temperatures in the combustion zone may exceed 1700°C. The gases, CO_2, H_2, CO, and CH_4 are given off by the retorting but may be burned to provide an energy source.

Spent shale residue from the MIS process is very different than the material left by surface retorting. Not only are the temperatures higher but the insulating properties of the surrounding rocks keep the spent shale hot for months (Hoffman 1981). Some of the spent shale from above ground retorting may be used to grout the underground retorts to reduce groundwater movement (Persoff and Fox 1979).

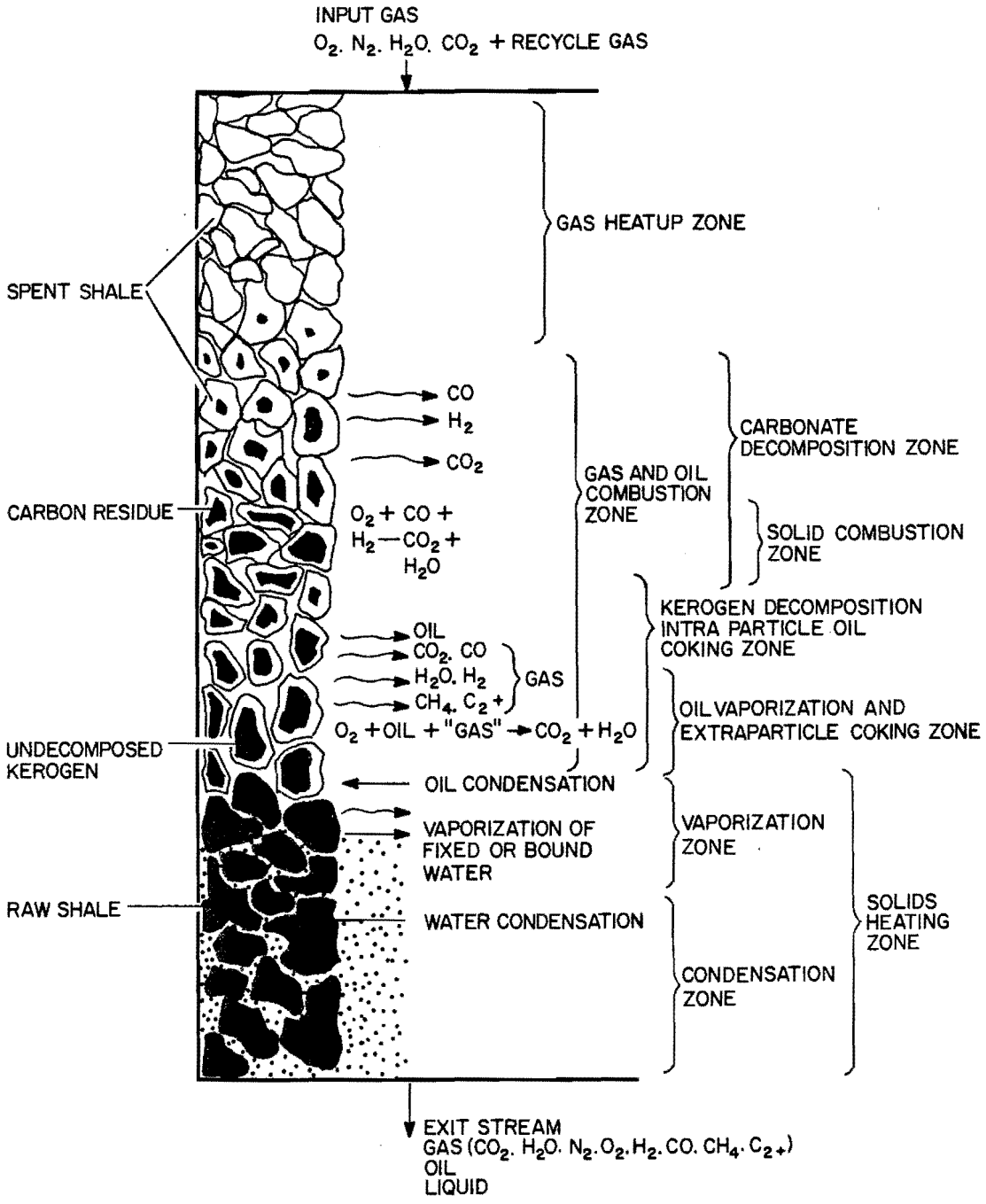

Figure 1. Schematic of a typical direct combustion oil shale retorting process (from Nowacki 1981).

Occidental Petroleum's plans for a 57,000 bbl/day MIS facility at Cathedral Bluff's C-b site were postponed in late 1981 after the mine shaft construction was completed. The future of the Oxy MIS process is under study by the Cathedral Bluffs Oil Shale Company (Callahan 1982).

Surface Retorting

The two favored types of surface retorting are those which use gas to transfer heat and those in which circulated solids are used as the heat carrier. The two most studied processes, the Paraho and the TOSCO II, are examples of gas and solids circulation processes respectively. At this time there are no operating commercial retorts in the United States. It is not clear, if and when shale oil production does begin, which processes will be used. Other types of surface retorting being considered include the NTU, Union Oil B and SRG-3, circular grate, TOSCO II, Lurgi-Hygas, HYTORT, Petrosix, and SPHER processes (Nowacki 1981).

The Paraho process was first used in 1967 and has since produced more than 32,000 m^3 of shale oil in several demonstration runs. The current facility is a pilot plant scale semi-works operated by Development Engineering at Anvil Points, Colorado (Nowacki 1981).

The Paraho retort is an above ground continuously operated vertical kiln (Figure 2). Shale flows down from the top. The cool shale is warmed by ascending hot gas in the mist formation zone. The preheated shale then passes through the retorting zone where the kerogen is decomposed into oil, gas, and coke. When in the direct combustion mode, coke remaining on the retorted shale serves as fuel for the process in the combustion zone. The shale particle size must be at least 1.25 to 7.60 cm in diameter to prevent excessive headloss across the retort.

The Paraho process is operated at temperatures up to 650°C. However due to the combustion of the residue, the spent shale may be subjected to higher temperatures (Nowacki 1981). The Paraho process operates at the highest temperature of any of the above ground processes now being proposed. Greater temperatures produce a higher degree of carbonate destruction, a higher leachate pH, and a lower organic content in the spent shale residue.

The TOSCO II process was first utilized in 1957. The original plant produced 1.9 m^3/day of shale oil. The present semi-works began operation in 1967 and is now capable of producing 15,000 m^3/day of shale oil.

The TOSCO II process flow diagram is shown in Figure 3. Retorting is achieved by direct contact between hot ceramic balls and preheated oil shale. Raw shale that has been crushed to less than 1.3 cm is preheated by hot flue gas from the ball heater. The preheated shale is then fed to the pyrolysis drum. Retorting of the oil shale is achieved by solid-to-solid heat transfer between the shale and hot ceramic balls in a rotating drum. The process results in an extremely fine particulate spent shale. Ward et al. (1971a) reports a mean particle size of 0.07 mm.

The spent shale produced by the Paraho process is quite different than that from the TOSCO II process. Because of the lower temperatures of the TOSCO II process, the coke is not burned off the spent shale and an organic residue remains. Whitcombe and Vawter (1976) found that the TOSCO II process spent shale contained 4.5 percent organic residue and had a lower leachate pH than did higher temperature processes.

The spent shale solids pose a major disposal problem. Redente et al. (1981) estimates that although the mass of the oil shale is reduced 12 to 15 percent

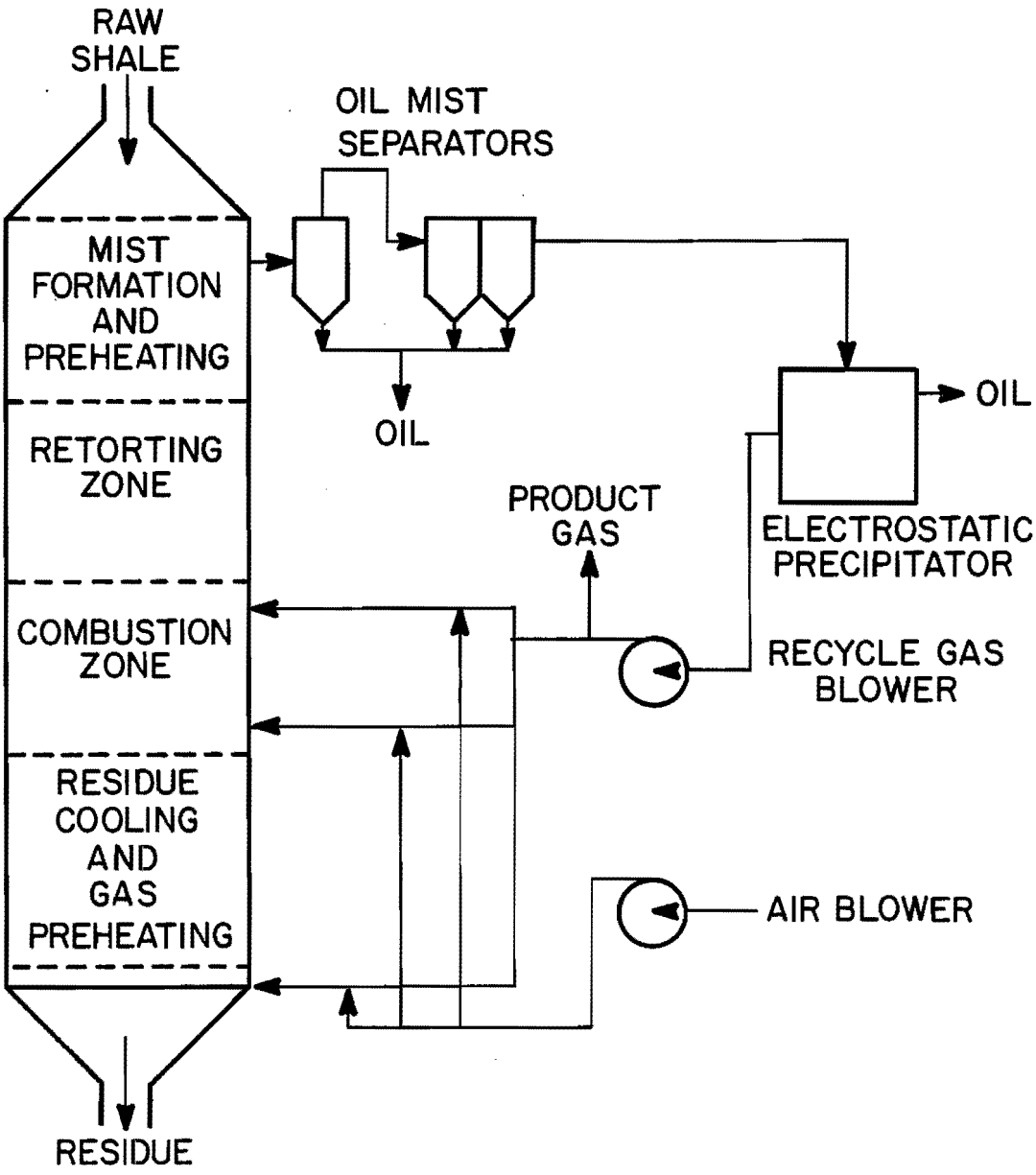

Figure 2. The Paraho oil shale retorting process (from Nowacki 1981).

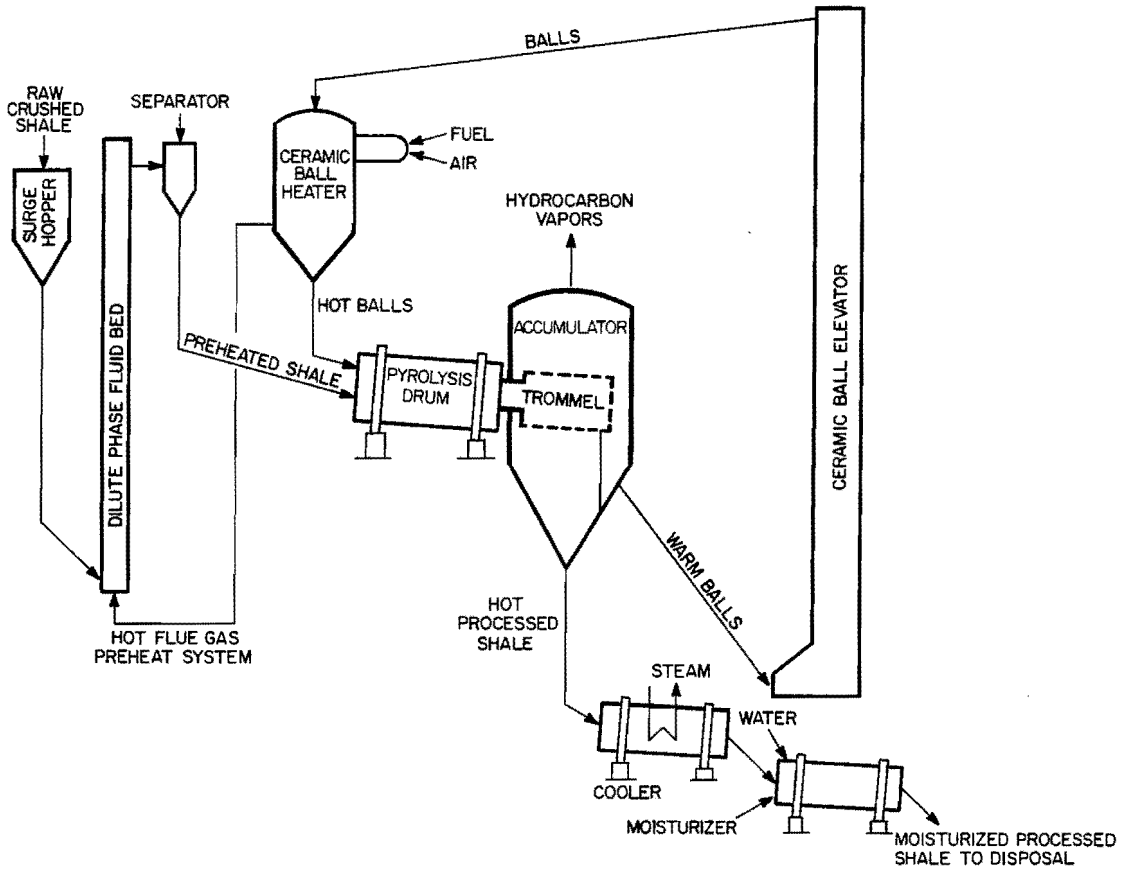

Figure 3. The TOSCO II oil shale retorting process (from Nowacki 1981).

during retorting, its volume will increase by as much as 30 percent. From 50 to 85 percent of the spent shale from surface retorts may be returned to the mine (Earnest et al. 1977; Routson et al. 1979; and Ward and Reinecke 1972). The Superior Oil Company has had proprietary design and economic studies performed for returning spent shale to the mine. The two methods considered were a shale slinging system and a slurry method (Nowacki 1981). The result would be return of a wetted shale to an abandoned mine or abandoned portions of a mine.

Oil Shale Mine Accural Water

At this time no active oil shale mines are discharging water. The Cathedral Bluffs mine shaft is currently discharging about 5000 m^3/day of accural water (Cathedral Bluffs Shale Oil Company 1981). Waters from active mines should have about the same composition as the water in the surrounding aquifers, except that mine drainage may also include wastes from drilling and mine operations and thus may contain higher suspended solids and oil and grease. Table 1 indicates the waters from these mines will be high in dissolved solids (TDS), flourides (F), and in some cases total or dissolved organic carbon (DOC or TOC). The kerogen in the oil shale is a probable source of the organic carbon.

In situ retorting changes the composition of the accrual water.

Table 1. Potential composition of oil shale mine accrual water.

Parameter	Units	Water Quality			
		Cathedral Bluffs (1981)	Fox and Phillips (1981)	Jensen (1981)	McWhorter (1981)
Al	mg/l	0.1	<0.001	<0.1-0.2	<0.05-7.54
Ag	mg/l	0.01-0.02	-[a]	-	-
Alkalinity	mg/l as $CaCO_3$	90.-415.	70-4,500	0.6-0.9	-
As	mg/l	0.2-0.3	<0.001	-	<0.005
Au	mg/l	-	-	<0.01	-
B	mg/l	0.1-0.5	<0.001	-	<0.025-2.75
Ba	mg/l	0.5	<0.001	-	0.038-0.495
Be	mg/l	-	-	-	<0.025
BOD	mg/l	1.0-78.0	-	-	-
Br	mg/l	0.1-0.4	-	-	-
Ca	mg/l	3.0-59.0	-	-	18.-1550.
Cd	mg/l	-	-	<0.01	-
Cl	mg/l	7.2-65.0	-	-	0.3-560.
Co	mg/l	0.01-0.03	<0.001	-	-
COD	mg/l	1.0 -40.0	-	-	-
Conductivity	µmho/cm	700-1590	600-45,000	-	125.-37,000.
Cr	mg/l	0.01-0.02	<0.001	-	<0.025-0.68
Cu	mg/l	0.01-0.04	<0.001	<0.02	<0.025-0.69
DOC	mg/l	1.0-23.0	-	-	-
F	mg/l	0.1-36.0	-	17.4-18.1	<0.5-75.
Fe	mg/l	0.02-0.08	<0.001	0.3-0.02	<0.03-0.89
Ga	mg/l	-	<0.001	-	-
$HCO_3 + CO_3$	mg/l	550.-2000.	70.-4500.	-	3.0-1026.
Hg	mg/l	0.0001-0.020	<0.001	<0.0002	<0.001-0.0035

[a]A dash signifies that no data are available.

Table 1. Continued.

Parameter	Units	Water Quality			
		Cathedral Bluffs (1981)	Fox and Phillips (1981)	Jensen (1981)	McWhorter (1981)
I	mg/l	-	<0.001	-	-
Li	mg/l	0.05	<0.001	-	-
K	mg/l	1.0-60.0	-	-	0.4-640.
Mg	mg/l	3.8-12.0	-	-	0.675-1050.
Mn	mg/l	-	<0.001	-	<0.05-3.2
Mo	mg/l	-	<0.001	-	0.075-5.18
Na	mg/l	410.0-1009.0	-	-	4.3-7710.
Ni	mg/l	-	<0.001	-	<0.025-0.60
NH_4-N	mg/l	0.03-13.0	0.1-200.	0.8-1.3	-
NO_3-N	mg/l	-	-	-	<0.3-245.
TKN-N	mg/l	0.1-15.0	-	-	-
Oil and Grease	mg/l	1.0-16.0	-	-	-
Pb	mg/l	0.02-0.05	<0.001	-	<0.04-1.9
Phenols	mg/l	0.001-0.120	-	<0.001-0.002	-
pH		7.1-9.3	6.3-9.3	-	6.8-11.98
Rb	mg/l	-	<0.001	-	-
Sc	mg/l	-	<0.001	-	-
Se	mg/l	-	<0.001	-	<0.01
Si	mg/l	0.02-0.002	-	-	1.2-23.28
Sn	mg/l	-	-	-	<0.025-1.28
SO_4	mg/l	8.-500.	<4.-900.	-	5.-6100.
Sr	mg/l	0.6-6.6	-	-	-
Ti	mg/l	-	<0.001	-	-
TDS	mg/l	-	1,800.-24,000.	1300-1400	70.-30,130.
TOC	mg/l	-	1.-70.	-	-
V	mg/l	-	<0.001	-	-
Zn	mg/l	0.1-0.06	<0.001	<0.02	<0.01-6.8

The water which is bound or held in the oil shale and released during the retorting process contains high levels of both inorganic and organic compounds (Helper et al. 1979) including organic mutagens (Rao et al. 1981).

After retorting, the spent shale remains below ground where groundwater leaches out both organic and inorganic contaminates (Fox 1980). In laboratory experiments, Amy et al. (1980) found that spent oil shale from the center of an underground retort contained up to 15 mg/l of TOC in its leachate. Fox (1980) believes that the composition of leachate from less thoroughly retorted zones, such as the bottom of an MIS retort, may contain more organic contaminates than that from portions of the retort which have reached higher temperatures.

Spent oil shale from surface retorting, returned to the mines for disposal or to grout an abandoned in situ retort, will alter the composition of the leachate water. Table 2 gives the water quality found in leachates from various spent shales. The composition of the water is similar to that contacting raw shale, but the concentrations are generally higher. The variation among shales is a result of different shales being leached under different conditions. As a shale is leached, most compounds solubilize rapidly; and the concentrations drop off exponentially with further leaching. A notable exception is fluoride. Stollenwerk and Runnells (1981) found the fluoride concentration to remain relatively stable in the 10 to 20 mg/l range through a leaching of more than 20 pore volumes. This was attributed to the limited solubility of CaF_2, pk_{so} = 10.3 (Stumm and Morgan 1981).

The hydraulic properties of spent shale as a porous medium have been investigated by Bloomfield and Stewart (1981), Heistand (1981) and Ward et al. (1971a, 1971b, and 1972). Table 3 compares important hydraulic properties of spent oil shales with natural soils. The TOSCO shale tabulated was very similar to TOSCO II spent shale. The USBM sample was similar to Paraho spent shale. The permeability of spent shale appears to be in the range between sand and silt, indicating it could conduct groundwater if returned to an abandoned mine shaft. Compaction of spent shale greatly reduces its permeability (Bloomfield and Stewart 1981, Heistand 1981). In situ spent shales which have been fractured by explosives will have much larger particle sizes and much higher hydraulic conductivity than spent shales from surface retorts, which require crushing prior to retorting.

Spent Shale Piles

It will be necessary to dispose of most, if not all, surface retorted spent shale above ground. Numerous approaches to above ground disposal have been proposed (Heistand 1981, Nowacki 1981, USGS 1981, and White River Oil Shale Company 1980). The general approach is to dispose of the shale either on relatively flat terrain, in a canyon, or by returning it to an open pit mine. At the selected site, the topsoil will be removed and stockpiled. The shale will be wetted and cooled before disposal. The lower layer of shale may be machine compacted to reduce permeability, but sufficient compaction may be achieved as the result of driving over it with the disposal equipment along with the weight of a deep pile. For optimal compaction and dust control a water content of 10-20 percent will be required (Nowacki 1981).

The spent shale will be spread by trucks or land-moving equipment in 46 - 61 cm layers in a terrace arrangement. On flat ground, the pile will be surrounded by an embankment of high strength impervious shale. In a canyon, the stream course will be diverted around the spent shale pile. On the down canyon side of the pile, a dam of compacted retorted shale will be built

Table 2. Potential composition of oil shale mine accural water contacting surface retort spent shale.

Parameter	Units	Water Quality				
		Ward et al. (1971b)	Cleave et al. (1979)	Stollenwerk and Runnells (1981)	Fransway and Wagenet (1981)	McWhorter (1981)
Al	mg/l	-	-	-	-	<0.05-0.1
As	mg/l	-	<0.001	-	-	<0.005
Ag	mg/l	-	<0.009-0.015	-	-	-
B	mg/l	-	0.97	0.6-13.	-	0.165-0.39
Ba	mg/l	-	<0.078-0.207	-	-	0.028-0.35
Be	mg/l	-	-	-	-	<0.025
Ca	mg/l	609.-3,150.	2.4-747.	400.-540.	0.3-21.9	10.-39.
Cd	mg/l	-	0.016-0.021	-	-	-
Cl	mg/l	80.-3,080.	2.0-92.5	60.-475.	0.11-3.0	1.1-15.0
Conductivity	µmhos/cm	3,850.-78,100.	1601.-10,046.	500.-71,000.	200.-66,000.	300.-1300.
Cr	mg/l	-	<0.011-0.015	-	-	<0.025-0.71
Cu	mg/l	-	<0.011-0.016	-	-	<0.025-0.33
F	mg/l	-	-	5.1-35.0	-	5.5-6.6
Fe	mg/l	-	<0.001-0.025	-	-	<0.05
HCO_3 and CO_3	mg/l	-	14.1-1483.	40.-180.	-	167.-600.
Hg	mg/l	-	-	-	-	<0.0001
K	mg/l	-	8.1-455.	35.-1100.	0.2-13.8	9.2-74.
Mg	mg/l	536.-4,720.	0.02-475.	26.-3000.	0.2-242.4	30.-108.
Mn	mg/l	-	<0.007-0.016	-	-	<0.05
Mo	mg/l	-	-	0.7-5.5	-	0.065-0.45
Na	mg/l	502.-35,200.	19.-2704.	1100.-8400.	0.2-243.3	12.-75.
Ni	mg/l	-	-	-	-	<0.05
NO_3-N	mg/l	-	-	-	-	0.5-8.
Pb	mg/l	-	-	-	-	0.05-0.16
pH		-	8.26-8.33	7.9-9.6	8.5	7.20-8.81
Se	mg/l	-	<0.001-0.0022	-	-	<0.005
Si	mg/l	-	-	-	-	11.0-20.7
SO_4	mg/l	4,450.-90,000.	57.8-6600.	5600.-16,800.	0.4-496.7	9.-128.
TDS	mg/l	-	-	10,800.-29,700.	-	460.-1200.
TOC	mg/l	-	2.0-11.3	-	-	-
Zn	mg/l	-	0.025-0.056	-	-	0.02-0.15

Table 3. Hydraulic properties of spent shale and natural soil.

Media	Permeability cm/sec	Porosity	Bulk Density (g/cc)	Solids Density (g/cc)	Source
TOSCO Spent Shale					
Loose fill	1.9×10^{-5}	0.47	1.30	2.49	1
Compacted	$2.5\text{-}3.2 \times 10^{-7}$	0.32-0.41	-	-	2
Paraho Spent Shale					
Compacted	$10^{-2}\text{-}10^{-7}$	0.31-0.41	-	-	2
Lightly compacted	2×10^{-4}	-	1.47	-	3
Highly compacted	$3\text{-}6 \times 10^{-7}$	-	1.65	-	3
USBM Spent Shale					
Loose fill	2.6×10^{-4}	0.41	1.44	2.46	1
Union Oil Company Retorting Process Spent Shale					
Loose fill	-	0.33	1.80	2.71	1
Gravel	>1.0	-	-	-	4
Sand	$1.0\text{-}10^{-3}$	0.30-0.46	1.44-1.86	-	4
Silt	$10^{-3}\text{-}10^{-5}$	0.50	1.37	-	4
Clay	$<10^{-5}$	0.37-0.84	0.43-1.70	-	4

Sources: 1. Ward (1971b) (calculated at 10°C); 2. Bloomfield and Steward (1981); 3. Heistand (1981); 4. Dunn et al. (1980).

to hold the shale and leachate water. On top of the spent shale piles, a layer of coarse gravel-like material may be used to separate the spent shale from the topsoil. The top layer will be made up of the returned top soil, and possibly other material such as spent shale or soil amendments. The surface of the piles will then be revegetated.

The depth of a spent shale pile will vary according to local conditions, but may in open pit mines reach a depth of up to 300 m (Miller 1983). Disposal techniques affect a spent shale's porosity, permeability, and water content. It is possible that in a deep pile the stress will be sufficient to compress the spent shale, reducing porosity, to the point of saturation. Water will be added to the shale pile for dust control, to aid compaction, and as a result of precipitation.

The climate of the Utah and Colorado oil shale develpment areas is typical of a high desert environment. Annual precipitation in the vicinity of the Utah oil shale tracts is approximately 25 cm and potential evaporation approximately 120 cm. The Piceance Basin of Colorado is somewhat wetter with an annual precipitation of approximately 43 cm per year with approximately 99 cm of potential evaporation (Maase 1980). The amount of infiltration into spent shale piles is unknown. Efforts to reduce infiltration, such as barriers between the top soil and the shale and revegetation, will have some effect but will not entirely stop infiltration.

Mutagenicity Testing

The Ames test (Ames Salmonella microsome test) as described by Ames et al. (1975) and Ames (1981) is a fast, relatively inexpensive biological method for determining chemical mutagens. McCann and Ames (1976) found that 90 percent of 174 known carcinogens exhibited a mutagenic response in the Ames test.

In the Ames test, a strain of Salmonella bacteria without the ability to synthesize histidine, but in which a single mutation will allow histidine synthesis, is grown on a histidine poor medium. A number of the bacteria gain the ability to synthesize histidine by mutation (revertants). The resulting histidine independent colonies, which can be counted, represent the number of mutants. The addition of any chemicals which increase the mutation rate cause more revertant colonies to develop. The revertant ratio is the ratio of colonies which synthesize histidine in the treated culture to the control culture. A revertant ratio of two or more is an indication of mutagenicity (Yamasaki and Ames 1977). The Ames test is normally conducted with five different strains, both with and without microsomal enzyme activation (usually obtained from rat liver homogenate called S-9). The strains TA98, TA100, TA1535, TA1537 and TA1538 have been recommended for mutagen screening (Ames et al. 1975; and Claxton 1980). During 1982, a new strain TA97 became available and has been recommended as a replacement for TA1537 (Levin et al. 1982).

Five different tester strains are used to detect the different types of mutagens. The two types of mutation examined are base-pair substitutions and frameshifts. Base-pair substitution occurs when one nucleotide in the deoxyribonucleic acid (DNA) chain is substituted for another and changes the opposite nucleotide in the double helix. Tester strains TA100 and TA1535 mutate by this mechanism. Frameshift mutations are the result of a base-pair deletion or addition which shifts the entire DNA nucleotide sequence. Tester strains TA97, TA98, TA1537, and TA1538 mutate by this mechanism. The more subtle differences among mutation mechanisms require use of all five tester strains in a screening program (Hoffmann 1982).

Carcinogenicity and Mutagenicity of Oil Shale

Some potentially carcinogenic and mutagenic compounds have been identified in spent oil shale (Coomes 1976; Maase 1980; Maase et al. 1979; and Schmidt-Collerus et al. 1976). In their study, Schmidt-Collerus et al. (1976) found that roughly half of the benzene extractable compounds in spent oil shale were no longer benzene extractable after water leaching. They concluded that these compounds may, therefore, be leached from the spent shale. Maase (1980) found fluoranthene, benz(a)anthracene, and benzo(a)pyrene in a spent shale water leachate. In a companion study using the same extracts, Dickson and Adams (1980) found these compounds to be mutagenic. The concentration of these compounds in the leachate was found to be at detection limits and considerably below the compounds solubilities. Dickson and Adams (1980) were unable to detect mutagenicity in the organic fractions concentrated from the leachate water.

Much research has been done on the carcinogenicity and mutagenicity of shale oil, spent oil shale, and oil shale wastewaters. Work by Barkley et al. (1979), Eppler et al. (1978a, 1978b), Holland et al. (1979), Pelroy and Petersen (1979), Rao et al. (1979), and Stroud (1979) established the mutagenicity and carcinogenicity of shale oil. Rao et al. (1979 and 1981) have shown in situ product water and in situ groundwater from a simulated MIS retort to be mutagenic. Chen and Strinste (1982) and Strinste et al. (1981, 1982, 1983) investigated the mutagenicity of above ground oil shale retort water and found mutagenicity using both the Ames test and mammalian cells. Schmidt-Collerus et al. (1976) and Maase (1980) have shown carcinogenic compounds to be present in spent oil shale and in the leachate waters from the spent oil shale. Dickson and Adams (1980) tested extracts obtained from organic solvent soxhlet extractions of spent oil shale with the Ames test. They tested four different spent shales; two Paraho spent shales, a Union process spent shale, and a TOSCO II spent shale. All four exhibited mutagenic activity. Coomes (1976) exposed mice directly to TOSCO II spent shale used as bedding material. No significant carcinogenic response was found. Coomes (1976) did not report the quantities of shale to which the mice were exposed. In contrast to the bedding material, relatively small quantities of water can contact large quantities of spent oil shale, and if mutagens are solubilized, they can become concentrated.

Most of the work involving carcinogens and mutations in spent shale has focused on polycyclic aromatic hydrocarbons (PAHs), especially B(a)P. Coomes (1976), Schmidt-Collerus et al. (1976), and Maase (1980) all identified various PAHs in spent oil shale. Maase (1980) reported the presence of fluoranthene, benz(a)anthracene, perylene, B(a)P, triphenylene, benzo(ghi)perylene, 7,12 dimethylbenzo(a)anthracene, and dibenzanthracene, and aminopyrene, a primary amine of the PAH pyrene, all of which Dickson and Adams (1980) found to be mutagenic. Maase (1980) also found a trace of 2-aminofluorine, which Ames et al. (1975) have found to be mutagenic.

Dickson and Adams (1980) used the extracts prepared by Maase (1980) to perform Ames tests. Most of the samples were developed by soxhlet extraction of spent oil shale using some combination of benzene and methanol as solvents. The extracts were evaporated to dryness and redissolved in methanol. Extracts in methanol were then Ames tested. The extract used represented up to 8 grams of spent shale per plate.

Table 4 is a summary of the mutagenic compounds found in spent shale by Maase (1980) and Coomes (1979), the concentration per plate of these compounds in Dickson and Adams' (1980) Ames tests of the extracts, and the concen-

Table 4. Concentrations of mutagens found in dry spent shale and required concentrations of the compounds for a mutagenic response in the Ames test.

Compound	Concentration[a] in Spent Oil Shale (μg/kg)		Concentration[b] Ames Tested μg/plate		Concentration Required[c] for an Ames Test Revertant Ratio of Two or More	
	Max	Mean	Max	Mean	μg/plate	Salmonella Strain
2-Aminofluorene	Trace	-	-	-	10	TA1538, TA98, TA100
Fluoranthene	85	22	0.68	0.18	5-15	TA1537, TA98, TA100
Aminopyrene	Trace	-	-	-	-	-
Triphenylene Benz(a)anthracene Chrysene	54	10	0.43	0.08	15	TA1535
Benzo(e)pyrene Perylene Benzo(a)pyrene	56	1	0.45	0.008	1-5	TA1537, TA98
7/12 dimethyl-benzo(a)anthracene	Trace	-	-	-	-	TA1537, TA1538, TA98, TA100
Benzo(g,h,i)perylene	Trace	-	-	-	-	TA1537, TA98, TA100
Dibenz(a,h)anthracene	Trace	-	-	-	-	TA1537, TA100

[a]From Maase (1980).

[b]This is the amount of these compounds per plate used by Dickson and Adams (1980) in the soxhlet extracts based on Maase's (1980) data and 8 g of shale per plate.

[c]2-Aminofluorene from Ames et al. (1975). Other data from Dickson and Adams (1980).

trations of these compounds required for a mutagenic response in the Ames test. Dickson and Adams (1980) also tested mixtures of the various compounds found in the oil shale. No combination was found to be additive or synergistic. It can be seen in Table 4 that none of the identified mutagens was present in sufficient concentration in spent shale to cause a mutagenic response in the Ames test at the concentration Dickson and Adams (1980) tested. Even the shales with the highest concentrations of B(a)P, probably did not have sufficient B(a)P present to explain the observed response. Since all shales showed a mutagenic response, it is likely that compounds other than those identified were at least partially responsible.

Schmidt-Collerus et al. (1976) applied a benezene extract of water-leached salts of spent shale to a thin layer chromatography (TLC) plate. In addition to the PAH band, a strong azarine band was found. They estimated 20 percent of the benzene extractable organics in spent oil shale were removed by a water leachate. Maase (1980) found that only 0.3 to 10 percent of the benzene extractable PAHs were water extractable. Fox et al. (1980b) found that spent shale used as an adsorption column was able to remove up to 66 percent of the organic carbon from oil shale retort water. The retort waters used had very high organic carbon concentrations varying from 915 to 3300 mg/l. Fox et al. (1980b) also showed that the spent shale was most effective at adsorbing non-polar compounds. Since PAH compounds are relatively nonpolar, they are not likely to be easily released to a water leachate.

Coomes (1979), working with three TOSCO II samples, found somewhat different concentrations of the PAH compounds than did Maase (1980). Table 5 summarizes the concentrations of these PAHs in TOSCO II spent shale and the dose required for a revertant ratio of 2.0 or greater in the Ames test.

Clark et al. (1980) and Guerin et al. (1981), working with two crude shale oil samples, found that polycyclic aromatic primary amines are responsible for roughly half the mutagenic activity of the shale oil. It logically follows that compounds found in shale oil may be found in spent oil shale.

Addition of an amine increases the polarity and solubility of an organic compound. The solubility of benzene is 0.07 g per 100 g of water (CRC 1972); however, the solubility of aniline in water is 3.7 g per 100 g (Morrison and Boyd 1972), which is greater than a 50-fold increase. The ionized salt of an amine is much more soluble than the unionized amine. The K_B's of most aromatic amines are quite low (Morrison and Boyd 1972), indicating that they will probably not be ionized. For each unit pH change, the amine group will undergo a 10 fold ionization change, affecting its solubility.

It is difficult to estimate the solubility of the polycyclic aromatic amines present in spent oil shale. However their solubilities are likely to be higher than those found for the PAH compound without an amine group. Large polycyclic compounds will be less affected by the presence of an amine group. The solubilities of any polycyclic aromatic amines may be pH dependent due to ionization of the amine group. At the high pH of most oil shale leachates, these compounds would be less soluble, however, pH differences may still have some effect on solubility.

Carbonate minerals present in raw shale decompose to oxides and hydroxides to varying extents during retorting (Wildung and Zachara 1981). The degree of carbonate destruction affects the pH of the leachate from a spent shale. Values ranging from a pH of about 8 to over 12 have been reported. Spent shale which is exposed to air may

Table 5. Concentrations of known mutagens found in TOSCO II spent shale and resulting spent shale and sample sizes necessary for a positive Ames test of results.

	Concentration in[a] TOSCO II Spent Shale (μg/kg)	Spent Shale Required[b] for a Revertant Ratio of 2.0 or Greater (g/plate)
Fluoranthene	0 - 33	152
Triphenylene	8 - 22	
Benz(a)anthracene	10 - 13	211
Chrysene	25 - 36	
Benzo(e)pyrene	18 - 29	
Perylene	3 - 9	11
Benzo(a)pyrene	28 - 55	
Benzo(ghi)perylene	12 - 24	24

[a]From Coomes (1979).

[b]This was calculated using the maximum level of the PAH found in any sample and the minimum dose required for a revertant ratio of 2.0 or greater for any salmonella strain as reported by Dickson and Adams (1980).

recarbonate (Stollenwerk and Runnells 1981), reducing the pH of the leachate. The lower pH and resulting different leachate composition may affect the mobility of the potential mutagens in the spent shale. PAH compounds such as B(a)P, with no functional groups, will probably not be affected. If mutagenic compounds such as polycyclic aromatic amines are present in the spent shale, recarbonation could affect their solubility.

In general, as the number of rings in the PAH compounds tested by Dickson and Adams (1980) increased, the solubility decreased and the mutagenicity increased (Table 6). The more soluble, smaller PAH compounds, though present at concentrations much below their solubility (Maase 1980), were present in the aqueous leachate at higher concentrations than were the larger PAHs. Only the highly insoluble five ring compounds were present in concentrations near their solubility. These aqueous leachates were developed in a variety of ways by Maase (1980). The shale to water ratio was approximately 1:2 by weight. It can be seen in Table 6 that these compounds are present in spent shale in sufficient quantities to saturate the leachate water, but the PAH compounds apparently have a higher affinity for spent shale than they do for water.

The kerogen matrix in Green River oil shale permeates the entire rock (Yen 1976). Retorting and removal of the kerogen results in a very porous spent shale. Ramirez and Morelli (1981) found the average radius of internal pores in TOSCO spent shale to be approximately 1.0 micron. The surface area of retorted shale is approximately two orders of magnitude larger than for equivalent diameter spheres, and is

Table 6. Relative mutagenicity, solubility, concentrations in aqueous leachate developed from spent oil shale (1:2 weight ratio of spent shale to water), concentration in spent oil shale, and number of rings for selected PAH compounds.

	Revertant Ratio[a]		Solubility[b] (µg/l)	Concentration[c] in an Aqueous Leachate of Spent Shale (µg/l)	Concentration[c] in Spent Oil Shale (µg/kg)		Number of Rings
	TA1537	TA98			Max	Mean	
Anthracene	1.0	1.0	73.	-	62	20	3
Phenanthrene	1.0	1.0	1129.	1.0	483	165	3
Pyrene	1.0	1.0	135.	1.0	97	34	4
Fluoranthene	2.53	2.32	260	1.0	85	22	4
Benz(a)anthracene	4.6	1.94	14	0.01 - 1.0[d]	54[d]	10[d]	4
Benz(a)pyrene	22.43	8.08	0.31	0.01 - 0.1[e]	56[e]	1.0[e]	5
Perylene	45.14	2.17	0.4				5

[a]From Dickson and Adams (1980).
[b]From Futoma et al. (1981).
[c]From Maase (1980).
[d]This is the combined concentrations of benz(a)anthracene, chrysene, and triphenylene.
[e]This is the combined concentrations of benzo(a)pyrene, benzo(e)pyrene, and perylene.

usually independent of particle size (Fox et al. 1980a). Above ground retorting removes approximately 70 percent of the organic carbon in the shale leaving the spent shale with a residue of 1 to 5 percent organic carbon.

Shale Leaching Dynamics

The leaching of solutes from shale has been quantitatively described by the classical one-dimensional advective-dispersive equation (Hall 1982):

$$\frac{\partial C}{\partial t} = D \frac{\partial^2 C}{\partial x^2} - v \frac{\partial C}{\partial X} - R - \rho/mn \frac{\partial S}{\partial t} \qquad (1)$$

in which

- C = concentration (M)
- t = time (t)
- X = distance in direction of flow (L)
- D = dispersion coefficient (L^2/t)
- v = interstitial fluid velocity (L/t)
- R = chemical or biological reaction rate (M/L^3t)
- ρ = solids density (M/L^3)
- m = ratio of macro-pore to micro-pore volumes (dimensionless)
- n = porosity (dimensionless)
- S = mass of solute adsorbed to the solid phase (dimensionless)

The terms on the right side of Equation 1 represent dispersive and convective transport, chemical reaction, and internal mass transfer.

Dispersion is the spreading of solute due to differential interstitial velocities and molecular diffusion due to concentration gradients. Dispersion coefficients are affected by velocity and pore configurations. Relationships between mechanical dispersion coefficients and the Reynolds numbers have been developed. For example, Bouwer (1978) reports the relationship between longitudinal dispersion and Reynolds number to be:

$$D_L = 1.8\ \gamma N_R 1.205 \quad . \quad . \quad . \qquad (2)$$

where

γ = kinematic viscosity (L^2/t)

N_R = Reynolds number ($v\ D_{50}/\gamma$) (dimensionless)

In a one-dimensional system, the longitudinal dispersion is the only dispersion so D_L in Equation 2 is equivalent to D in Equation 1.

Numerous investigators have explored the internal mass transfer term. Sorption theory (Weber 1972) proposes three mechanisms for desorption of organics from the shale: 1) desorption of water soluble organic compounds from the shale surface, 2) internal diffusion within the shale pores, and 3) external diffusion from the boundary layer immediately adjacent to the shale particle to the bulk leachate. The mass transfer dynamics are usually determined by the slowest process.

Amy et al. (1980) investigated TOC leaching dynamics from several simulated in situ retorted spent oil shales. It was found that for a combustion retorted shale, internal diffusion was the rate limiting step. The controlling mechanism was not as clear for the inert gas retorted shale. It appeared that initially external diffusion was limiting. After an initial leaching time of 10 hours, internal diffusion or the desorption process became the rate limiting step.

McWhorter (1980, 1981) examined the EC of leachate from raw oil shale and concluded that simple linear desorption could not explain the leachate breakthrough curve. He believed the nature of the leachate was dependent on several

mechanisms including 1) several time dependent chemical reactions, 2) internal diffusion, and 3) dispersion from micro-spaces to macro-spaces within the shale. This third mechanism results in what McWhorter calls the bi-modal nature of raw shale.

Kuo et al. (1979) studied leaching of inorganics from modified in situ spent shale. They explained their results in terms of a "shrinking-core model" in which internal diffusion is considered the rate limiting process.

The high surface area and organic content of spent shale permits the adsorption of some organic compounds. Fox et al. (1980a, 1980b) investigated using spent oil shale as a treatment medium for retort water. TOSCO II spent shale was found to remove up to 66 percent of the organic carbon or 1.3 mg of dissolved organic carbon per gram of spent shale. It was found that the amount of organic carbon sorbed by the shale was largely a function of the concentration of organic carbon but was also affected by the nature of the organic carbon. Sakaji et al. (1981a,b) found that TOSCO II spent shale preferentially adsorbs nonpolar organic constituents.

The problem of an uncontaminated groundwater or rain water entering a spent shale pile is similar. Some organic compounds, the more polar and soluble ones, will be rapidly released into the water, other nonpolar organic molecules including PAH, may be more tightly held by the spent shale. The slightly more polar primary aromatic amines may be held less tightly. This phenomenon is similar to adsorption/desorption of organic groundwater contaminates by the organic components of the soil.

The n-octanol water partition coefficient is the equilibrium distribution of an organic compound between an aqueous phase and the nonpolar organic n-octanol phase (Chiou et al. 1977). It can be related to a compound's affinity for soil and its likelihood to move with groundwater (Roberts et al. 1982), indicating that compounds which are more soluble in a nonpolar organic solvent are more likely to be adsorbed by soil or spent shale particles than are compounds which are more soluble in water.

MATERIALS AND PROCEDURES

Shale Samples

The samples used in this study were a combined coal sample, a raw shale sample, and two spent shale samples. The raw shale sample (RS) was collected in June, 1981, at the Paraho semi-works site. The RS sample from Paraho's Colorado mine was approximately 2 years old and "representative" of Paraho's 20 to 30 gallon per ton shale (Jones 1981a). The raw shale was collected in approximately fist sized (5 - 15 cm diameter) pieces and was crushed to a smaller size to increase the surface area available for contact during leaching. After crushing and a sieve analysis, the RS sample was found to have an effective particle size of 0.23 mm with a uniformity coefficient of 8.70. The approximate bulk density was 1.13 gm/cc, and the solids density of 2.20 gm/cc indicated a porosity of 0.49. Field capacity of the RS sample was approximately 10 percent.

Two spent shale samples were available for this work. The first, a Paraho spent shale (PSS), was collected at the same time as the RS sample. It was retorted in January of 1981 and placed in sealed 30 gallon drums. At the time the sample was collected, Jones (1981a) stated he believed the sample to be representative of the Paraho process Colorado retorted shale. Subsequent conversations with Jones (1981b) revealed that the shale had been subjected to higher temperatures than the Paraho process normally reaches. The Paraho spent shale was not crushed; the shale was used as collected. Fox et al. (1980a) found the surface area of Paraho spent shale was independent of particle size. This is due to extensive internal porosity of spent shale resulting from the kerogen extraction. Sieve analysis of a PSS sample showed it to have an effective particle size of 0.15 mm with a uniformity coefficient of 38.0. The approximate bulk density of the shale was 0.96 gm/cc, the solids density 2.33 gm/cc, and the porosity 0.59. Field capacity of the PSS was approximately 21 percent. The concentration of organic carbon was found to be 1.2 percent.

The second sample of spent shale was a TOSCO II process spent shale (TSS). It was retorted in November of 1981, not wet down, and sealed in 55 gallon drums after cooling (Marino 1981). Marino (1981) described the shale as a typical product from retorting Dow property oil shale in the TOSCO II Rocky Flats pilot plant. The shale was shipped to Logan and arrived 16 November 1981. Sieve analysis was difficult due to the extremely small particle size (22 percent passed through the smallest sieve, with an opening size of 0.038 mm). Extrapolation of the log probability plot of the sieve analysis data gave estimates of a 0.0036 mm for effective particle size and a uniformity coefficient of 36.6. Field capacity of the TSS sample was 26 percent. The porosity was difficult to determine; however, it appeared to be about 0.35 to 0.40.

The physical properties of the samples used in this study, porosity, and density were measured in the laboratory under loose fill conditions

with no packing. No attempt was made to simulate actual field conditions, therefore these values should only be used in intrepreting the leachate data and not projected to field conditions.

The coal sample was a composite of freshly mined coals from the Wasatch Plateau. The coal mines were located at 2100 to 2400 m elevation. Coal used in the organic soxhlet extractions had been passed through a 2.0 mm sieve. Aqueous leachates were performed on unsieved coal.

The coal samples were stored in a nitrogen atmosphere. The spent shale samples were kept sealed in the barrels in which they came. The raw shale was stored in a teflon-lined sealed barrel.

Upflow Columns

Raw shale TSS and PSS were leached in an upflow column, as shown in Figure 4. Upflow columns help prevent short circuiting (Cleave et al. 1979, Maase et al. 1975). The columns were filled to within approximately 2 cm of the overflow port with shale (approximately 2500 g). The top of the column was packed with glass wool to prevent the shale from passing out the overflow. The PSS and raw shale samples were packed dry. Because the TSS sample would not wet when the column was filled with water from the bottom, it was packed wet.

When Mok (1981) mixed PSS and reagent grade water (DDW) in a 1:4 ratio on a shaker table and measured total organic carbon (TOC), electrical conductivity (EC), and pH, he found that the mixture came to a quasi equilibrium after 48 hours. Runnells and Esmaili (1981) found that inorganic constituents of water in contact with spent and raw shales slowly increased in concentration up to a contact time of 41 days. At least 90 percent of the TDS found in the water on day 41 was present on day 7. Stollenwerk and Runnells (1981) found a slight increase in TDS to continue for up to 13 months in a TOSCO II spent shale water mixture. Seven days was the longest feasible contact time for these experiments, and it was believed this would be sufficient to approach equilibrium.

Water used to leach the columns was DDW which had been bubbled with air to insure CO_2 saturation. The water was allowed to flow at a rate which closely approximated 1/7 of a pore volume per day (about 200 ml/day). The flow rates varied slightly but were adjusted as appropriate to insure uniformity.

Initially three columns of each material, RS, TSS, and PSS, were used. The columns were sampled every day for two weeks (approximately every 1/7 pore volume for two pore volumes). The EC and pH were measured separately, and the samples were then combined to provide sufficient samples for TOC and other parameter measurements. The leaching characteristics, pH, and EC of the replicate columns were very similar. After the first two weeks, the column which had EC measurements nearest the mean was retained, and the other two columns were disassembled. The columns were then sampled twice weekly for four more weeks (approximately each 1/2 pore volume for four more pore volumes).

Extractions and Concentrations

Prior to use, all organic solvents except absolute ethanol were redistilled in glass. All laboratory work was carried out in the dark or under low light conditions. Samples in organic solvents were stored in the dark at -76°C. Water samples were stored in the dark at 4°C. Water samples used to obtain organics for Ames testing were not stored for more than 48 hours prior to extraction or condensation. All laboratory equipment was made of glass, metal, or teflon. All reusable glass-

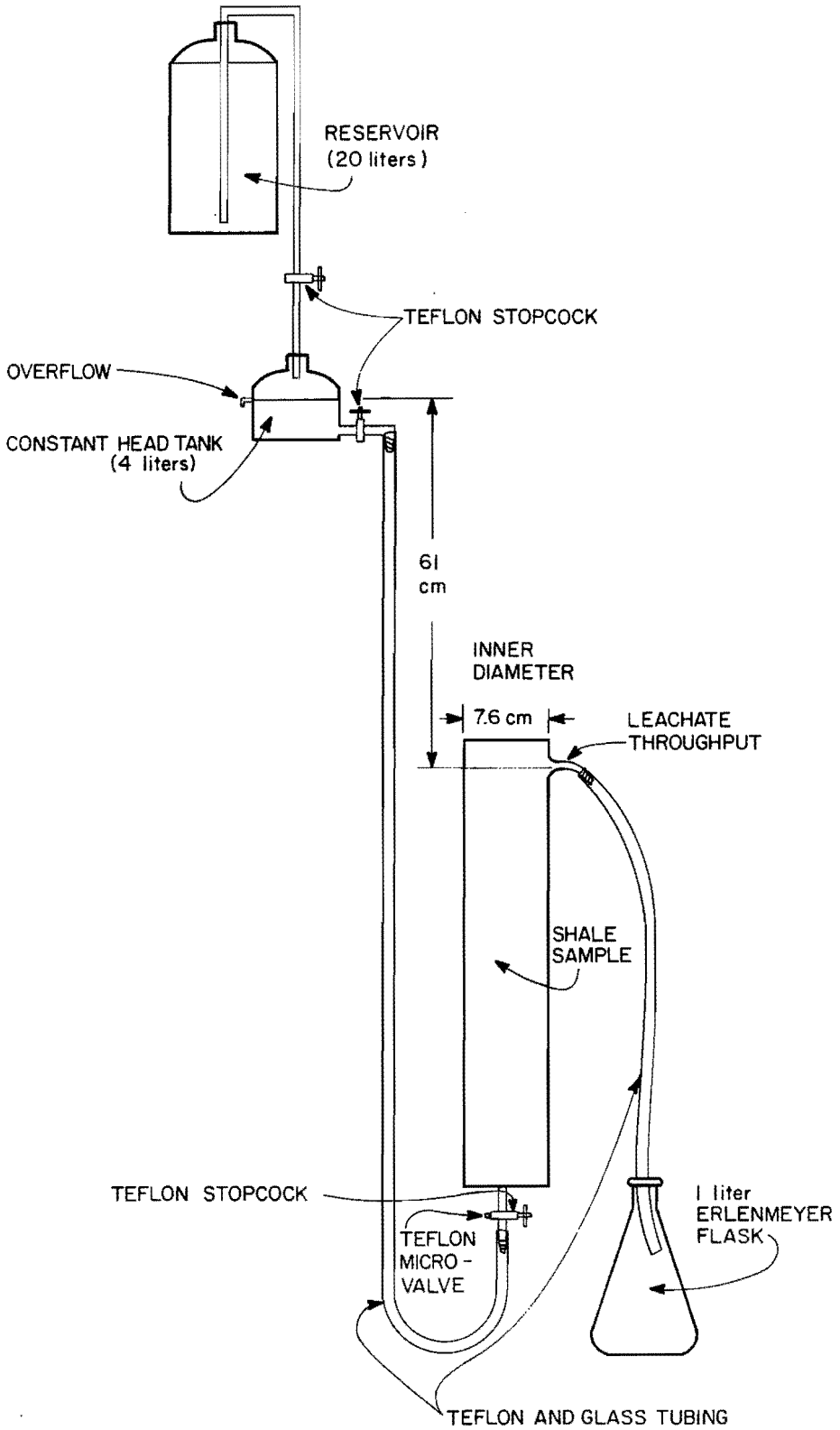

Figure 4. The up-flow column for leaching oil shale.

ware was acid washed, distilled water rinsed, and either heated for one hour at 550°C or washed in three organic solvents of increasing polarity (iso-octane, acetone, ethanol). The samples obtained in organic solvents were flash evaporated to complete dryness and redissolved in either ethanol or dymethyl sulfoxide (DMSO) for use in the Ames test.

Water Extractions

Two aqueous leaching processes were used. In the first (described by Maase 1980), shale or coal was placed in a 208 ℓ (55 gal) teflon lined drum in a ratio of 1:4 solid:water by weight. The mixture was vigorously stirred for 48 hours using a steel paddle and then allowed to settle for 24 hours at 4°C. After settling, the clarified liquid was removed for concentration. The supernatant was not filtered. The water sample was generally clear in appearance, however some slight turbidity usually remained.

The other leachate was prepared by mixing shale and water in a 2.5:1 solid:water by weight. The mixture was stirred vigorously by hand, until the shale was thoroughly wetted, in a teflon lined 104 ℓ (27.5 gal) barrel. After sitting 24 hours, the mixture was again thoroughly hand stirred. At the end of 48 hours the mixture was once more manually stirred and allowed to slowly drain for 24 hours.

The 1:4 leachate generally yielded more than 80 percent of the water originally added. The 2.5:1 generally yielded less than 10 percent because the 2.5:1 ratio is only slightly above the field capacity of the shale. As a result large quantities of shale were required to produce 2.5:1 leachate, more than 25 kg per liter of leachate. The 2.5:1 leachate was only prepared from the TSS sample.

Numerous methods have been used to extract mutagens from water for Ames testing. The most common method is the use of XAD resin columns (Cheh et al. 1980; Douglas et al. 1980; Honer et al. 1980; Tabor et al. 1980; Rao et al. 1981; and Dickson et al. 1979). The process was described in detail by Junk et al. (1974) and Glaze et al. (1977). The method involves passing water containing organic impurities over an XAD micromolecular resin. The resin is then eluted with an organic solvent. Junk et al. (1974), working with XAD-2, found the technique recovered an average of 78 percent of 110 different organic compounds tested. The concentration of the compounds was in the 10 to 100 µg/l level. High recoveries were found for polynuclear aromatic compounds, organic alcohols, aldehydes, ketones, organic nitrogen compounds, and halogenated organics. Stephan and Smith (1977) have shown that XAD-2 is more efficient at removing non-polar compounds and XAD-7 is more efficient for removing polar compounds. Yamasaki and Ames (1977) successfully isolated mutagens from urine using XAD-2. XAD extraction is the most common technique identified in the literature for concentrating mutagens from drinking water. However, the XAD resins are very selective. Kool et al. (1981a) found that although XAD extraction was very efficient at removing mutagens from Rhine River water, it did not have a measurable effect on the total organic carbon in the sample.

XAD-2 was used to concentrate the 1:4 leachate from all the solid samples. The resin was purified by repeated soxhlet extraction, consecutively, for 16 hours each in methanol, diethylether, acetonitril, and methanol again. The resin was stored at room temperature in methanol.

The 1:4 leachate was passed through 20 cm^3 of XAD-2 resin in a 25 x 1.5 cm column at a flow rate of approximately 30 ml/min. The adsorbed organic material was then eluted with the solvent used in the Ames test, usually dimethyl

sulfoxide, DMSO. The concentration of organics generally was 4 ℓ of leachate to 1 ml of solvent (4000:1).

Cascade distillation, a process in which the volatile organics are removed from a water sample, was described by Renk et al. (1978). This technique involves a series of distillations in which the first fraction to come off is retained and the process repeated. Cascade distillation has apparently not been previously used to concentrate organics for Ames testing. It is, however, an efficient technique for isolating organic compounds which may not be isolated by other concentration or extraction processes.

Cascade distillation was used to concentrate the 1:4 leachate from all the solid samples. The resulting concentration was the same as for the XAD procedure (4000:1). The residue produced by cascade distillation is the most volatile organic component of the leachate. The cascade distillation sample was the only sample tested in water with the Ames assay. The glassware was wrapped in aluminum foil and heated to 550°C for one hour prior to use. It was assumed this was sufficient for sterilization for Ames test purposes. The samples were handled using aseptic techniques, and no contamination was detected. All the cascade distillation work was done under low light conditions.

Liquid-liquid extraction using an organic solvent is a more conventional method of extracting organic compounds from water. This technique has also been used to concentrate organics for Ames testing. Grabow et al. (1980, 1981) compared XAD extraction to liquid-liquid extraction using wastewater and found the methods to be comparable for extraction of mutagens for Ames testing. The greatest difficulty in using liquid-liquid extraction is the large volume of water involved. This technique was used to extract both 1:4 and 2.5:1 TSS leachate samples.

Lyophilization (freeze-drying) has also been used to concentrate organics for Ames testing. Kool et al. (1981a) used this technique for extracting mutagens from Rhine River water. Maase (1980) attempted to use lyophilization on oil shale leachate with little success. Schmidt-Collerus et al. (1976) used lyophilization followed by soxhlet extraction of the remaining salts to identify organic compounds in spent oil shale leachate. This technique was used on 2.5:1 TSS leachate.

Soxhlet Extractions

A soxhlet extraction method (Roberts et al. 1969) was used to develop samples from solid samples for Ames testing. A 60 x 180 mm single thickness cellulose thimble was packed with 400 g of solid sample. Approximately 1.5 ℓ of solvent was placed into the boiling flask. The heat was regulated to cause the extraction chamber to fill and siphon approximately every 30 minutes. All soxhlet extractions were done in the dark and run 48 hours providing approximately 96 solvent washes. The PSS sample was extracted with ethanol, methanol, benzene, and a (1:1) methanol benzene mix. The TSS sample was extracted using ethanol, benzene, and a benzene-methanol mixture. The raw shale and coal samples were extracted using only ethanol. Absolute ethanol (100 percent) was used at the beginning of the extractions, to minimize salt leaching. It is probable that the ethanol picked up some water in the extraction process. After the initial testing, larger quantities of TSS ethanol extract were developed by using the same solvent for two 400 g fillings (48 hours each) of the soxhlet.

The soxhlet extraction samples were concentrated using a rotating flash evaporator (Buchler Instruments) in a 500 ml flask. Dickson and Adams (1980) compared flash-evaporation to a Kuderna-Danish heat evaporation technique for

concentrating spent shale extract for Ames testing. They concluded the techniques had little or no effect on the concentration and activity of the chemical mutagen. The samples were evaporated to dryness and redissolved in either DMSO or ethanol for Ames testing. The only exception was the methanol extract of PSS which was redissolved in methanol.

The extracts were concentrated to a residue representing 100 g of original solid sample per ml of concentrate. In some of the residues, noticeable particulates were present and removed by centrifugation. The supernatant liquid was decanted and retained. Samples were stored in the dark in teflon or aluminum foil capped vials at -76°C. All of the soxhlet extraction work was carried out in the dark or under low light conditions to prevent photooxidation.

Mutagenicity Testing

The salmonella/mammalian-microsome mutagenicity test or Ames test (Ames et al. 1975; Ames 1981; and Dickson 1980) was used for mutagenicity testing. The initial screening was carried out using the tester strains TA98, TA100, TA1535, TA1537, and TA1538 (provided by Professor B. N. Ames, U. C. Berkeley). A new strain, TA97 became available during the course of the study. In the interest of continuity, TA1537 was retained as a tester strain, but TA97 was also used after it became available.

The microsomal enzyme fraction (S-9) was obtained by inducing male Sprague-Dawley rats with Aroclor 1254. The liver extract was prepared according to the method described by Dickson and Adams (1980). Initially the optimal S-9 concentration in the S-9 mix was determined to be 50 µl/plate using benzo(a)-pyrene and TA100. After a mutagen was identified in the spent shale, the optimal S-9 concentration was found to be 25 µl/plate for that mutagen and tester strain TA98. All earlier Ames tests with S-9 activation used 50 µl/plate of S-9. The later tests used 25 µl of S-9 per plate. The revertant ratio was not significantly lower at 50 µl/plate than 25 µl/plate (8.7 vs 8.2), indicating that the results of the studies should be comparable.

All assays were carried out using the standard plate incorporation technique. Mutagens were dissolved in DMSO, methanol, water or ethanol and 0.1 ml of the solvent was then added to the top agar. After 48 hours of incubation, the colonies were counted on an Artek Model 880 autocounter. Each test was carried out at least in triplicate and the means were reported.

The most common criterion for assigning a positive response in the Ames test is to look for a doubling in the mutation rate, a revertant ratio of 2.0. A dose-response relationship is also used to determine the significance of a mutagenic response (Claxton 1980). To insure reproducibility, samples exhibiting a mutagenic response were tested at least twice when possible.

A model for analyzing Ames test data statistically was developed by Stead et al. (1981), and a copy of the FORTRAN listing was obtained from Hasselblad et al. (1980). Attempts to make the program operational on the VAX system at Utah State University were only partially successful. A portion of the program which operated properly was used to analyze the data. The Ames test data listing in the appendix is output from this program. The program calculates the mean and standard deviation of the plate counts at each dose, and performs a linear regression on the data. A problem with Ames test data is that frequently the sample being tested is toxic as well as mutagenic. As a result, the mutation rate begins to decline at higher doses. The program handles this by discarding the data from

any dose level which is more than one standard deviation below the previous count. At least three non-zero doses are required for the program to calculate the slope. The 95 percent confidence interval about the slope is calculated from the standard error. The slope is the concentration of potential mutants per unit measure of tested material.

In this study, the criteria used to determine mutagenicity were 1) revertant ratio exceeding 2.0, 2) a positive slope of the dose-response regression line, and 3) all samples passing these two tests at least twice. That is each sample which showed mutagenicity was tested for reproducibility.

Mutagenicity was also evaluated by streaking revertant colonies on a histidine deficient minimal agar. In addition, toxicity was evaluated by stero-microscopic comparison of the background lawn on test and negative control plates.

Determining non-mutagenicity is much more difficult. Some of the data from this study meets the criteria established for assigning a negative response (Claxton 1980). However, all of the samples were not pursued this far. It was not within the scope of this study to prove non-mutagenicity. Therefore, care must be taken in interpreting the negative results of this study.

Recarbonation

In order to determine the importance of atmospheric recarbonation in affecting leachate pH, a 35 kg sample of PSS and two 35 kg samples of TSS were placed in 208 ℓ (55 gallon) teflon lined barrels with an air port in the bottom. Sufficient compressed air was forced into the barrel to fluidize approximately 10 percent of the spent shale. Every day the barrels were vigorously shaken to insure homogeneous exposure. At periodic intervals, small samples were removed and a 1:4 leachate developed on a shaker table. The leachate EC and pH were then measured. All EC values reported were adjusted to 25°C.

RESULTS AND DISCUSSION

The general approach used in this study was to first determine through column experiments the general behavior of leachable materials in the samples, then use the Ames test to determine mutagenicity of the leachates and the materials leached. The leaching mechanisms were studied, and finally the relationship between these mechanisms and potential for transport of mutagenic compounds to groundwater was examined.

Upflow Columns

EC Leaching

The electrical conductivities declined with the volume of leachate waters (measured as multiples of the pore volumes) extracted from the three shale columns as shown in Figure 5. The curves all show an exponential decline in EC with increasing pore volume, with the exception that the top segment of the TSS curve is relatively flat. This was due to the TSS column being packed wet. Reagent grade water (DDW) was allowed to equilibrate with the TSS for 7 days, then the water was allowed to flow. The first pore volume had been packed with the shale and had a relatively constant EC (a little above 14,000 μmhos/cm). Its drainage was followed by an exponential decrease in EC. The fact that the EC remained constant until the initial pore volume drained indicates that short circuiting was not occurring.

The effective surface area of oil shale is greatly increased by retorting (Fox et al. 1980a). The larger surface area provides more contact between the water and soluble salts, accounting for the higher ECs of the spent shales as compared to the raw shale. The smaller particle size also reduces the distance an ion has to diffuse to reach the water, increasing the leaching rate.

Table 7 compares the average EC measurements for the first pore volume of leachate among this study and two others. It can be seen that there is some variability in TOSCO shales and that the leachate from the TSS used in this study had a lower EC than that reported from the other TOSCO shales. The depth of the leaching column and the water velocity have some effect on EC of the first pore volume.

As seen from the last four samples in Table 7, the first pore volume EC of Paraho leachate tested by Fransway and Wagenet (1981) showed a general increase in EC with decreasing flow rate. All four have higher values than the 10,700 obtained in this study. Finally and perhaps most importantly, the leachate water quality varies according to the mineralogic make up of the shale. Although all Colorado oil shales are marlstones, their mineral contents vary widely.

Relationships between TDS and EC for spent shale leachate have been developed and are shown in Table 8. Based on an average relationship, the TDS of the first pore volumes of the PSS, TSS, and RS samples were 10,699 mg/l, 13,700 mg/l, and 1,393 mg/l respectively.

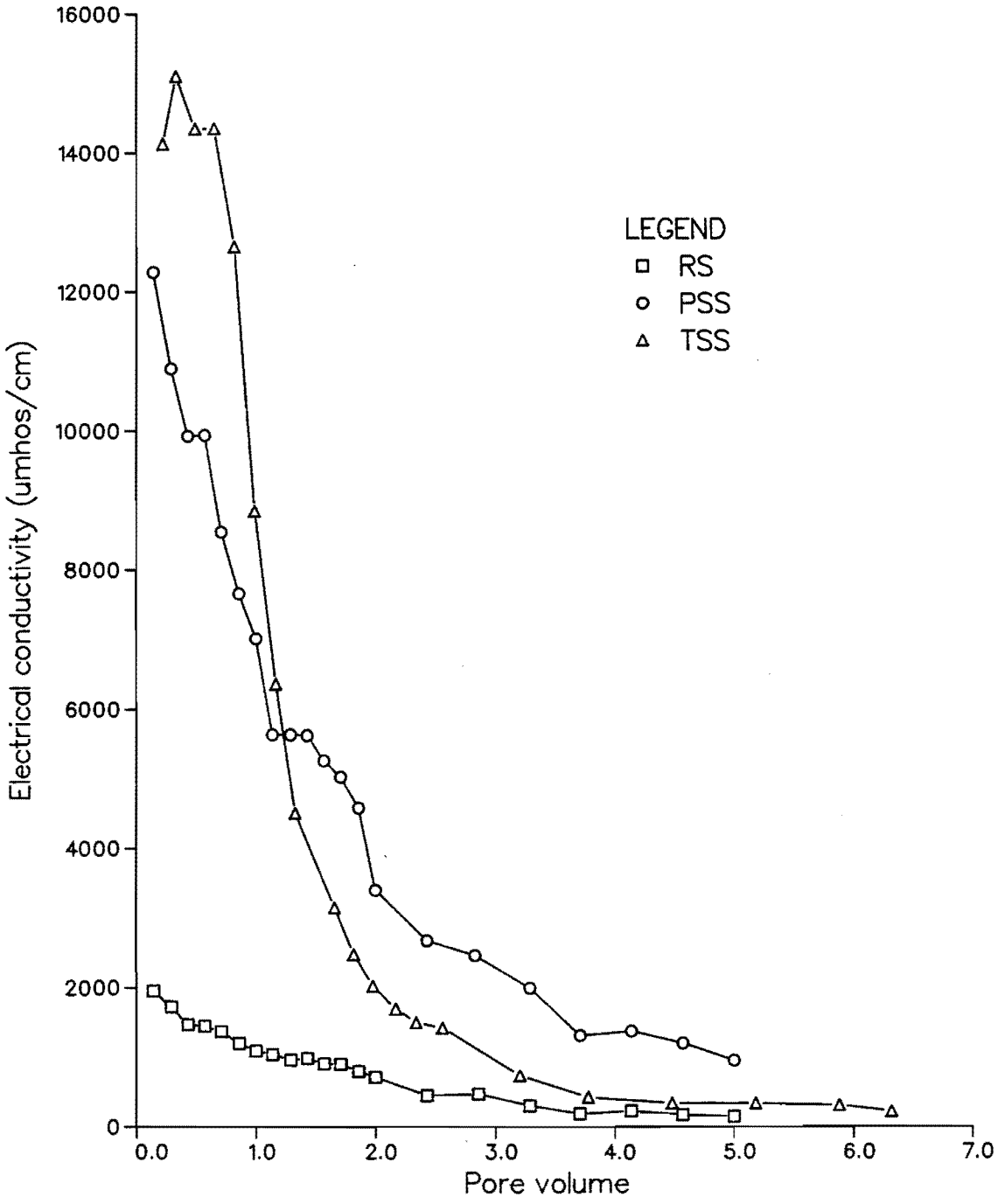

Figure 5. Change in EC (adjusted to 25°C) vs pore volume of effluent from leachate columns (detention time of 7 days).

Table 7. Electrical conductivities of various shale leachates.

Sample	EC (μmho/cm)	Flow Rate (Pore Volumes Per Day)	Column Depth (cm)	Source
RS	1,440	0.14	50	This study
PSS	10,700	0.14	50	This study
TSS	13,686	0.17	50	This study
TOSCO II[a]	19,870	NR[b]	30	Stollenwerk & Runnells
TOSCO II[a]	19,610	NR	30	(1981)
TOSCO	17,200	NR	120	Ward et al. (1971a)
Paraho[c]	21,063	0.44	60	Fransway & Wagenet (1981)
Paraho[c]	34,000	0.22	60	Fransway & Wagenet (1981)
Paraho[c]	26,875	0.15	60	Fransway & Wagenet (1981)
Paraho[c]	39,563	0.073	60	Fransway & Wagenet (1981)

[a]These TOSCO II samples were identical except the first, with the higher EC was allowed to equilibrate for 10 days followed by rapid leaching. The second sample was rapidly leached without equilibration.

[b]NR = Not reported.

[c]These Paraho samples were identical.

Table 8. Relationships between TDS and EC of several spent shales (for the first pore volume of leachate).

Equation	Shale	Source
$TDS^a = EC^b\ (0.900) - 116$	Paraho, Union B, TOSCO II	Maase (1980)
$TDS = EC\ (0.923) - 48.6$	TOSCO	Ward et al. (1971a)
$TDS = EC\ (1.193) + 1.7$	Paraho	Fransway & Wagenet (1981)[c]
$TDS = EC\ (1.005) - 54$	Average	

[a]TDS is in mg/l.

[b]EC is in μmmho/cm.

[c]Fransway and Wagenet (1981) correlated cation concentration with EC, and Ward et al. (1971a) correlated cation concentration with TDS; this TDS, EC correlation is a combination of the two equations.

TOC Leaching

Figure 6 shows the TOC as leachate waters passed through the shales. Similar exponential declines in concentration are seen with TOC as with EC. The average first pore volume TOC concentration is highest for TSS and lowest for PSS with the raw shale plotting between the two. Maase (1980) and Jackson et al. (1975) found correlations between EC or TDS and TOC. Figure 7 shows the correlations between EC and TOC for the three shale leachates examined in this study. The correlation is caused by a fairly large amount of soluble organic matter with similar leaching dynamics to the soluble inorganics. The relationship varies considerably from one shale to the next, and the regression results should not be applied to any shale other than the one for which it was developed.

Cleave et al. (1979) examined the TOC of a Paraho leachate and found very different results. For 12 pore volumes, TOC remained relatively constant declining only from 8.27 mg/l for approximately the first 1.25 pore volumes. Cathedral Bluffs Shale Oil Company (1981) found the TOC of leachate from piles of raw oil shale varied with the source of the shale. The highest TOC measured was 228 mg/l in the initial sample leached from Horse Draw Saline oil shale, after three pore volumes the TOC dropped to 11 mg/l. Several raw shale leachates were found to contain no measurable TOC. Amy et al. (1980) found the TOC of leachate from simulated in situ retorted oil shale to be high during the initial leaching period but to drop rapidly to a relatively constant TOC during leaching. The TOC of spent in situ shale leachates was in general much lower than for surface retort shale. From a high of 15-25 mg/l, the in situ leachate water rapidly dropped to about 2 mg/l TOC. Hall (1982), also working with simulated in situ spent shale, found initially that leachate TOC concentrations, varyed from 64 to 19 mg/l, decreased during the first pore volume to 17 to 2 mg/l.

Observations

Mutagenic compounds make up only a small fraction of the total TOC. The mutagenic fraction is likely to be more non-polar and, therefore, have a higher affinity for the shale than does the majority of the organics making up the TOC of the leachate water. If this is the case, the surface reaction, desorption, may be the controlling mechanism for mutagen leaching. However, surface reactions are severely complicated by competing compounds. As the shale is leached, the more soluble organic compounds are removed making sites available for remaining less soluble compounds.

Surface reactions are affected by the rapidly changing EC. Jurinak (1980) has found an approximate relationship:

$$I = (1.27 \times 10^{-5})\ EC \quad . \quad . \quad (3)$$

where

I = ionic strength (molar)

EC = electrical conductivity (μmmhos/cm) at 25°C for soil water solutions in the western United States.

Based on the values shown on Figure 5, TSS leachate would therefore decline from an initial ionic strength of approximately 0.18 to 0.004 after five pore volumes of flow had passed through the spent shale. The activity of typical nonelectrolytes, such as the mutagenic fraction in oil shale may be, increases with increasing ionic strength resulting in "salting-out" (Moore 1972). Therefore, as the salts are leached, nonelectrolytes may become more soluble.

Mutagenicity Testing

Coal

Leachate (1:4) was extracted from the coal by using both the XAD and

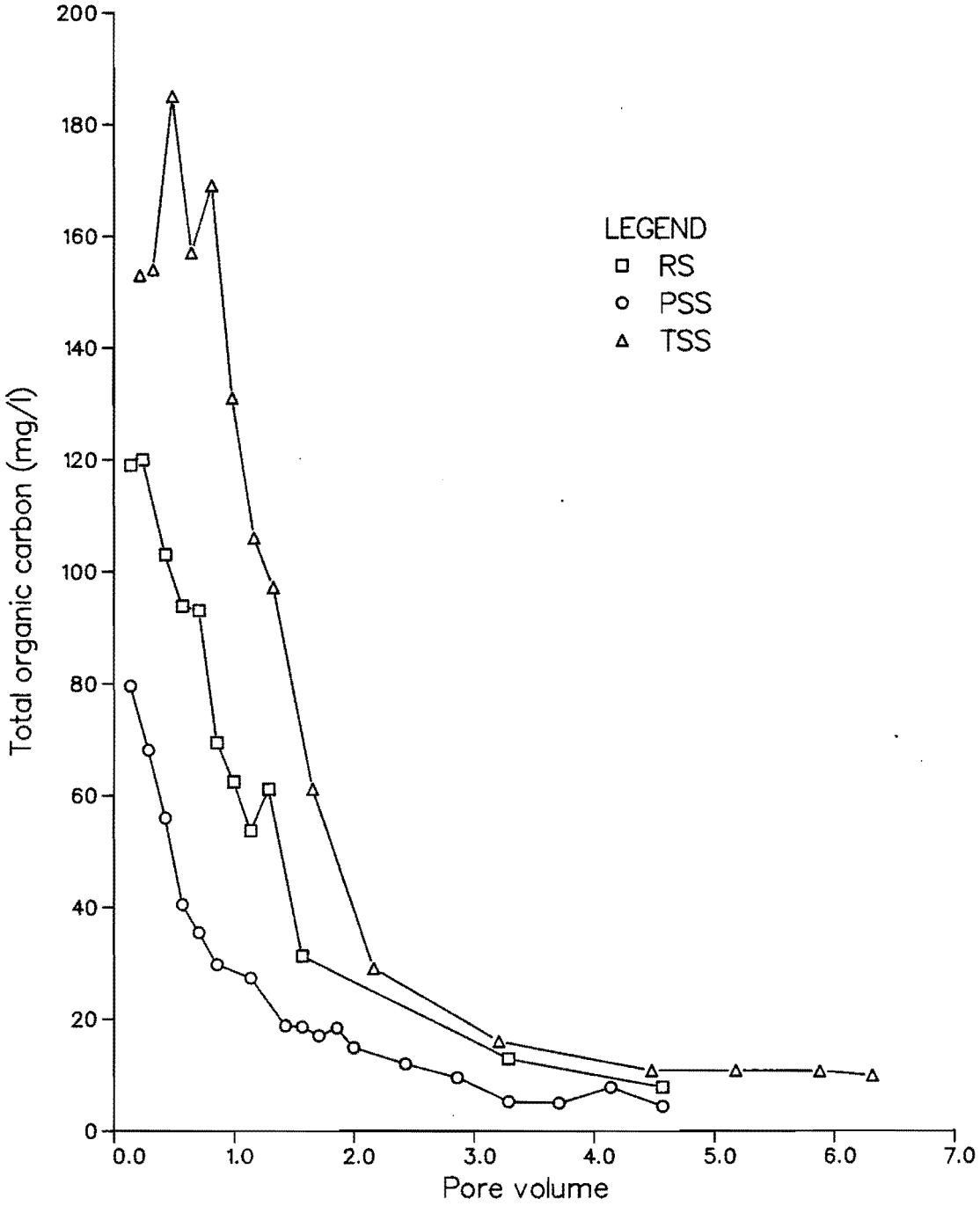

Figure 6. Change in TOC vs pore volume of effluent from leachate columns (detention time of 7 days).

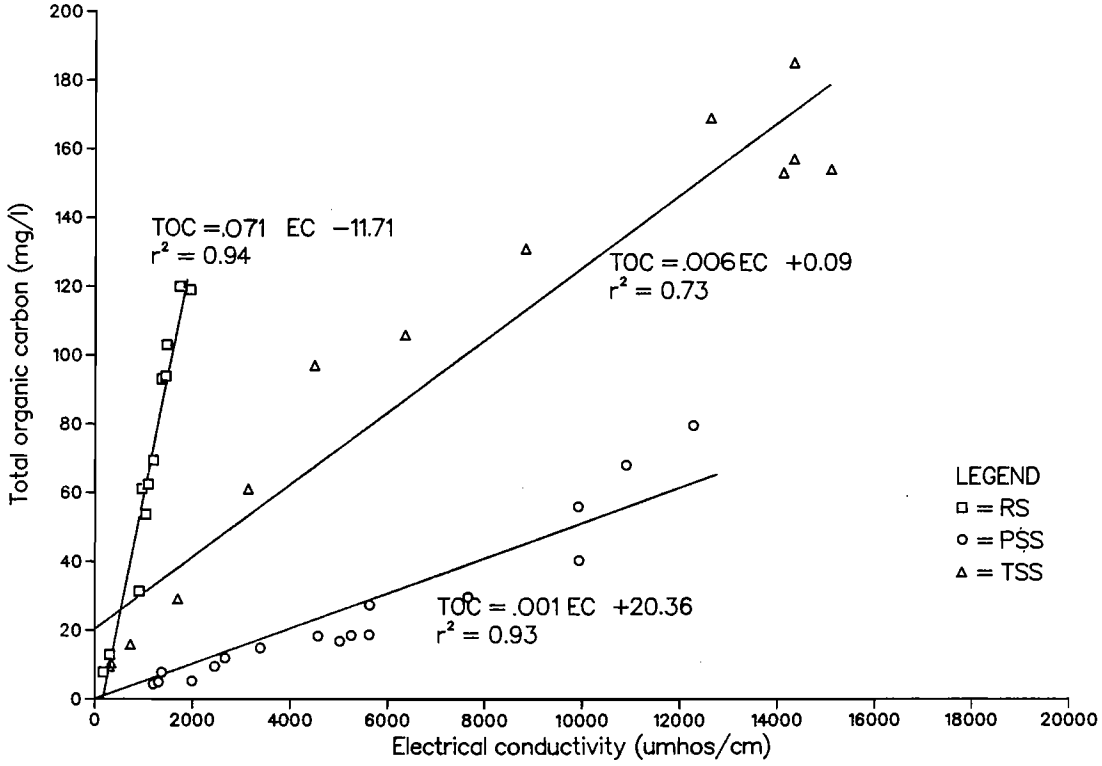

Figure 7. Relationships between TOC and EC of effluent from leachate columns (EC adjusted to 25°C).

cascade techniques. Both extracts were Ames tested with all five strains with the results shown in Table 9. The highest concentrations for each test represented 600 grams of shale per plate. The TA1538 test, of the cascade sample without S-9 activation, showed a toxic response at the highest concentration but showed no toxicity or mutagenicity at the 600 grams per plate dose. The TA1538 test, of the XAD sample without S-9 activation, showed a toxic response at all doses.

The composite coal samples from soxhlet extracts also exhibited no mutagencity, as shown in Table 10. Ethanol was used as the solvent. The resulting residue was quite thick and tarry; it did not disperse well in the top agar. Plates at the higher concentrations had visible undissolved tar particles. If these particles had contained mutagens, a ring of mutant colonies may have been visible around them. This was not the case. At all lower concentrations (1.0 and 0.1 grams per plate), the extract appeared to dissolve well in the agar.

Raw Shale

The RS aqueous leachate showed no mutagenic activity in doses up to 600 g per plate. The results are shown in Table 11. The aqueous leachate concentrates, both XAD-2 and cascade distillation, appeared to be less toxic than did those of either of the spent shales or of the coal. None of the concentrations of RS leachate extract appeared toxic to Ames test Salmonella.

The ethanol soxhlet extract also showed no mutagenic activity (Table 12).

Table 9. Mutagenicity testing results of 1:4 coal leachate concentrates.

Tester Strain	S-9 Activation	XAD Extract		Cascade Distillation Extract	
		Dose[a] (Grams)	Revertant[b] Ratio	Dose[a] (Grams)	Revertant[b] Ratio
TA98	Yes	600.	0.96	600.	1.38
TA98	No	600.	1.49	600.	1.12
TA100	Yes	600.	1.09	600.	0.81
TA100	No	600.	1.44	600.	0.80
TA1535	Yes	600.	1.51	600.	0.63
TA1535	No	600.	0.70	600.	0.91
TA1537	Yes	600.	1.11	600.	1.06
TA1537	No	600.	1.50	60.	1.32
TA1538	Yes	600.	1.69	600.	0.93
TA1538	No	Toxic at all doses		600.	0.71

[a]The dose is grams of shale extracted per plate, these are the highest non-toxic doses tested.

[b]Revertant ratio with values exceeding 2.0 indicates mutagenicity.

Table 10. Mutagenicity testing results of coal ethanol Soxhlet extracts.

Tester Strain	S-9 Activation	Dose[a] (Grams)	Revertant[b] Ratio
TA97	Yes	10.0	1.17
TA97	No	10.0	1.50
TA98	Yes	10.0	1.00
TA98	No	10.0	0.93
TA100	Yes	10.0	1.30
TA100	No	1.0	0.82
TA1535	Yes	1.0	0.70
TA1535	No	1.0	0.91
TA1537	Yes	1.0	0.86
TA1537	No	10.0	0.86
TA1538	Yes	0.1	0.88
TA1538	No	0.1	0.89

[a]The dose is grams of shale extracted per plate, these are the highest non-toxic doses tested.

[b]Revertant ratio with values exceeding 2.0 indicates mutagenicity.

Table 11. Mutagenicity testing results of 1:4 RS leachate concentrates.

Tester Strain	S-9 Activation	XAD Extract		Cascade Distillation Extract	
		Dose[a] (Grams)	Revertant[b] Ratio	Dose[a] (Grams)	Revertant[b] Ratio
TA98	Yes	600.	0.98	600.	1.59
TA98	No	600.	0.98	600.	1.32
TA100	Yes	600.	1.18	600.	0.81
TA100	No	600.	1.31	600.	0.73
TA1535	Yes	600.	0.99	600.	0.75
TA1535	No	600.	1.12	600.	0.71
TA1537	Yes	600.	1.14	600.	1.41
TA1537	No	600.	0.84	600.	1.40
TA1538	Yes	600.	1.17	600.	0.97
TA1538	No	600.	0.83	600.	1.13

[a]The dose is grams of shale extracted per plate, these are the highest non-toxic doses tested.

[b]Revertant ratio with values exceeding 2.0 indicates mutagenicity.

Table 12. Mutagenicity testing results of RS ethanol soxhlet extracts.

Tester Strain	S-9 Activation	Dose[a] (Grams)	Revertant[b] Ratio
TA97	Yes	10.	0.78
TA97	No	10.	0.47
TA98	Yes	1.0	1.95
TA98	No	1.0	1.40
TA100	Yes	10.	1.18
TA100	No	1.0	0.88
TA1535	Yes	1.0	1.69
TA1535	No	1.0	1.75
TA1537	Yes	1.0	0.95
TA1537	No	0.1	1.50
TA1538	Yes	Toxic at all doses	
TA1538	No	Toxic at all doses	

[a]The dose is grams of shale extracted per plate, these are the highest non-toxic doses tested.

[b]Revertant ratio with values exceeding 2.0 indicates mutagenicity.

It was toxic at 10 g per plate to strains TA98, TA100 without S-9, TA1535, and TA1537 without S-9. It was toxic at the 10 and 1 g per plate to TA1538 without S-9. RS ethanol extract was toxic to TA1538 at all doses. RS soxhlet concentrate was difficult to work with. It had a tar-like light brown appearance and was very difficult to dissolve into the top agar used in the Ames test.

Rao et al. (1979) found a benzene soxhlet extract of raw oil shale mutagenic to both TA98 and TA100 with S-9 activation. Neither the concentration nor the source of raw shale is mentioned. Raw shales are highly variable, and the apparent discrepancy between the Rao et al. study and this one serves to point out the difficulty in dealing with oil shales.

Paraho Spent Shale

The PSS aqueous leachates showed no mutagenic activity in doses up to 900 g per plate (Table 13). Many of these tests were repeated numerous times, although an occasional mutagenic response was observed, none were reproducible. Revertant ratios shown in the tables are typical values. Complete test data are in the appendices.

The PSS sample was subjected to extensive soxhlet extraction and Ames testing. Extractions were made with ethanol, methanol, benzene and a methanol/benzene mixture. Results are shown in Table 14. None of the extracts tested resulted in a mutagenic response. The benzene extract was toxic at most higher doses in the Ames tests without S-9 activation. The S-9 apparently detoxified the extract.

Dickson and Adams (1980) found methanol, and benzene/methanol mix soxhlet extracts of two different Paraho spent shales mutagenic. Differences are probably attributable to higher than typical retort temperatures at which the PSS was processed.

TOSCO Spent Shale

The TSS leachate was concentrated for mutagenicity testing using XAD-2 and cascade distillation first. Concentrations representing up to 600 g of shale per plate of the 4:1 leachate showed no significant mutagenicity (Table 15).

The organic solvent soxhlet extractions of the TSS did show mutagenic activity in levels below 1 g per plate. The tester strains TA98 and TA1537 (the frameshift mutants) with S-9 activation both showed mutagenic responses. Figures 8 and 9 are dose response curves for the three solvent systems used for the TSS extracts (benzene, ethanol, and a benzene/methanol mix). Ethanol extracts generally had the highest mutagenic activity. Benzene extracts were quite often toxic to the Salmonella. This problem has also been encountered by others (Dickson 1981, and Rao et al. 1979). TA98 showed a greater mutagenic response to ethanol and benzene/methanol extracts than to benzene extract. This is probably due to wetting ability of the alcohols. If any moisture had remained in the shale, the highly hydrophobic benzene would not have penetrated the shale. Soxhlet extract with the solvent schemes using alcohols appeared much darker than benzene alone. These observations combined with Dickson and Adams' (1980) problems with methanol induced mutagenicity led to the decision to use only ethanol for further soxhlet extractions.

The units of mutagenicity, potential mutants, are an index of mutagenicity. Mutagenicity in potential mutants reflects the number of mutants which would be expected above the background mutation rate, in an Ames test. The residue recovered by ethanol soxhlet extraction of 100 g of shale weighed approximately 46 mg, and was usually concentrated into 1 ml of ethanol. Although potential mutagenicity is an index of mutagenic hazard it can be related back to mass of extract and it

Table 13. Mutagenicity testing results of 1:4 PSS leachate concentrates.

Tester Strain	S-9 Activation	XAD Extract		Cascade Distillation Extract	
		Dose[a] (Grams)	Revertant[b] Ratio	Dose[a] (Grams)	Revertant[b] Ratio
TA98	Yes	900.	1.18	600.	1.34
TA98	No	900.	1.27	600.	0.73
TA100	Yes	600.	1.70	600.	1.12
TA100	No	600.	1.20	600.	0.87
TA1535	Yes	600.	1.53	600.	0.54
TA1535	No	600.	1.01	600.	1.74
TA1537	Yes	600.	1.67	600.	1.19
TA1537	No	600.	1.48	600.	0.48
TA1538	Yes	600.	1.16	600.	0.84
TA1538	No	600.	1.95	600.	0.93

[a]The dose is grams of shale extracted per plate, these are the highest non-toxic doses tested.

[b]Revertant ratio with values exceeding 2.0 indicates mutagenicity.

Table 14. Mutagenicity testing of PSS organic solvent Soxhlet extracts.

Tester Strain	S-9 Activation	Ethanol		Methanol		Benzene		Methanol/ Benzene	
		Dose[a]	RR[b]	Dose[a]	RR[b]	Dose[a]	RR[b]	Dose[a]	RR[b]
TA98	Yes	16.00	1.07	10.0	1.35	16.00	1.53	8.00	1.68
TA98	No	16.00	1.18	10.0	0.90	0.16	0.94	8.00	1.13
TA100	Yes	16.00	1.01	10.0	0.96	16.00	0.85	8.00	1.07
TA100	No	16.00	0.95	10.0	1.00	0.16	0.94	8.00	0.41
TA1535	Yes	16.00	0.77	10.0	1.26	16.00	0.78	8.00	1.75
TA1535	No	16.00	0.84	10.0	1.09	0.16	16.67	8.00	0.45
TA1537	Yes	16.00	0.96	10.0	1.52	16.00	1.36	8.00	0.65
TA1537	No	16.00	1.00	10.0	1.10	0.16	1.88	8.00	0.74
TA1538	Yes	16.00	1.04	10.0	0.76	Toxic at all doses		8.00	1.23
TA1538	No	16.00	0.90	10.0	0.88	Toxic at all doses		8.00	0.75

[a]The dose is grams of shale extracted per plate, these are the highest non-toxic doses tested.

[b]Revertant ratio with values exceeding 2.0 indicates mutagenicity.

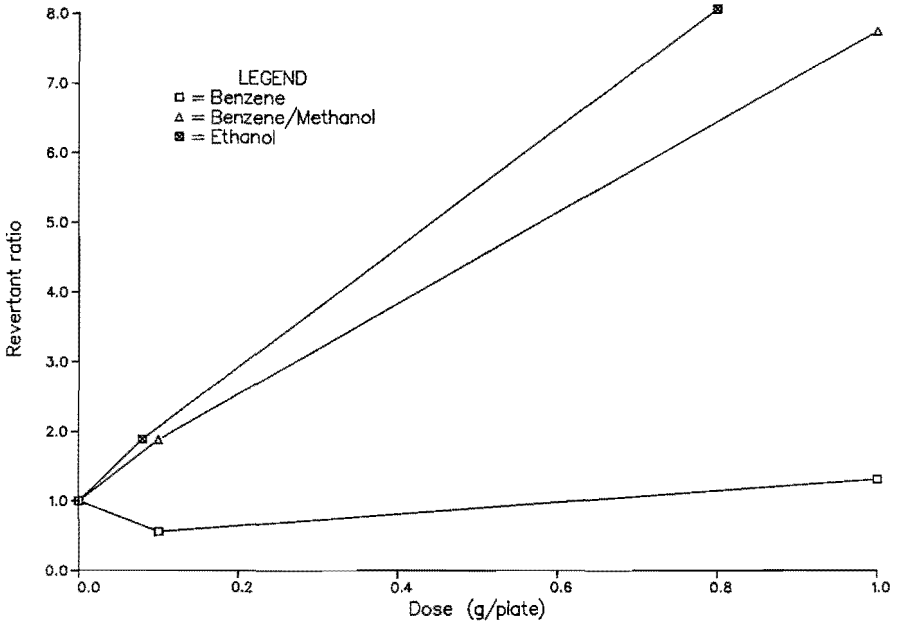

Figure 8. Dose response curves for TSS soxhlet extracts with Ames test strain TA 98 (with S-9 activation).

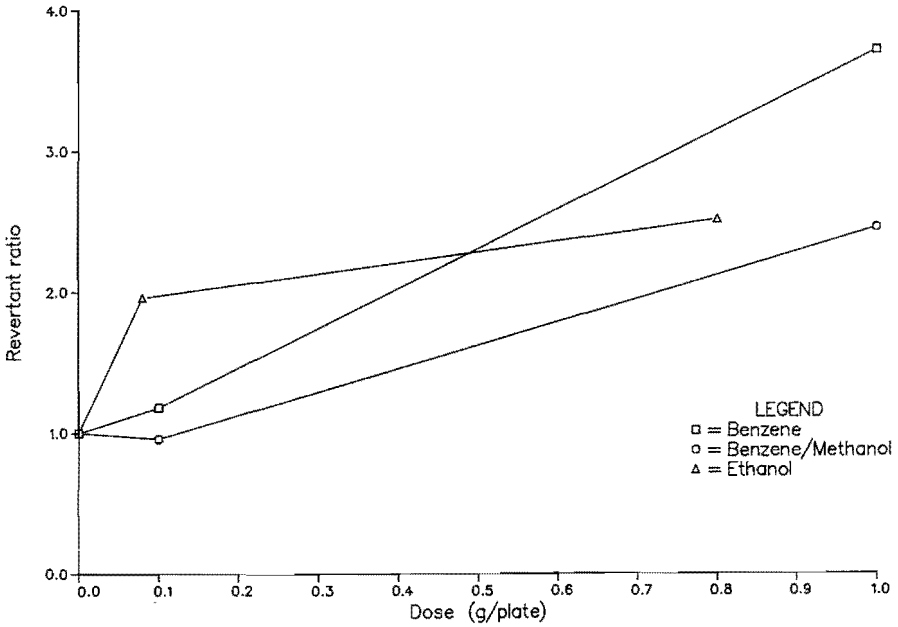

Figure 9. Dose response curves for TSS soxhlet extracts with Ames test strain TA 1537 (with S-9 activation).

Table 15. Mutagenicity testing results of 1:4 TSS leachate concentrates.

Tester Strain	S-9 Activation	XAD Extract		Cascade Distillation Extract	
		Dose[a] (Grams)	Revertant[b] Ratio	Dose[a] (Grams)	Revertant[b] Ratio
TA98	Yes	600.	1.79	600.	0.92
TA98	No	600.	1.48	600.	0.71
TA100	Yes	600.	1.70	600.	1.05
TA100	No	600.	0.96	600.	0.79
TA1535	Yes	600.	1.55	600.	1.14
TA1535	No	600.	1.00	600.	0.91
TA1537	Yes	600.	1.15	600.	0.83
TA1537	No	600.	1.11	60.	1.11
TA1538	Yes	600.	0.95	600.	0.80
TA1538	No	600.	1.26	60.	0.74

[a]The dose is grams of shale extracted per plate, these are the highest non-toxic doses tested.

[b]Revertant ratio with values exceeding 2.0 indicates mutagenicity.

will be considered here to have dimensions of mass.

Figures 10 and 11 show the first four dose response curves for the TSS ethanol extract using TA98 and TA1537, both with S-9 activation. Tester strain TA98 generally exhibited a stronger mutagenic response, and gave more reproducible results than the TA1537 strain. For this reason TA98 with S-9 activation was used for all further mutagenicity testing.

The TSS 2.5:1 leachate was concentrated using both lyophilitation and liquid/liquid extraction (isooctane). The lyophilized sample resulted in a salt cake which was soxhlet extracted with ethanol and Ames tested. The ethanol extract produced a sticky yellow substance which was tested using strains TA98, TA100, and TA1537 with S-9 activation. The residue did not show a mutagenic response in concentrations representing up to 98 g of shale per plate (Table 16).

Two liters of the 2.5:1 leachate was isooctane extracted three consecutive times using 150 ml per liter of leachate. The leachate was then acidified to pH 1.0 and extracted again. The isooctane extracts were combined and then flash evaporated. The samples were then fractionated into basic, acidic, neutral, and PAH components (using the technique described for mutagen fractionation). The dry residue was washed first with DMSO, then with ethanol. All the samples were then Ames tested using tester strains TA98, TA100, and TA1537 with S-9 activation. Ames testing of concentrations representing up to 130 g per plate resulted in no mutagenic response (Table 16).

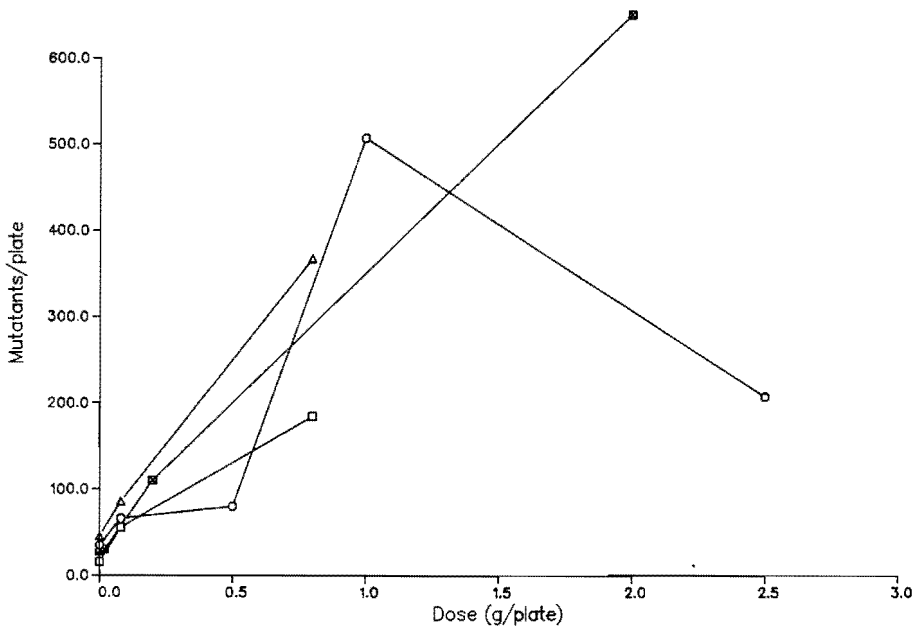

Figure 10. Dose response curves for four different Ames tests of the same TSS ethanol extracts with Ames test strain TA 98 (with S-9 activation).

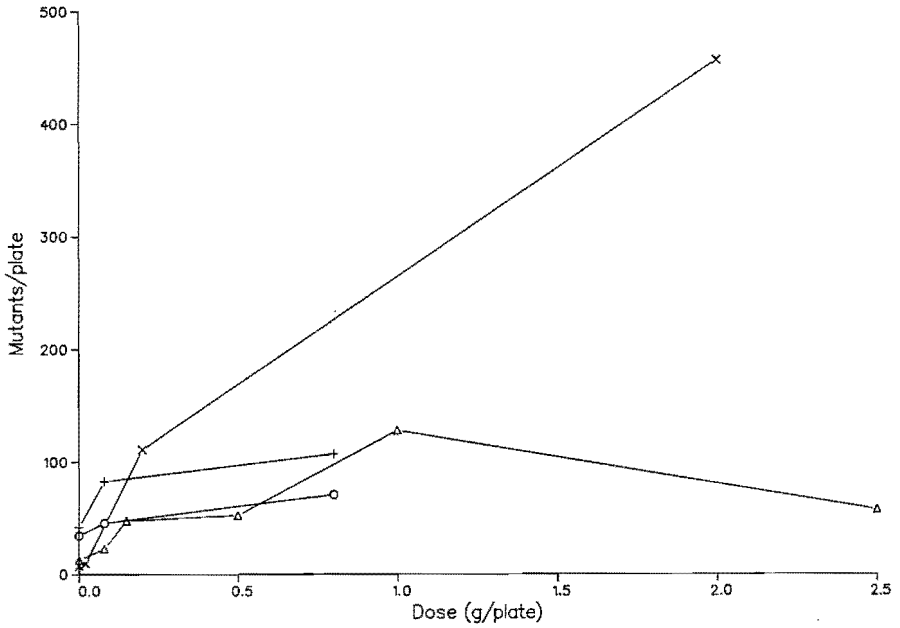

Figure 11. Dose response curves for four different Ames tests of the same TSS ethanol extracts with Ames test strain TA 1537 (with S-9 activation).

Table 16. Mutagenicity testing results of TSS 2.5:1 leachate concentrates.

Tester Strain	S-9 Activation	Lyophilized Extract		Liquid/Liquid Extract							
				Acidic[b]		Basic[b]		Neutral[b]		PAH[b]	
		Dose[a] (Grams)	Revertant[c] Ratio	Dose[a] (Grams)	Revertant[c] Ratio	Dose[a] (Grams)	Revertant[c] Ratio	Dose[a] (Grams)	Revertant[c] Ratio	Dose[a] (Grams)	Revertant[c] Ratio
TA98	Yes	98	1.41	130	1.48	130	0.91	130	1.89	71.5	1.25
TA100	Yes	98	1.09	130	0.69	130	1.04	130	0.81	13.0	0.34
TA1537	Yes	98	1.42	71.5	0.61	130	0.63	130	0.81	71.5	0.42

[a]The dose is grams of shale extracted per plate, these are the highest non-toxic doses tested.

[b]The revertant ratios shown here are for the concentrate of the ethanol wash.

[c]Revertant ratio with values exceeding 2.0 indicates mutagenicity.

Extraction efficiency. To determine the efficiency of the ethanol soxhlet extraction, a TSS sample was extracted for 48 hr. This was followed by a second 48 hr extraction of the same shale. Both extracts were tested for mutagencity using tester strains TA97, TA98, and TA1537 with S-9 activation. Figure 12 shows the results of the two tests. None of the second extractions showed significant mutagenicity. However TA98 did show a dose response with a positive slope of 34.8 mutants/g. The slope of the first extract is 455 mutants/g. If it is assumed that all of the mutant was removed by the two extractions, the first extraction removed 93 percent of the mutagenic fraction.

After a mutagenic fraction was isolated from the TSS sample, experiments were performed to determine the efficiency of the extraction techniques. To test the XAD-2 extraction efficiency 120 ℓ of DDW was spiked with mutagen extracted from 300 g of TSS. The water was then treated exactly the same as the 1:4 water to shale extraction experiments, 48 hr of mixing followed by settling overnight at 4°C. The water was then passed over XAD-2 as the leachate was, and XAD-2 eluted with DMSO. The residue was then Ames tested.

The measured concentration of potential mutagen (TA98 with S-9) was 199 potential mutants/ℓ, of the 914 potential mutants/ℓ added by spiking. This is a recovery efficiency of 22 percent. The loss may be due to a number of reasons. Some of the mutagen may volatilize while being stirred, but this amount is probably small because most PAH compounds have very low vapor pressures. A second possibility is that

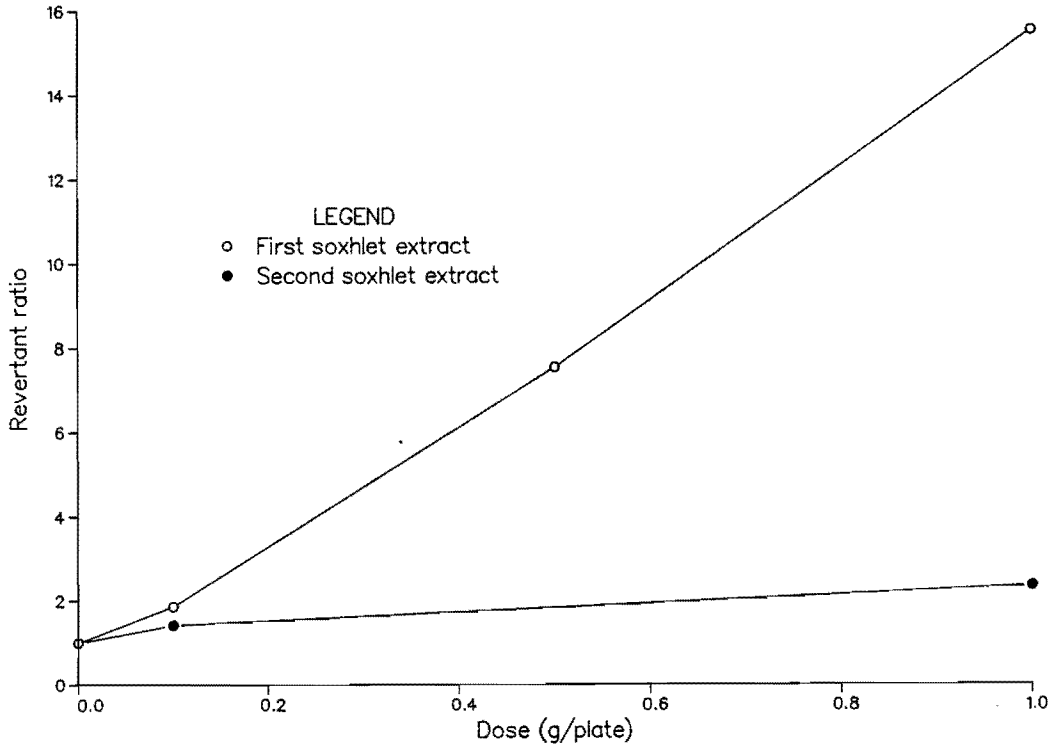

Figure 12. Dose response curves for the first and second ethanol soxhlet extractions of the same TSS sample (TA 98 with S-9 activation).

the mutagen may adsorb on the apparatus used for extraction. The barrel was teflon lined, however, the bottom of it showed scratches, and an uncoated metal paddle was used to stir the mixture. Before extraction the water had been transferred to a 20 ℓ glass jar and portions of the siphon and the XAD column were glass. It is also possible that some of the mutagen passed through the XAD-2 without being adsorbed, or that the DMSO failed to elute some of the mutagen.

The liquid/liquid isooctane extraction efficiency was determined by spiking 2 ℓ of leachate with TSS mutagen extracted from 200 g of shale. The mixture was then condensed and Ames tested with strains TA98, and TA1537 with S-9 activation. Figure 13 shows the results of these tests. It can be seen the leachate extract, although somewhat less mutagenic, follows much the same dose response curve as does the positive controls. Toxicity appears to affect the results at a dose of approximately 2 g per plate. If the slope of the curves to a dose of 1.0 g per plate is used to determine concentration, the extraction efficiency can be estimated. Using tester strain TA98 the extraction appeared to be 72 percent efficient, while with tester strain TA1537 it was 89 percent efficient.

Mutagen fractionation. The TSS mutagen was subjected to a fractionation scheme similar to the technique used by Pelroy and Petersen (1979) and shown in Figure 14. Initially 2 ml of the TSS mutagen (representing 200 grams of TSS)

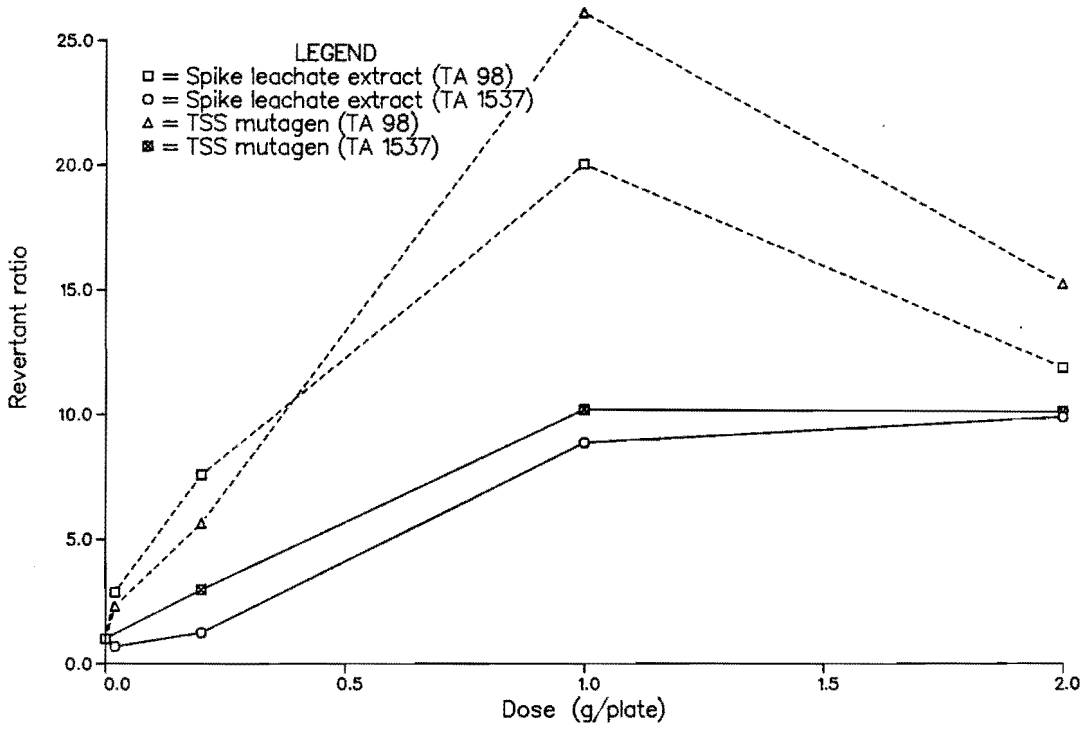

Figure 13. Dose response curves showing isooctane liquid-liquid extraction efficiency (with S-9 activation).

was shaken with 300 ml of isooctane and allowed to stand 2 hours. The isooctane was then serially extracted, three times, with 100 ml 1 N HCl. The extracts were then combined, the pH of the solution was adjusted to above 11 with 10 N NaOH, and the extraction was repeated three times with 100 ml of isooctane. The remaining isooctane solution (without the basic components) was extracted three times with 1 N NaOH. These extracts were combined, and the pH adjusted to below 3 with 10 N HCl. The samples were then extracted using the same technique as described for the basic extracts. The remaining isooctance fraction was extracted three times with 150 ml of DMSO. The DMSO extracts were combined, diluted with 450 ml of distilled water, and extracted three times with 100 ml of isooctane to recover the PAH fraction (Natusch and Tomkins 1978). The remaining isooctane fraction was retained for the neutral components. All isooctane samples were evaporated to dryness on a rotary evaporator and then redissolved in ethanol and DMSO. The ethanol samples were then Ames tested using strains TA97, TA98, and TA1537, with S-9 activation. The DMSO sample was tested with TA98 only. Two ml of the TSS mutagen was added to 300 ml DDW and extracted three times with 100 ml of isooctane. The isooctane was then evaporated and the residue redissolved and used as a positive control.

The residue from rotoevaporation of the isooctane samples did not appear to redissolve in DMSO. Ames testing of the DMSO sample did not result in any mutagenic responses. After fractionation, a dark residue appeared in the basic and neutral fractions. This residue may have provided a matrix which held the mutagens, preventing them from solubilizing in DMSO.

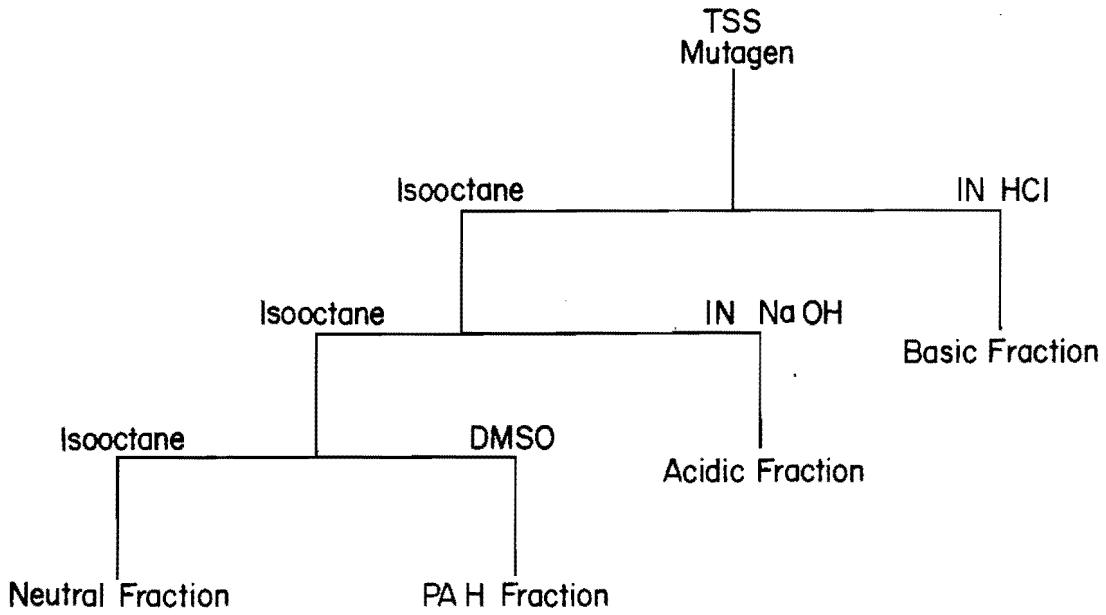

Figure 14. TSS mutagen fractionation scheme (after Pelroy and Petersen 1979).

Ames test results of the residues redissolved in ethanol are shown in Table 17 and Figure 15. With both TA98 and TA1537, the mutagenic activity of the basic fraction was the highest, 79 percent of the mutagenicity measured by TA98 and 70 percent as measured by TA1537. The strain TA97 did not show any significant mutagenic response. The results are similar to the findings of Pelroy and Petersen (1979) who found the basic fraction of shale oil to have the highest mutagenic activity. Rao et al. (1981) working with an oil shale product water (retort water) found the basic fraction accounted for 94 percent of the mutagenicity. Guerin et al. (1980) found the 2, 3, 4, and 5 ring polycyclic aromatic amines in the basic fraction of shale oil to be the most important mutagens. No identification of the compounds present in the mutagenic TSS fraction was attempted in this study. However they appear to be of the same class of compounds identified as active mutagens in shale oil. Previous work on mutagens in spent oil shale has centered primarily on PAH compounds such as B(a)P. Maase (1980) identified an unquantified trace of aminopyrene in spent oil shale, a compound which Guerin et al. (1980) found to have a mutagenic activity of 2,600,000 revertants per mg using TA98 with S-9 activation. Schmidt-Collerus et al. (1976) found a large band of azarines in TLC separation of a benzene leachable fraction of a TOSCO process spent shale. Rao et al. (1979) working with this sample found the azarine band to be the most mutagenic portion of the TOSCO spent shale benzene leachables. Rao et al. (1979) believed this to be roughly analogous to a basic fraction produced by an acid-base extraction technique.

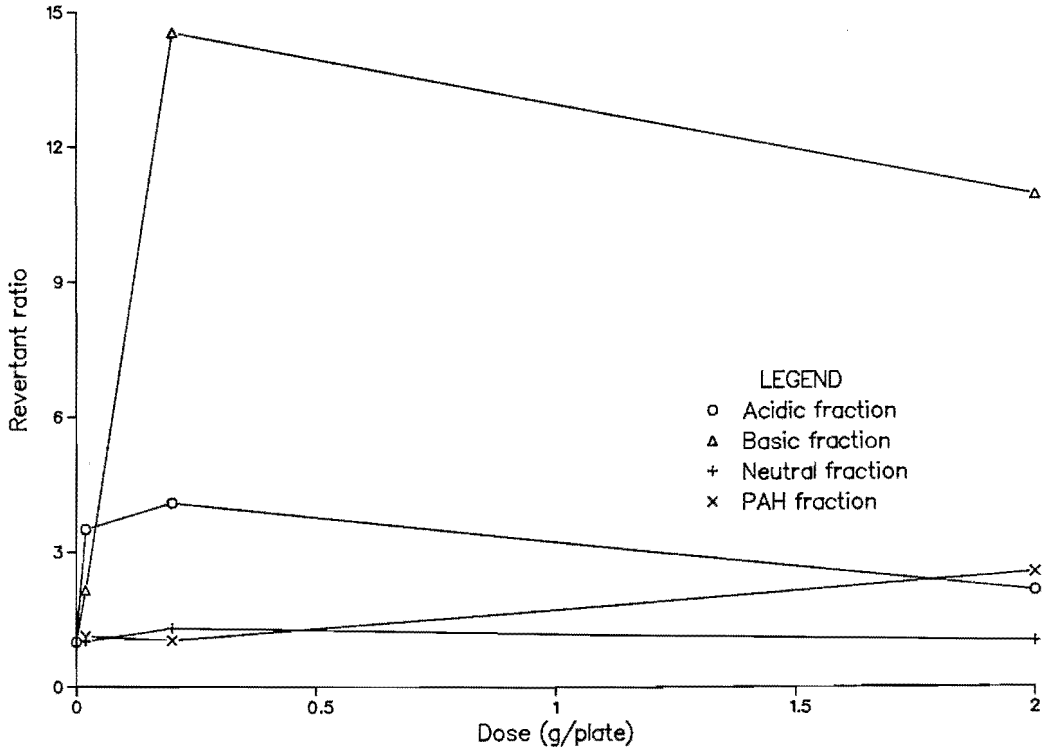

Figure 15. Dose response curves for the fractions of the TSS mutagenic extract (TA 98 with S-9 activation).

Table 17. Mutagenic activity in fractions of TSS mutagenic extract (with S-9 microsomal activation).

Fraction	Revertants Per Gram		
	TA1537	TA97	TA98
Acidic	NM[a]	NM	25.5[b]
Basic	33.4	T[c]	151.5[d]
Neutral	6.6	NM	NM
PAH	8.0	NM	13.9

[a]Test showed no significant mutagenicity.

[b]Average of two tests only one of which showed significant mutagenicity.

[c]Toxic response at all doses.

[d]Average of two tests both of which showed significant mutagenicity.

Mutagen/shale sorption. A series of isotherm adsorption experiments was performed using TSS spent shale and the TSS mutagenic extract in ethanol. Aqueous leachate (2.5:1) and DDW were spiked with TSS extract of a concentration equivalent to 100 g of shale extracted per liter. TSS which passed through a 0.038 mm sieve (approximately 22 percent passes through the sieve) was added to these spiked samples. The mixture was placed on a shaker table for 48 hr at 50 rpm. Sakaji et al. (1981a) found TOSCO II spent shale, in an isotherm adsorption experiment with retort water, had achieved 95 percent of equilibrium within 24 hr. Mok (1981) found the TOC leached from PSS came to an equilibrium within 48 hours in batch extractions.

After shaking, the shale was removed from the samples by centrifugation at 700 rpm for 10 minutes. This was repeated three times for each sample.

The DDW became cloudy after the addition of TSS mutagen. The TSS mutagen did not appear to entirely dissolve. When the mutagenic residue was added to the leachate water, the color of the water obscured any observable changes. Following the 48 hours of shaking and centrifugation, the spiked DDW appeared clear with only some color evident as the result of the mutagenic spike. As a control in both the DDW and leachate experiments, one treatment involved no spent shale addition.

The clarified samples were then extracted using isooctane (three times with 300 ml isooctane/liter of sample). In the process of extraction, all the glassware which had come in contact with the sample was washed with the isooctane. A sample of 2.5:1 leachate was spiked with the TSS mutagen with no spent shale and treated as above, except the aqueous sample was discarded. All glassware was washed first with ethanol and then with isooctane. The organic solvents were rotoevaporated to dryness, and the residue redissolved in ethanol for Ames testing.

In the first set of experiments, tester strains TA97, TA98, and TA1537

with S-9 activation were used. However, TA98 with S-9 activation was found to give the most satisfactory results and was used throughout this series of experiments as the primary tester strain.

Originally concentrations of 1.0, 10.0, 50.0, and 100.0 g/l of shale were used. The mutation rate of TA98 with S-9 was 277 mutants per gram of shale extracted with no shale added, and 60 mutants per gram with 1.0 g/l of shale added. At the higher spent shale doses no mutagenic activity was detected. Based on this initial data, it was apparent that the spent shale was effective at removing the mutagen at doses near 1 g/l. For the second set of experiments using both DDW and leachate, spent shale concentrations of 0.25, 0.50, 1.0, 2.5, 5.0, and 10.0 g/l were used. Figures 16 and 17 are dose response curves showing the results of these experiments.

The spent shale apparently adsorbs the mutagens removing them from the leachate water. This is probably not a simple adsorption process. The TOC of the leachate water is approximately 110 mg/l, and a large number of different compounds are competing for nonuniform sites. Fox et al. (1980b) found TOSCO II spent shale adsorbed 1.3-60.6 mg of dissolved organic carbon per gram of oil shale from retort water. The nature of organics in retort water, however, is probably different than it is in the spent shale leachate. The organics in the leachate have been solubilized in contact with oil shale and are not likely to have a high affinity for the spent shale. The

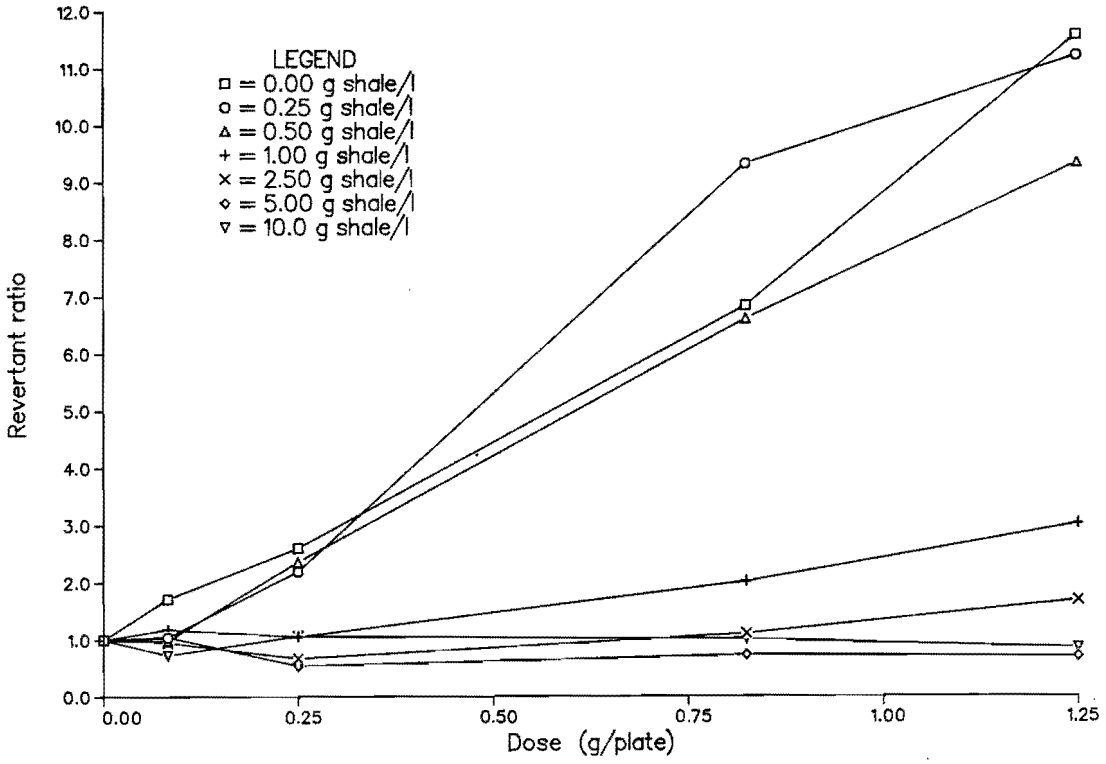

Figure 16. Dose response curve for DDW sorption experiments (TA 98 with S-9 activation).

organics in the TSS mutagen have been extracted using ethanol and may be less nonpolar and have a higher affinity for the spent shale than TOC removed by aqueous leaching. Figure 18 is the resulting partition isotherm between the spent shale and leachate water. The mutagen concentrations are expressed as potential mutagens per liter. This is based on the slope of the dose response curve of TA98 with S-9 activation. The 95 percent confidence intervals about the points are shown. At higher mutagen concentrations the confidence intervals increase greatly.

The two most commonly used isotherms are the Langmuir:

$$S = (A\ b\ C)/(1 + bC) \quad . \ . \quad (4)$$

where

S = mass of solute absorbed onto the solid phase (dimensionless)

A = concentration of solute absorbed at saturation (dimensionless)

b = constant (L^3/m)

C = concentration of solute in the aqueous phase (m/L^3)

and the Freundlich:

$$S = KC^N \quad . \ . \ . \ . \ . \quad (5)$$

where

K = constant (L^3/m)

N = constant (dimensionless)

The Langmuir isotherm can be developed theoretically based on the assumptions (Moore 1972) that: 1) the energy of adsorption is constant and independent of surface area, 2) the adsorption is on localized sites and there is no interaction among adsorbate molecules, and 3) the adsorption is a monolayer phenomenon. The first assumption, that the energy of sorption is constant, requires a homogeneous surface with no variation in binding sites. This is a poor assumption in any case and especially for spent shale in which there is no doubt large variation in the nature of the surface. The second assumption that there is no interaction between adsorbate molecules is also not likely to hold for the case of spent shale.

Although it is generally assumed the Langmuir isotherm has a better theoretical basis and the Freundlich isotherm is only used as an empirical equation to fit data, at low concentrations relative to saturation the Freundlich isotherm can be justified on a thermodynamic basis. Making the assumption that the energy of sorption declines logarithmically with increasing concentration of adsorbate, Halsey (1952) derived the Freundlich isotherm. Halsey also showed the Freundlich isotherm may be used when there is cooperative adsorption between adsorbate molecules. These conditions of declining energy of sorption with concentration and cooperative adsorption are probably good approximations of the condition in a spent shale/leachate mixture.

The Freundlich isotherm was fit to the data shown in Figure 18. Because of the high variability of the two upper estimates they were not used. The resulting equation was:

$$S = 72.3\ C^{0.57} \quad . \ . \ . \ . \ . \quad (6)$$

$$(r^2 = 0.78)$$

where

S = mutagen adsorbed onto TSS (potential mutants/g, dimensionless)

C = mutation concentration in leachate (potential mutants/ℓ) M/L^3)

An expression for the total concentration of potential mutagen can be written:

$$TC = C + M_S S \quad . \quad . \quad . \quad . \quad . \quad (7)$$

where

TC = total concentration of potential mutagens per liter of water (M/L^3)

M_S = mass of shale per liter of water (M/L^3)

The total concentration of mutagen per liter is the concentration of mutagen initially adsorbed on the shale, as determined by the ethanol soxhlet extraction. This expression solved simultaneously with the isotherm relationship will give predicted mutagen concentrations at any given shale to water mixture. The results of this calculation predicts a concentration of 17.2 potential mutants per liter in both leachates; rounding to a whole number this becomes 17.

In order to obtain some feel for the sensitivity of this model to variations in the isotherm, a "worst case" estimate was developed. The lower isotherm on Figure 18 was obtained by calculating the lower 95 percent confidence interval for the isotherm regression equation. This calculation was based only on six slopes of lines with at least 15 data points per line. Therefore, due to wider confidence limits based on six points than on more points, the actual 95 percent confidence limit would be less than this estimate. However for the purposes of this calculation the more conservative confidence limit will be used.

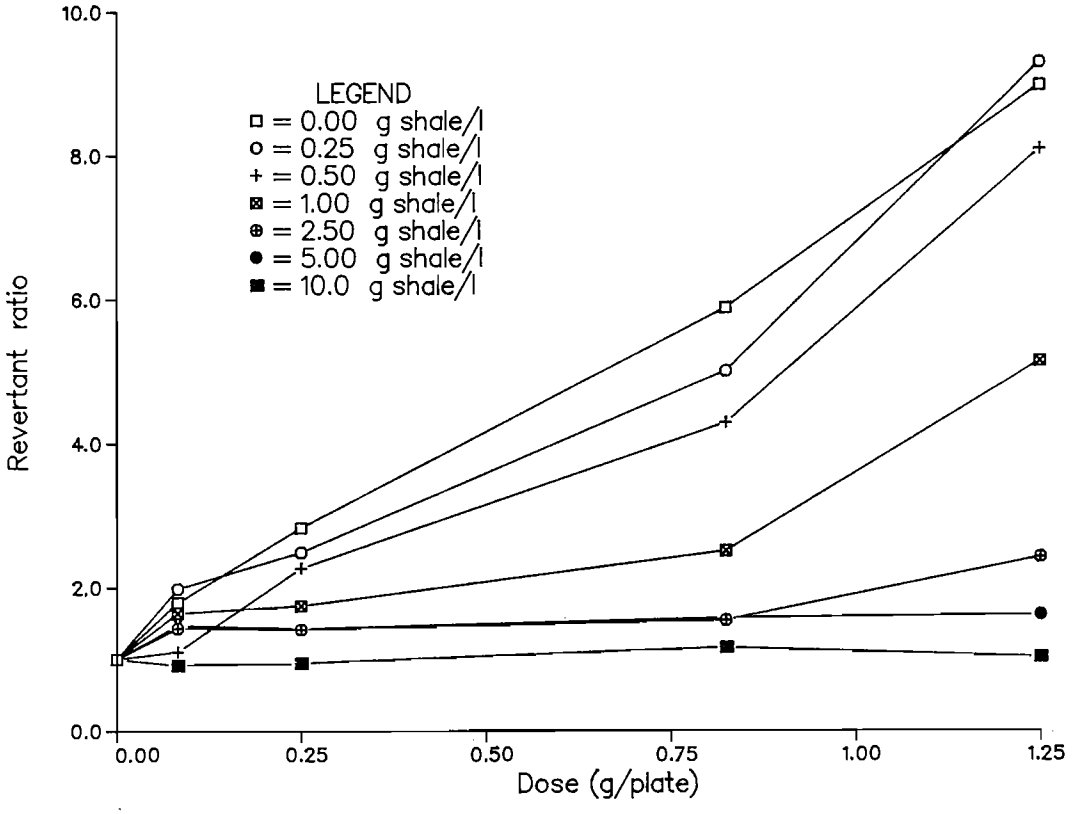

Figure 17. Dose response curves for leachate sorption experiments (second experiment, TA 98 with S-9 activation).

The resulting isotherm was:

$$S = 28.3\ C^{0.51} \quad . \quad . \quad . \quad . \quad . \quad (8)$$

Using this worst case isotherm and solving Equations 7 and 8 simultaneously, a predicted concentration of 151 mutants/ℓ in the 2.5:1 leachate is obtained.

In the 1:4 leachate experiments, the highest dose usually represented 2.4 liters of leachate per plate. If 17 potential mutants/ℓ were present, approximately 41 potential mutants per plate should have been found. This may have resulted in a revertant ratio of greater than 2.0 and should have been detected.

Isooctane liquid/liquid extraction of 21 ℓ of 1:4 leachate was used to determine low level mutagenic activity. The resulting data had a revertant ratio, at the highest dose (157.5 g/plate) of only 1.83, however the slope was slightly positive and significant at the 95 percent criteria. This slope presents a concentration of 33 potential mutants/ℓ in the 1:4 leachate. Sufficient shale was not available to repeat this test. Although the revertant ratio was below 2.0, the significantly positive slope cannot be ignored. The difference between a predicted concentration of 17 potential mutants per liter and the 33 measured is probably within the natural variation of the Ames test.

The use of adsorption isotherms to predict concentration under these conditions makes the assumption that the shale particles and the solute are in equilibrium. This would of course be a

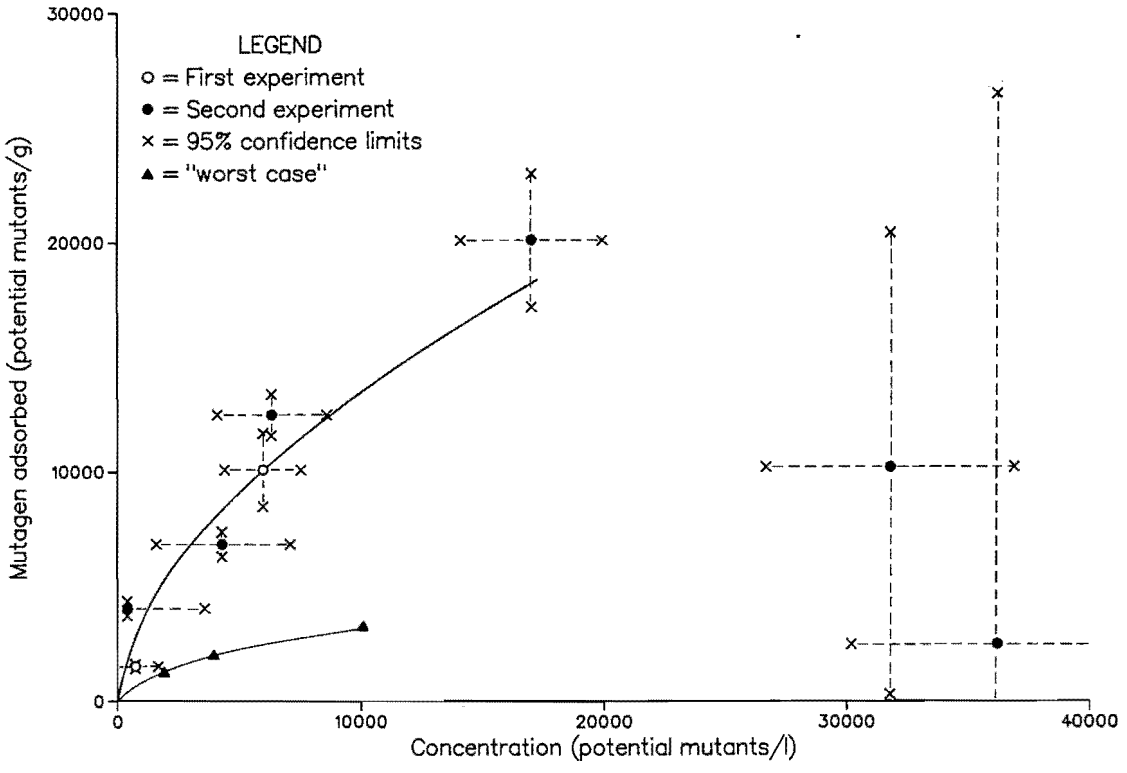

Figure 18. Adsorption isotherm resulting from leachate sorption experiments (second experiment and 2 points from the first experiment).

time dependent phenomenon. In the isotherm studies the shale was in contact with the leachate for 48 hours. If it is assumed that internal diffusion within the shale pores is the rate limiting step, the rate equation becomes:

$$dS/dt = K_G (S^* - S) \quad . \quad . \quad (9)$$

where

K_G = internal diffusion rate coefficient (1/t)

S = mass of solute adsorbed to the solid phase (dimensionless)

S* = mass of solute adsorbed to the solid phase at equilibrium (dimensionless)

Amy et al. (1980) and Hall (1982) have estimated K_G values for TOC leaching from simulated in situ spent shale. Their values range from approximately 10^{-1} to 10^{-4}/hr. The shale they used had an average diameter approximately 100 times greater than the TSS sample used in this study. The rate of diffusion in the internal pores is proportional to the concentration gradient. In the much smaller TSS particles the diffusion rate becomes much higher, diminishing the importance of internal pore diffusion in leaching dynamics.

Due to the small particle size in the TSS sample, pore diffusion is likely to be reached quickly. Empirically, Mok's (1981) data showed that in a 1:4 batch mixture, the TOC of a PSS leachate came to equilibrium within 36 to 48 hours.

Sorption column experiment. Using the Freundlich isotherm developed for the TSS mutagen system and a technique developed by Weber (1972), a breakthrough curve was estimated for a shale column and leachate spiked with TSS mutagen. The pore diffusion was assumed to be equilibrium and the breakthrough concentration was taken to be 500 potential mutants/ℓ. Weber's (1972) numerical technique allows the use of the Freundlich isotherm, and the data shown in Figure 18 indicate that the isotherm follows a power curve, Freundlich isotherm, in the concentration range of the TSS mutagen spiked leachate used.

An experiment was designed to validate this breakthrough curve. Seven liters of the 2.5:1 leachate were spiked with 3.5 ml of the mutagen solution (representing 350 g of TSS). This solution was then passed through a 1.2 x 4.4 cm column of TSS at a rate of 0.125 ℓ/hr. The effluent water was sampled approximately every hour, isooctane extracted, evaporated, dissolved in ethanol, and Ames tested using tester strain TA98 with S-9 activation.

The dose response curves for the various samples are shown in Figure 19. The mutagenic activity of the effluent samples was well below the positive control, indicating that complete breakthrough did not occur. Figure 20 shows the predicted breakthrough curve along with the points plotted from the measured data. Sufficient sample was not available to continue the experiment and determine the shape of the remainder of the curve.

None of the early points had a revertant ratio greater than 2.0. The fact that the actual breakthrough came after the predicted breakthrough indicates that the adsorption model based on batch isotherm experiments may be conservative in its estimation of the adsorptive capacity of TSS. This is to be expected because the isotherm was based only on sorption of the mutagen and was followed by centrifugation to remove the spent shale. It is possible that colloidal material with mutagen adsorbed remained in the aqueous fraction of the leachate. In a column or spent shale pile, the shale itself would act as a filter removing mutagen by

entrapment and impingement. Use of an adsorption isotherm to predict breakthrough will, therefore, be conservative because the filtration effect of spent shale slows the breakthrough.

Ames test spontaneous revertant results. Table 18 shows the spontaneous reversion rates measured for the tester strains along with spontaneous reversion rates reported by Ames et al. (1975) and Levin et al. (1982). The spontaneous reversion rates measured were lower for TA98 and TA100, and greater for TA1537 than those reported. Strains TA97, TA1535, and TA1538 showed higher than reported spontaneous reversion rates with S-9 activation and lower without. The S-9 microsomal activation normally increases spontaneous reversion rates as observed.

The differences between the spontaneous reversion rates found in this study and those reported in the literature are not unusual. Cheli et al. (1980) and Peak et al. (1980) describe the inherent variability of the Ames test. Even under identical conditions, it is not possible to obtain reproducible data. The greatest potential problem is inadvertant introduction of an unknown mutagen. A high spontaneous reversion rate can occur as the result of the use of ethylene oxide as a sterilizing agent or from some nutrient broth batches (Ames 1981). The data indicate mutagen contamination was not a problem in this study.

Mutagen/Shale Leaching Dynamics

The problem of a pile of spent shale releasing mutagenic compounds into groundwater is somewhat different than the breakthrough of a mutagen in a spiked sample. The mutagen is distri-

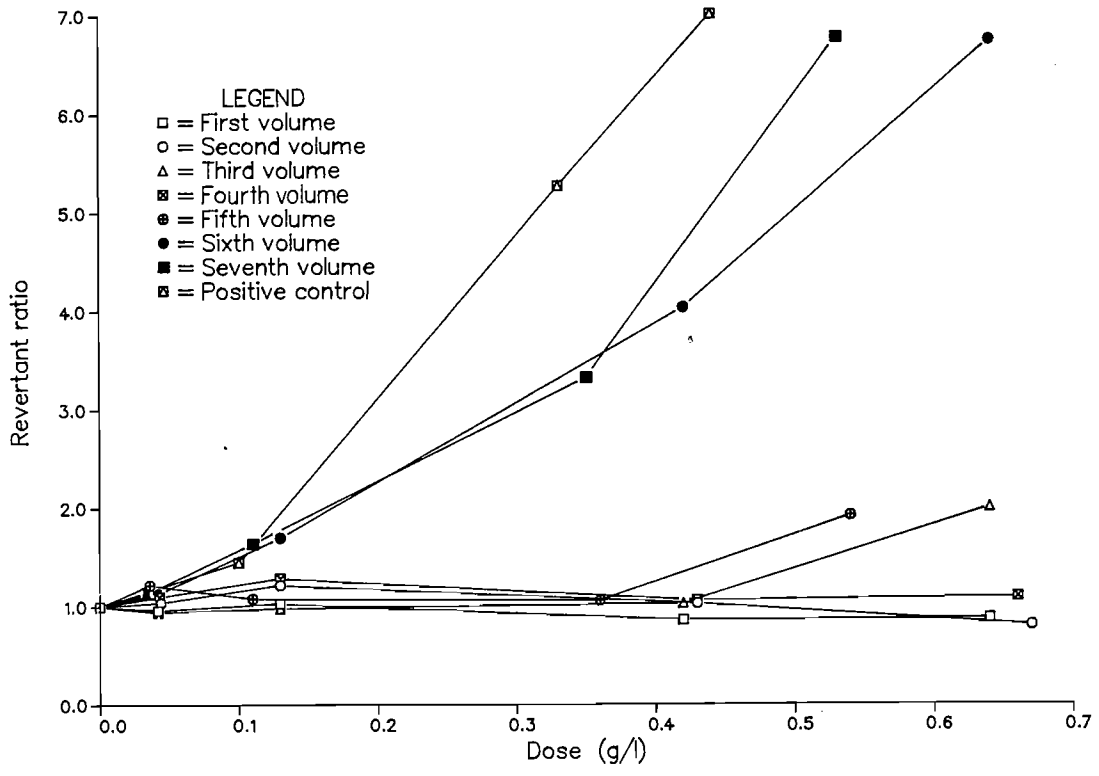

Figure 19. Dose response curve for the sorption column experiment (TA 98 with S-9 activation).

Table 18. Spontaneous reversion rates obtained for the Ames test tester strains in this study and reported rates.

Strain	S-9 Activation	Measured Rates $\bar{X}$	S	n	Reported Rates
TA97	Yes	106.69	18.76	27	95[a]
TA97	No	78.22	24.68	9	
TA98	Yes	33.24	13.39	133	40[b]
TA98	No	18.77	9.66	62	
TA100	Yes	93.43	26.45	87	160[b]
TA100	No	79.81	23.61	68	
TA1535	Yes	24.84	12.68	51	20[b]
TA1535	No	15.38	7.60	53	
TA1537	Yes	17.30	12.25	88	7[b]
TA1537	No	11.05	7.11	63	
TA1538	Yes	43.20	20.22	51	25[b]
TA1538	No	13.02	7.59	42	

[a]Levin et al. (1982).
[b]Ames et al. (1975).

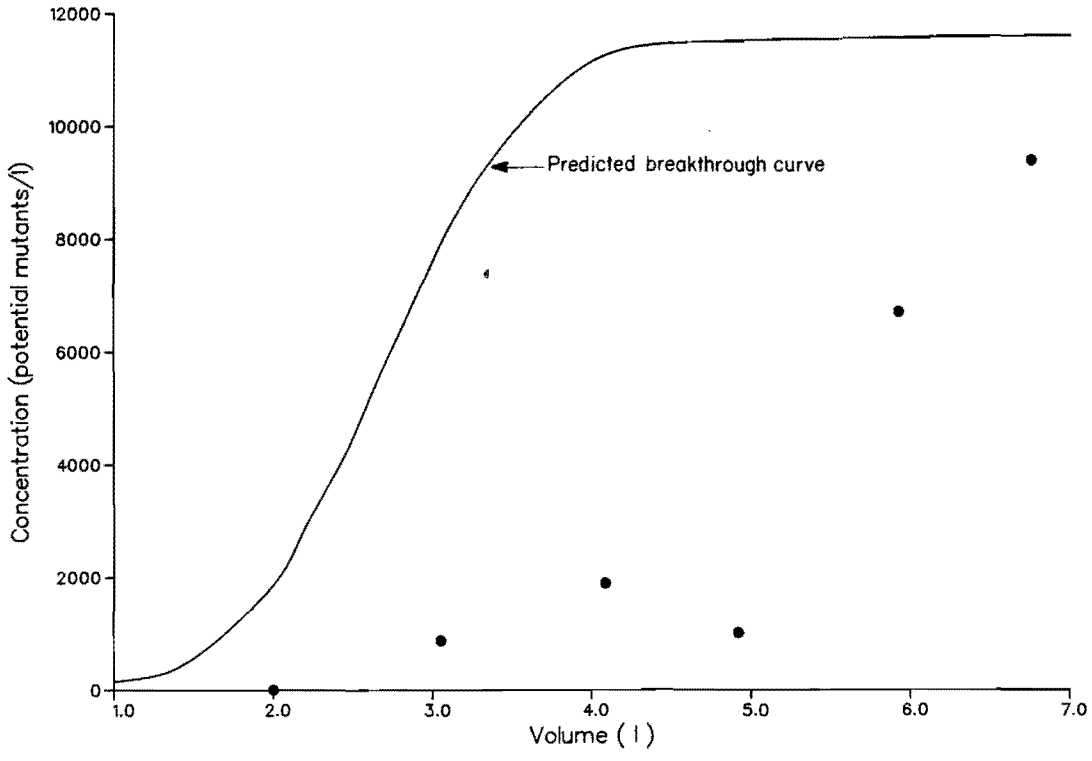

Figure 20. Breakthrough curve calculated (after Weber 1972) and measured for sorption column study (TA 98 with S-9 activation).

buted throughout the column of shale and slowly released into the water. Beginning with the basic one-dimensional advective-dispersive transport equation (Bear 1972):

$$\frac{\partial C}{\partial t} + \rho/n \frac{\partial S}{\partial t} = D\frac{\partial^2 C}{\partial x^2} - v\frac{\partial C}{\partial x} \quad . \quad . \quad . \quad (10)$$

where

- C = solute concentration (M/L^3)
- t = time (t)
- ρ = solids density of medium (M/L^3)
- n = porosity of medium (dimensionless)
- S = mass of solute adsorbed per unit mass of media (dimensionless)
- D = dispersion coefficient (L^2/t)
- v = fluid velocity (L/t)

The relationship between adsorbed and soluble solute (S and C) can be expressed as the Freundlich isotherm:

$$S = KC^N \quad . \quad . \quad . \quad . \quad . \quad (11)$$

differentiating 11 with respect to time:

$$\frac{\partial S}{\partial t} = K\,N C^{N-1} \frac{\partial C}{\partial t} \quad . \quad . \quad . \quad . \quad . \quad (12)$$

Substituting 12 into 10 and solving:

$$\frac{\partial C}{\partial t} = D/Rf \frac{\partial^2 C}{\partial x^2} - v/Rf \frac{\partial C}{\partial x} \quad . \quad . \quad . \quad (13)$$

where

$$Rf = 1 + \rho/n\ KNC^{N-1}$$ (retardation factor)

Equation 13 cannot be solved in closed form, however numerical solutions are available (Ho 1982, Van Genuchten and Wierenga 1974).

Equation 13 is the same as Equation 1 except that R, the chemical or biological reaction rate term and m, the ratio of macro-pore to micro-pore volume, have been eliminated. Although some chemical or biological degradation may take place, it is not likely to be too significant in the short run. Sorensen (1982) found very low levels of microbial activity in a spent shale pile. The environment in a spent shale pile will be anaerobic, due to the reduced salts present (Burnham et al. 1978), and highly saline, not conducive to biological degradation. The desorption source term used by Hall (1982) in Equation 1 was for a nonequilibrium desorption process in which internal pore diffusion was the rate limiting process. If desorption is assumed to be in equilibrium the internal diffusion rate and pore ratio become unimportant.

In soil water systems a linear isotherm of the form

$$S = K_d C \quad . \quad . \quad . \quad . \quad . \quad (14)$$

where

- S = mass of solute adsorbed to the solid phase (dimensionless)
- K_d = distribution coefficient (L^3/M)
- C = concentation of solute (M/L^3)

is often used (Karickhoff et al. 1979, Means et al. 1979). This is a simplification of the Freundlich isotherm in which the exponential term, N, is set equal to one. If a linear isotherm is used the retardation factor becomes

$$Rf = 1 + \rho/n\ K_d \quad . \quad . \quad . \quad . \quad (15)$$

and Equation 13 can be solved using a Laplace transform (Ogata and Banks 1961). For the boundary conditions:

$$C(x,o) = Ci,$$

$$-D\ \partial C/\partial X + vC \Big|_{x=0} = 0$$

and

$$\partial C/\partial X\ (\infty,t) = 0$$

the solution is (Van Genuchten and Alves 1982):

$$C_{(x,t)} = C_i \left[1 - 1/2 \left[\operatorname{erfc}\left[\frac{Rf\ x - vt}{2(D\ Rf\ t)^{1/2}} \right] + 2 \left(\frac{v^2 t}{\pi DRf}\right) \right.\right.$$
$$\exp\left(-\frac{(Rfx - vt)^2}{4DRft} \right) - \left(1 + \frac{vx}{D} + \frac{v^2 t}{DRf}\right) \exp(vx/D)$$
$$\left.\left. \operatorname{erfc}\left[\frac{Rf\ x + vt}{2(D\ Rf\ t)^{1/2}} \right] \right] \right] \quad . \ . \ . \ . \ . \ . \ . \ . \ . \quad (16)$$

where

- x = distance (L)
- t = time (t)
- C_i = initial solute concentration (M/L^3)
- v = fluid velocity (L/t)
- erfc= complementary error function

This equation was solved using the computer program listed in Appendix B. The two important parameters in affecting the shape of a breakthrough curve are retardation factor (Rf) and dispersion (D).

The retardation factor is a function of a compound's affinity for the medium and the density and porosity of the medium. Hydrophobic compounds, which probably make up the spent shale mutagen, have been found to follow an approximately linear isotherm at low concentrations (Means et al. 1979). Karickhoff et al. (1979) working with sediments have found the following relationship between the linear isotherm constant or distribution coefficient (K_d) and the octanol water partition coefficient (K_{ow}):

$$K_d = 0.63\ K_{ow}\ (O_c) \quad . \ . \ . \quad (17)$$

where

- O_c = fraction of organic carbon in sediment (dimensionless)

Using an organic carbon content of 0.045 for the spent shale this relationship becomes:

$$K_d = 0.0284\ K_{ow} \quad . \ . \ . \quad (18)$$

Table 19 shows compounds found in spent oil shale or shale oil which may be in the mutagenic fraction, the corresponding K_d values based on Equation 18 and the predicted concentration in a 2.5:1 batch leachate. These predicted concentrations are well below the solubilities of the compounds, and well below any concentration which would produce a mutagenic response in the Ames test.

At the concentration predicted in 2.5:1 leachate, 17.2 potential mutants/liter, the slope of the isotherm can be determined by evaluating the derivative of the adsorbed concentration with respect to the aqueous concentration:

$$S = 72.3\ C^{0.57} \quad . \ . \ . \quad (6)$$

differentiating:

$$dS/dC = 41.2\ C^{-0.43} \quad . \ . \ . \quad (19)$$

at C = 17.2:

$$dS/dC = 12.1 \quad . \ . \ . \ . \ . \quad (20)$$

This is equivalent to the distribution coefficient, K_d, in the linear isotherm. Another estimate of K_d can be made by simply drawing an operating line through

Table 19. Literature values of K_{ow} and resulting predictions of Kd and solubility in leachate.

PAH Compound	Structure (number of rings)	Highest Identified Concentration in Shale[a] (µg/kg)	K_{ow}	Kd	Predicted Concentration in 2.5:1 leachate[d] (µg/l)
Fluorene	3	33	$10^{4.18}$[b]	430	0.077
Benz(a)anthracene	4	13	$10^{5.61}$[b]	11,570	0.0011
Chrysene	4	36	$10^{5.61}$[b]	11,570	0.00311
Benz(a)pyrene	5	55	$10^{6.04}$[b]	31,140	0.0018
Benzo(ghi)perylene	6	24	$10^{7.23}$[b]	482,302	0.000050
Pyrene	4	-	$10^{5.32}$[b]	5,900	-
Aminopyrene	4	trace	$10^{0.80}$[c]	-	-

[a]From Maase (1980) and Coomes (1979).
[b]From Versar (1979).
[c]From Leo et al. (1971)
[d]Based on Equations 7 and 14.

the lower points on the curve in Figure 18 and the origin, the resulting slope is equivalent to K_d. If this is done, the resulting K_d is approximately 3 - 6 (using the lower 2 points).

These estimates are much smaller than the K_d values of the PAH compounds listed in Table 19. There could be any of a number of reasons for a small K_d. The compounds in the mutagenic fraction extracted from TSS could have a lower affinity for the shale than the types of compounds listed in Table 19. This is possible, however, it is likely that the K_d would be lower than for an unsubstituted PAH. The addition of a primary amine group greatly decreases the K_{ow} of pyrene, a 4-ring PAH. The pH and therefore the ionization of the amine group would affect the K_{ow} of a compound making it difficult to compare a published K_{ow} of a primary amine to its K_d in spent shale leachate (pH $\simeq$ 12.0).

Another likely explanation is that small colloids containing mutagens were formed in the leachate used for the isotherm tests and not removed by centrifugation. The leachate water was not filtered prior to extraction because it was believed mutagenic compounds would be lost by adsorption to the filter apparatus. If mutagen was attached to colloids, the batch isotherm experiments would underestimate K_d. These colloids will probably not pass through a pile of spent shale because of the filtration effect of the spent shale itself.

Another possible explanation is that the solvent properties of leachate waters are affected by high TOC. Soluble organics have been found to result in micelle formation increasing PAH solubility. Caffeine in water at concentrations of 10-50 mg/l can increase PAH solubility 10 times (Lee et al. 1981). This explanation is supported by the apparently higher affinity of the mutagen found in the isotherm experiment for deionized water rather than leachate (Figure 16). Using a K_d of 3 - 12 and assuming ρ/n = 3.0, the retardation factor becomes 10 - 37.

The importance of dispersion is related to the magnitude of D in Equation 4. Dispersion under field conditions is very difficult to estimate from either laboratory experimentation or theoretical constructs (Roberts et al. 1982). The dispersion term is actually made up of both molecular diffusion and mechanical dispersion. An approximate value of diffusion in water is 1.7 cm^2/day (Sverdrup, Johnson, and Fleming 1942). Diffusion is a function of temperature, fluid viscosity, and molecular size. Using the Stokes-Einstein equation (Hobler 1966) the diffusion constant for pyrene, a 4-ring PAH in water is estimated to be 0.43 cm^2/day. Under conditions of very low water velocity, short distances or low mechanical dispersion, the diffusive portion of the dispersion term may dominate.

Dispersion results in the spreading of a plume with distance. The magnitude of the dispersion effect can be estimated from the Peclet number (Pe) (Fried 1975):

$$Pe = vx/D \quad . \quad . \quad . \quad . \quad (21)$$

where

v = fluid velocity (L/t)
x = distance (L)
D = dispersion coefficient (L^2/t)

In general, for Pe > 1000 dispersion can be neglected and for Pe < 5 the system approaches a complete mix (Ogata and Banks 1961). As the Peclet number approaches 1.0, diffusion becomes the predominant means of transport (Gillham and Cherry 1982). Figure 21 shows plots of solutions to Equation 16 for various Peclet numbers. At high values the breakthrough curve approaches plug flow at lower values, a completely mixed system.

Table 20 shows the Peclet numbers which can be expected from a variety of different spent shale leaching conditions. It can be seen that the Peclet number may vary from just above 1.0 to nearly 10,000. This covers nearly the entire range of flow regimes shown in Figure 21.

The retardation factor is inversely related to the length of time required to breakthrough in any given flow regime. The time to 50 percent breakthrough is approximately equal to the time required for a single pore volume divided by the retardation factor. In Table 20 the times to $C/C_o = 0.9$ (beginning of breakthrough) are given for a retardation factor of 10.0. It can be seen that with the exception of shallow (10 m) loose fill or lightly compacted Paraho spent shale, more than 10 years are required for initial breakthrough. More realistic flow regimes with deeper piles, and higher compaction have much slower infiltration rates. The initial breakthrough may come only after hundreds to thousands of years.

The problem in making these long term predictions is that the assumption is made that there will be no degradation or change in the leaching conditions with time. Although microbial activity will probably be low in spent shale piles, it is difficult to predict what may happen over a period of tens to thousands of years. With micelle formation or competition for binding sites due to the high TOC in leachate waters, K_d may increase with time resulting in a lower but longer term mutagenicity of the leachate. The important point here is that the higher K_d (and Rf) the lower the concentration, but more chronic the problem.

Potential Mutagenic Hazard of Leachate

The TSS sample was found to contain a mutagenic fraction which is slowly released into leachate waters over a long period of time. Possible sources of error in estimations of the mutagenic potential include:

1. Random variation in the Ames test.

2. Incorrect isotherm calculations due to nonequilibrium conditions in the isotherm experiments.

3. Incorrect isotherm calculations due to failure to remove colloids from suspension in the isotherm experiments.

The first source of error is unavoidable. However, any variation should be within the confidence interval used for the "worst case" calculations. The second and third possible sources of error would result in an underestimation of the affinity of the mutagen for the shale and an overestimation of mutagenic potential of the leachate. The possibility of nonequilibrium in the isotherm experiments is even less than in other leaching experimentation due to the small sieve fraction of TSS used. If errors did occur in the estimates of K_d, it is most likely that K_d has been underestimated. Therefore predictions of concentrations would be expected to be on the conservative (high) side.

These numbers, however, do not address the most important question. That being the carcinogenic danger associated with the mutagen which is leached. McCann and Ames (1977) reported that mutagenic potency is generally related to carcinogenic potency but the relationship depends on interactions among the mutagens. Dickson and Adams (1980) found mixtures of PAH mutagens to be generally less mutagenic than an additive model would predict. Pelroy and Petersen (1979), working with a mutagenic shale oil fraction, found the addition of known mutagens could be either antagonistic or synergistic. The same phenomenon has been reported by others (Shahin and

Table 20. Hydraulic parameters for various spent shale leaching conditions.

	D_{50} (cm)	Macroscopic Velocity[a] (cm/day)	Depth (cm)	Reynolds Number[b]	Molecular Diffusion (cm2/day)	Mechanical Dispersion[c] (cm2/day)	Mechanical Dispersivity[d] α (cm)	D (cm2/day)	Pe	Detention Time (yr)	Time to $C/C_o = 0.9$ Rf = 10.0 (yr)
Paraho Spent Shale											
Saturated well drained[e]											
Loose fill	0.4	1.3×10^{3}	1,000	6.0×10^{-1}	1.0	8.4×10^{2}	6.5×10^{-1}	8.4×10^{2}	1.5×10^{3}	2.1×10^{-3}	1.9×10^{-2}
Lightly compacted	0.4	5.2×10	1,000	2.4×10^{-2}	1.0	1.7×10	3.1	1.8×10	2.9×10^{3}	5.3×10^{-2}	4.9×10^{-1}
Highly compacted	0.4	7.8×10^{-2}	1,000	3.6×10^{-5}	1.0	6.7×10^{-3}	8.6×10^{-2}	1.0	7.8×10	3.5×10	2.9×10^{2}
	0.4	7.8×10^{-2}	10,000	3.6×10^{-5}	1.0	6.7×10^{-3}	8.6×10^{-2}	1.0	7.8×10^{2}	3.5×10^{2}	3.2×10^{3}
Assuming an infiltration rate											
5 cm/yr	0.4	4.1×10^{-2}	10,000	1.9×10^{-5}	1.0	3.1×10^{-3}	7.6×10^{-2}	1.0	4.1×10^{2}	6.7×10^{2}	6.0×10^{3}
5 cm/yr	0.4	4.1×10^{-2}	1,000	1.9×10^{-5}	1.0	1.9×10^{-5}	4.6×10^{-3}	1.0	4.1×10	6.7×10^{3}	4.0×10^{4}
0.5 cm/yr	0.4	4.1×10^{-3}	1,000	1.9×10^{-6}	1.0	1.2×10^{-15}	2.9×10^{-4}	1.0	4.1	6.7×10^{4}	1.3×10^{3}
TOSCO Spent Shale											
Laboratory Column	0.008	7.1	50	6.5×10^{-5}	1.0	1.7	0.22	2.7	1.3×10^{2}	1.9×10^{-2}	1.7×10^{-1}
	0.008	7.1	50	6.5×10^{-5}	1.0	5.0	1.4	6.0	6.0×10	1.9×10^{-2}	1.3×10^{-1}
Saturated well drained[f]											
Loose fill	0.008	2.3	1,000	2.1×10^{-5}	1.0	5.1×10^{-1}	0.22	1.5	1.5×10^{3}	1.2	1.1×10
Compacted	0.008	1.3×10^{-1}	10,000	1.2×10^{-6}	1.0	2.9×10^{-2}	0.22	1.0	1.3×10^{3}	2.1×10^{2}	2.0×10^{3}
Assuming an infiltration rate (TOSCO)											
5 cm/yr	0.008	4.1×10^{-2}	10,000	3.8×10^{-7}	1.0	9.0×10^{-3}	0.22	1.0	4.1×10^{2}	4.1×10^{2}	6.0×10^{3}
5 cm/yr	0.008	4.1×10^{-3}	10,000	3.8×10^{-8}	1.0	9.0×10^{-4}	0.22	1.0	4.1×10	4.1×10	4.0×10^{4}

[a]Assumes a porosity of 0.33.
[b]Calculated based on $Nr = v\ D_{50}/\gamma$ at 20°C
[c]Paraho calculated based on $DM = 1.8\gamma\ N_R^{1.208}$, TOSCO calculated based on $D = \alpha v$.
[d]Paraho calculated based on $\alpha = D_{m/v}$, TOSCO values from Ramirez et al. (1982).
[e]Calculated assuming a hydraulic gradient of 1.0 and permeability from Table 3.
[f]Estimated by fitting Equation 16 to the EC data.

Fournier 1978; Stoltz et al. 1979). This points out the potential danger of comparing the mutagenic potency of complex environmental mixtures. Comparison is, however, the only approach to attempting to evaluate the potential risk of spent shale leachate. Table 21 and Figure 22 show the mutagenic activity of the TSS leachate to other waters which have been tested. The TSS leachate appears to have a slightly higher mutagenic potency than most drinking water tested, but at least six orders of magnitude below retort water and in approximately the same range as chlorinated secondary effluent. These figures may be somewhat misleading in that waters which are tested for mutagenicity are generally first suspected of being contaminated. There are no studies existing on mutagenicity of pristine spring waters. Nonetheless, the data from this study suggest mutagenic activity of TSS leachate water is not a more significant hazard than many other environmental sources of mutagens.

Recarbonation Studies

The results of the recarbonation experiments are shown in Table 22. After 59 days of exposure, no significant change in leachate EC or pH was found. Gas chromatographic examination of the air going into and out of the recarbonation barrels showed no detectable CO_2 uptake. The pH did drop slightly, but not sufficiently to affect the leachate.

When oil shale is retorted, the carbonate minerals decompose to varying degrees resulting in oxides or hydroxides and an increased pH (Burnham et al.

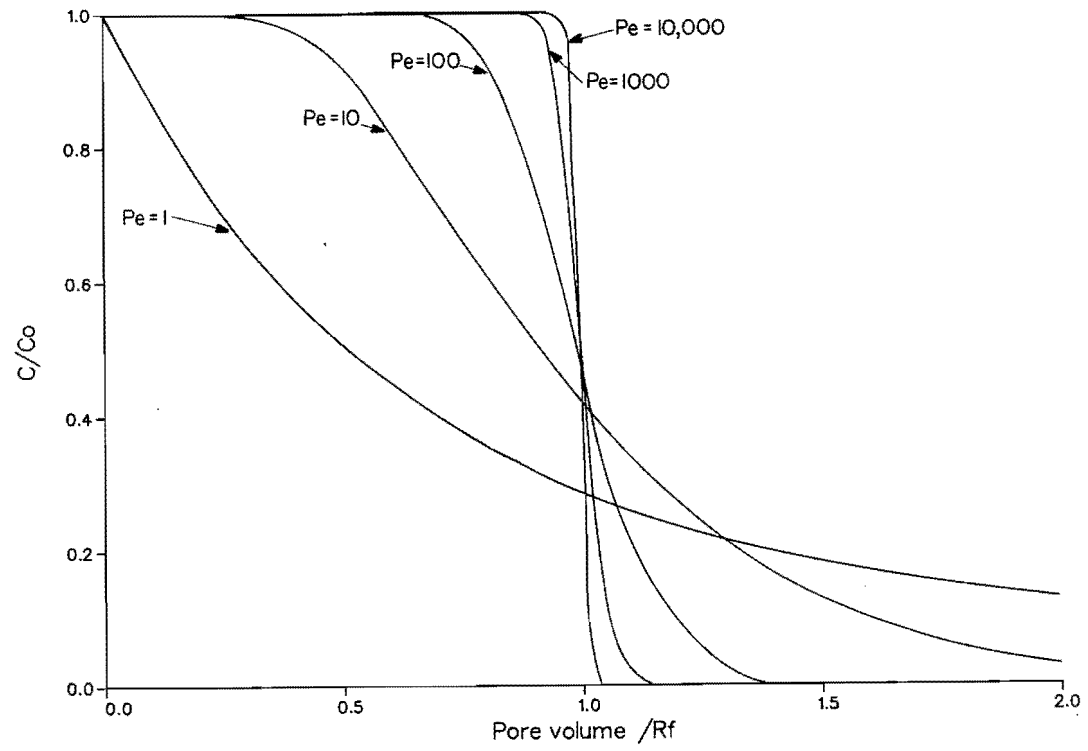

Figure 21. Predicted breakthrough curves based on various Peclet numbers.

Table 21. Mutagenic activity of spent shale leachate and other waters.

Source of Water Sample	Mutagenic Activity (Potential Mutagens/ Liter)	Ames Test Strain	S-9 Activation	Source
TOSCO II Spent Shale				
Best Prediction	17	TA98	Yes	This study
Worst Case Prediction	151	TA98	Yes	This study
Measured	33	TA98	Yes	This study
Groundwater Contaminated	11,120-23,360	TA98	Yes	1
by In Situ Oil Shale	26,240	TA98	No	1
Processing	8,160-17,680	TA100	Yes	1
	30,240	TA100	No	1
Paraho Process	103,000,000	TA98	Yes	2
Retort Water	11,000,000	TA100	Yes	2
Chlorinated Wastewater				
Tel Aviv, Israel	400	TA98	Yes	3
	380	TA100	Yes	3
Secondary Effluent.	1,000-3,000	TA100	Yes	4
Bay Park and	500-5,000	TA100	No	4
Piscataway, N.Y.	250-1,250	TA1535	Yes	4
	250-2,000	TA1535	No	4
Kempton, South Africa	9-394	TA98	Yes	5
	6-245	TA98	No	5
Advanced Wastewater	1,100-4,000	TA100	Yes	4
Treatment Plant Ef-	2,500-6,000	TA100	No	4
fluent, Bay Park and	600-2,000	TA1535	Yes	4
Piscataway, N.Y.	1,500-3,200	TA1535	No	4
Drinking Water	0-13	TA98	Yes	5
Pretoria, S.A.	0-23	TA98	No	5
Ontario, Canada	0-12	TA98	Yes	6
	0-31	TA98	No	6
	0-67	TA100	Yes	6
	0-68	TA100	No	6

Table 21. Continued.

Source of Water Sample	Mutagenic Activity (Potential Mutagens/ Liter)	Ames Test Strain	S-9 Activation	Source
Minneapolis, Minnesota	9-49	TA98	No	7
	738-898	TA100	No	7
	34-152	TA1535	No	7
Netherlands	0-15	TA98	Yes	8
	0-32	TA98	No	8
	0-103	TA100	Yes	8
	0-120	TA100	No	8
Swimming Pool Water, Victoria, Canada	10,000	TA100	Yes	9
	8,500-10,000	TA100	No	9
River Water				
Rhine River	420-645	TA98	Yes	10
Vaal River, South Africa	15-390	TA98	Yes	5
	13-213	TA98	No	5

Sources:

1. Rao et al. (1979).
2. Strinste et al. (1983).
3. Neeman et al. (1980).
3. Saxena and Schwartz (1979).
5. Grabow et al. (1980).
6. Nestmann et al. (1979).
7. Cheh et al. (1980).
8. Kool et al. (1981b).
9. Honer et al. (1980).
10. Kool et al. (1981b).

Table 22. Electrical conductivity and pH (adjusted to 25°C) of 1:4 leachate of PSS and TSS samples exposed to air.

Sample	EC (μmho/cm)		pH		Recarbonation Time (Days)
	Mean	Standard Deviation	Mean	Standard Deviation	
TSS	3270	363	11.69	0.04	0
TSS	2950	216	11.48	0.07	30
TSS	3015	416	11.37	0.04	59
PSS	3217	277	12.44	0.12	0
PSS	3193	29	12.36	0.07	30
PSS	3403	590	12.34	0.07	59

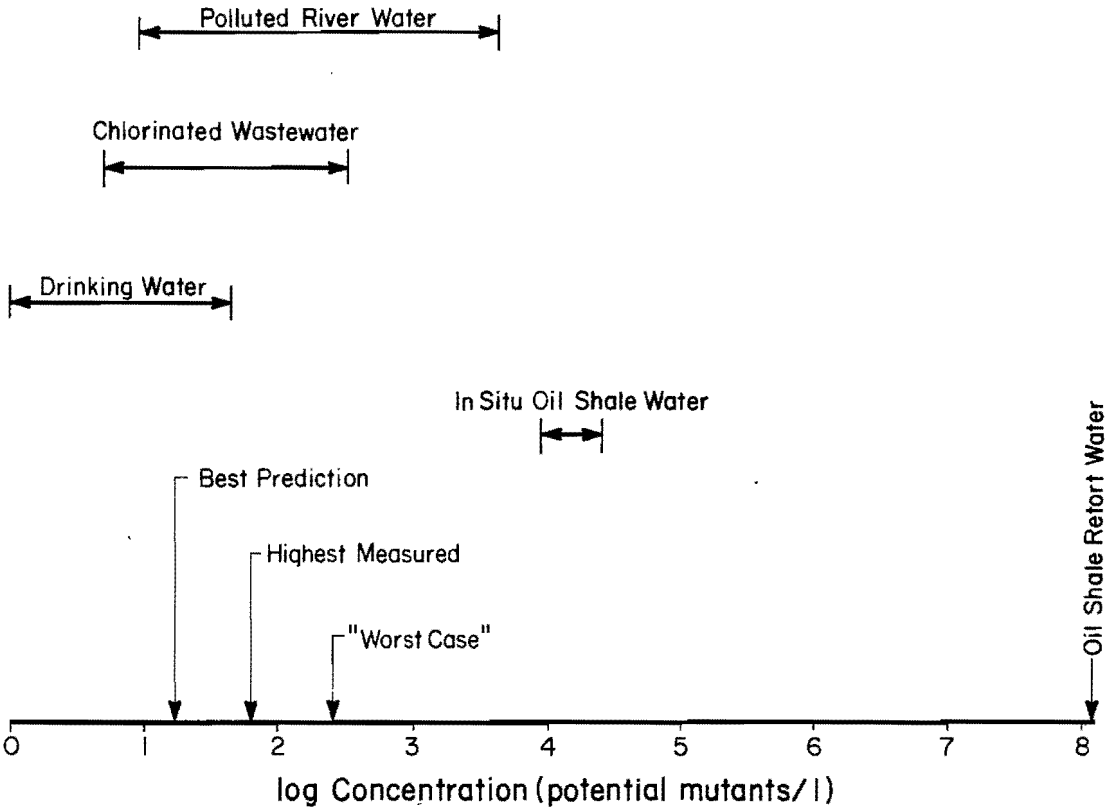

Figure 22. Relative mutagenicities of spent shale leachate and other waters (TA 98 with S-9 activation).

1978; and Campbell 1978). It has been assumed this process is reversed after cooling. That is, carbon dioxide in the atmosphere reacts with the spent shale to reform carbonates from the oxides and hydroxides, recarbonation. The pH in the spent shale leachate drops over time and exposure to the atmosphere. Harbert et al. (1979) found recarbonation of Paraho spent shale after repeated wetting and drying. The water may have accelerated the rate of CO_2 uptake. Stollenwerk and Runnells (1981) reported a pH drop from 11.7 to 9.5 during 18 months of storage of a Paraho spent shale sample; however, this experiment does not support atmospheric recarbonation as an important factor in changing spent shale leachate pH, in the time frame of this experiment.

SUMMARY AND CONCLUSIONS

At the start of this study the objective of determination of potential mutagenic contamination of groundwaters was to be accomplished by utilizing the Ames test to evaluate the mutagenicity of leachate waters. The specifics of this study are shown in Figure 23, a flow chart showing the principal test results. Starting in the upper left hand corner, the upflow column experiments showed that most of the compounds, both organic and inorganic, rapidly leached from the spent shale. This points out that due to rapidly changing leachate characteristics with leaching, for comparing shales it is necessary to obtain leachates under the same conditions. A recarbonation study was then performed to determine the potential pH change in the spent shales on exposure to the atmosphere. The results indicated that recarbonation was probably not important during the duration of this study.

Water extracts were then created for both the raw and the spent shales as well as coal at a 1:4 mixture (1 part water to 4 parts solids) in a 208 ℓ (55 gal) teflon lined drum. Of the water, 100 liters were concentrated using the XAD-2 and cascade distillation processes. The Ames test showed no measurable mutagenic response for doses of up to 600 g of shale or coal per plate for any of the samples. More concentrated aqueous extracts of TSS were then obtained. These were made to a ratio of 1:2.5 (1 part water to 2.5 parts shale). These water samples were extracted using isooctane liquid-liquid extraction and lyophilization followed by soxhlet extraction with absolute ethanol. The TSS aqueous extracts also failed to produce a positive mutagenic response with doses of up to 157.5 g of shale per plate.

The next step was to test a soxhlet extraction of the shale and coal samples. PSS and TSS samples were extracted with ethanol, benzene, and a benzene/methanol mixture. Of the two, the TSS sample showed a mutagenic response. The lack of a positive response by the PSS sample could have been due to the unusually high retort temperatures to which this shale was exposed. The RS and coal samples were also soxhlet extracted with ethanol and showed no positive mutagenic response.

The results of the soxhlet extractions indicated that only the TSS sample had an extractable mutagenic fraction. This conclusion led to a decision to further test only the TSS sample.

Using the soxhlet mutagen extract (in ethanol at a concentration of 100 g of shale extracted per ml of sample), aqueous leachates of TSS were spiked. The XAD concentration and the liquid-liquid extraction techniques were then repeated. The liquid-liquid extraction recovered a high percentage of the mutagen. The XAD technique recovered less of the mutagen but a mutagenic response was still detected. A liquid-liquid extraction of 21 ℓ showed a marginal mutagenic response.

The conclusion drawn from this testing was that the mutagen present in TSS appears to have a high affinity for

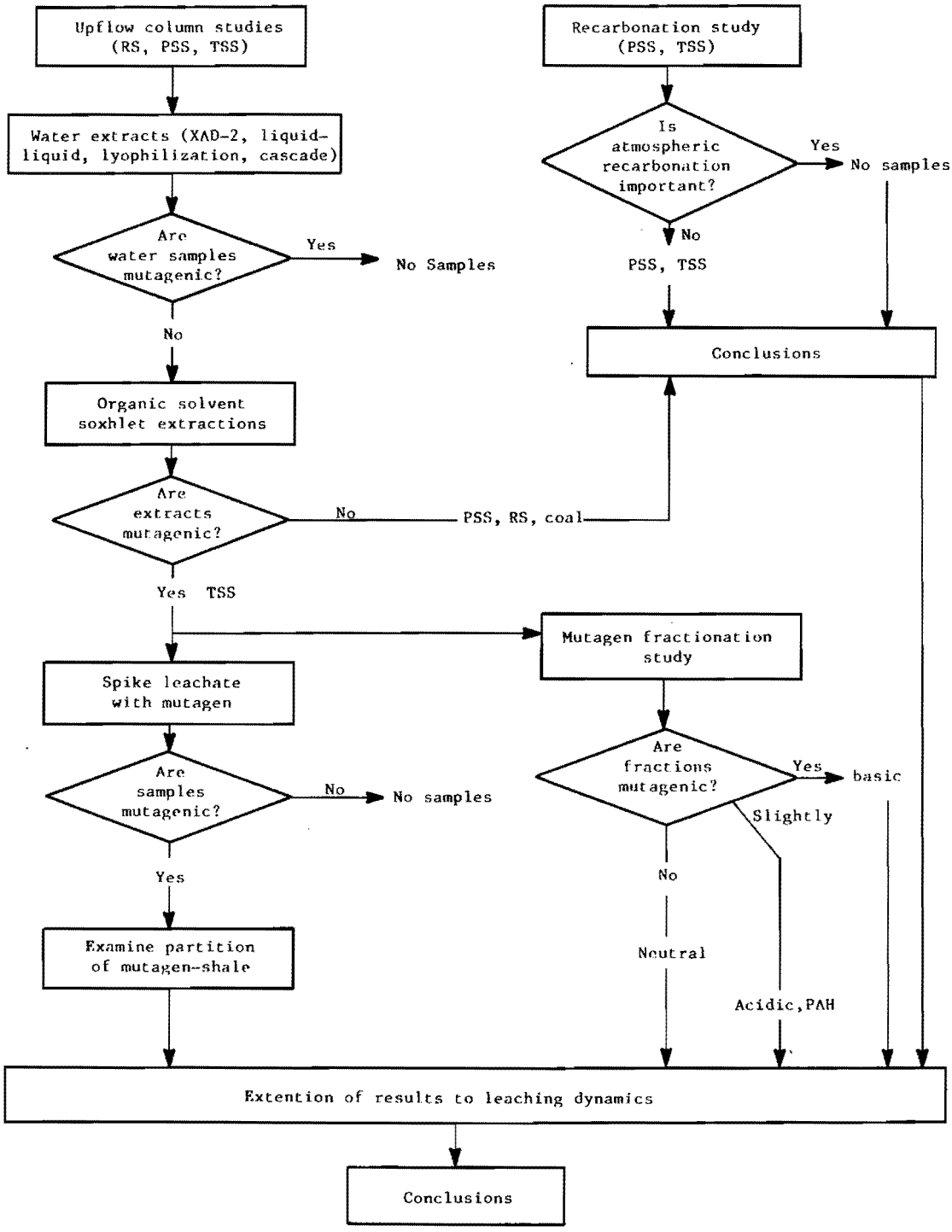

Figure 23. Summary of the methodology and results of this study.

the shale and is not readily solubilized. To further examine this point, a series of isotherm experiments were designed to determine the partition between the TSS and the aqueous leachate.

Only two of the five original strains used mutated in the presence of TSS extract. These strains, TA98 and TA1537, are both frame-shift mutants. Ames tests without S-9 activation showed no mutagenic response. During these experiments a new strain, TA97, became available. For all tests performed after this time in which TA1537 was used, TA97 was used also. The strain TA98 was the most sensitive strain used and gave more reproducible results.

The mutagenic extract recovered from the TSS sample was separated into basic, acidic, neutral, and PAH fractions. Ames testing of these fractions indicated that the basic fraction was the most mutagenic, as has been found for shale oil by other investigators.

Isotherm experiments using a 2.5:1 TSS leachate spiked with 1 ml/l (extracted from 100 g shale) of TSS mutagen extract were also performed. Based on these experiments, isotherms were estimated. A breakthrough curve was derived theoretically for a column of TSS shale with spiked leachate passing through it. The estimated breakthrough of the shale was found to be conservative. Actual breakthrough occurred later than predicted.

Using the results of the isotherm experiments, the distribution coefficient, K_d, between TSS and the leachate water for the mutagenic fraction was estimated. The potential mutagenicity of leachate from spent shale piles under a variety of hydraulic conditions was predicted based on a one-dimensional convective-dispersive equation assuming a linear retardation factor.

Based on this sequence of experiments, the following conclusions have been drawn:

1. Recarbonation of spent oil shale through contact with the atmosphere does not appear to be an important short-term phenomenon in regulating leachate pH.

2. Under the conditions described in this study the coal, RS, and PSS samples induced no measured mutagenic activity.

3. TSS induced mutagenic activity that was detected by Ames test strains TA97, TA98, and TA1535 (the frameshift mutagens), with S-9 activation.

4. The active mutagens in the TSS are primarily in the basic organic fraction as found from studies of shale oil.

5. The TSS mutagen is only sparingly soluble and has a higher affinity for the spent shale than for leachate water.

6. Under normal flow conditions, the mutagenicity of the TSS leachate will probably remain relatively constant for very long periods of time.

7. The mutagenic activity induced by the leachates produced from TSS is in the same range as reported for chlorinated wastewater

ENGINEERING SIGNIFICANCE

Both raw shales and extracting processes are highly variable. Any one sample of material cannot be considered representative of the industry as a whole nor predictive of future waste products from the industry. Contributing factors include the high inherent variability within samples associated with any given process or location, the variable nature of the various processes, and uncertainty as to the ultimate direction of an oil shale industry. Practical application of the results of the above tests must therefore rely more on observed mechanisms and principles than on specific concentrations or rates. Specific attention needs to be given to the behavior of potentially leachable contaminants in spent oil shale and mechanisms which affect leachability. Of the four materials tested (raw oil shale, a composite coal sample, and two samples of spent shale), only one of the two spent shales was found to contain mutagenic compounds. However, these compounds had a high affinity for the spent shale, and mutagenic properties were not detected in aqueous leachates from the shales. The probable causes for this high affinity for spent shale include the hydrophobic nature of the mutagen and the organic content and high specific surface area of the spent shale. These characteristics would probably be found in any spent shale. These results suggest that under a wide variety of leaching conditions some levels of mutagen will be leached from a spent shale pile. In contrast to TOC and EC, which drastically decline after only a few pore volumes, the mutagenicity of the leachate will probably remain stable for very long periods of time.

Since basic fraction organic compounds are often suspect mutagens, pH can affect their solubility and partition into leachate water. The potential for pH changes through recarbonation of the spent shale by atmospheric CO_2 was studied by passing air through dry spent shale. The pH did not change significantly. This experiment suggests that simple exposure to the atmosphere will not in the short term affect pH.

Engineering approaches to protecting ground and surface waters from mutagenic (and carcinogenic) compounds leached from spent shale need to be designed according to the mutagenicity of the particular spent shale. However, selection of an approach will require recognition that mutagenic compounds will continue to be released over a much longer period than do high discharge levels of most other pollutants. If the problem of mutagenic compounds in leachate water is addressed a longer term environmental protection effort is needed.

Because of the uncertain future of the oil shale industry and nature of its solid waste products it will be necessary to determine the potential mutagenic and carcinogenic risk of whatever waste is produced. Based on this study, it is recommended that as a minimum the following information should be obtained for any spent shale:

1. Factors effecting the hydraulic characteristics of a spent shale deposit including porosity, particle size-range, density, permeability, expected infiltration or inflow rate, and dispersivity.

2. Mutagenicity of the spent shale, utilizing an organic solvent extract (i.e., soxhlet).

3. Characteristics of the spent shale which would affect transport of the mutagenic compounds to leachate including specific surface area, internal porosity, and organic carbon content.

Based on this information a rough assessment could be made as to potential risk of spent shale leachate.

RECOMMENDATIONS FOR FURTHER RESEARCH

1. Future work on spent shale leaching should investigate the mechanism of leaching as well as the quality of leachate water. This will facilitate generalization of the findings from one shale to another.

2. Laboratory studies of the behavior of mutagens in different strengths of leachate should be instigated to determine what changes in K_d may occur as the EC and TOC of a leachate decreases.

3. Stability of the mutagenic fraction of spent shale with time needs to be investigated. That is it should be determined if the mutagens degrade with time, becoming less mutagenic, or are transformed to a more mutagenic compound?

4. Laboratory studies need to be conducted on spent shales with differing particle sizes, organic carbon content, specific surface areas and mineral content, in order to determine the effect of varying conditions of spent shale.

5. In future studies of leaching dynamics of mutagenic compounds from spent shale, tracer compounds need to be used. This will greatly simplify quantification of results and allow more extensive studies to elucidate transport mechanisms in spent shale.

6. Leaching dynamics of compounds found in retort water should be investigated to determine the effect on leachate of codisposal of retort water and leachate. This should include an examination of the compounds which are volatilized from the spent shale retort water mixture.

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APPENDICES

Appendix A

Supporting Data

RS Upflow Column Data

Pore Volume	EC (@25°C) (µmho/cm)	pH	TOC (mg/l)	TIC (mg/l)	SO_4 (mg/l)	F
0.14	1956	8.89	119	122	270	-
0.29	1726	8.95	120	109	-	35.4
0.43	1467	8.76	103	86.1	-	-
0.57	1446	8.41	93.8	76.4	294	-
0.71	1367	8.90	93.0	76.5	-	38.4
0.86	1197	8.86	69.4	63.6	-	-
1.00	1089	9.01	62.4	59.1	108	-
1.14	1038	8.94	53.7	64.4	-	30.1
1.29	960	8.96	61.1	60.1	-	-
1.43	983	8.95	-	64.2	-	-
1.57	904	8.97	31.3	61.2	-	-
1.71	899	9.33	-	61.3	-	-
1.86	794	9.01	-	-	-	26.3
2.00	712	9.10	-	55.5	35	-
2.43	449	8.85	-	23.3	-	-
2.86	472	8.30	-	19.2	-	-
3.29	303	8.95	12.9	-	-	-
3.71	191	8.94	-	-	-	-
4.14	230	9.04	-	-	-	-
4.57	173	8.15	7.8	-	-	-
5.00	146	8.30	-	-	8	-

PSS Upflow Column Data

Pore Volume	EC (@25°C) (µmho/cm)	pH	TOC (mg/l)	TIC (mg/l)	SO_4 (mg/l)
0.14	12,282	12.31	79.6	4.9	583
0.29	10,894	12.94	68.1	5.0	-
0.43	9,923	12.53	56.0	4.1	-
0.57	9,935	12.20	40.5	6.4	250
0.71	8,544	12.55	35.5	9.1	-
0.86	7,658	12.54	29.8	5.8	-
1.00	7,011	12.50	80.6[a]	3.7	180
1.14	5,630	12.41	27.4	6.5	-
1.29	5,630	12.40	-	8.3	-
1.43	5,618	12.39	18.8	10.0	-
1.57	5,256	12.31	18.6	7.7	160
1.71	5,021	12.28	17.0	8.4	-
1.86	4,578	12.14	18.4	-	-
2.00	3,396	12.32	14.85	6.8	143
2.43	2,673	12.14	11.98	34.3	-
2.86	2,460	12.03	9.54	44.9	-
3.29	1,992	11.94	5.28	-	80
3.71	1,310	11.90	5.03	-	-
4.14	1,372	11.74	7.82	-	-
4.57	1,199	11.74	4.40	-	-
5.00	951	11.24	-	-	52

[a]This value failed an outlier analysis.

TSS Upflow Column Data

Pore Volume	EC (@25°C) (µmho/cm)	pH	TOC (mg/l)	TIC (mg/l)	SO_4 (mg/l)	F
0.22	14,130	11.01	153.	35.6	-	-
0.33	15,101	11.64	154.	38.2	-	0.73
0.49	14,346	11.04	185.	48.9	-	-
0.65	14,351	11.63	157.	48.0	1319	-
0.82	12,649	11.85	169.	48.5	-	0.36
0.99	8,845	12.17	131.	22.3	486	-
1.17	6,364	12.19	106.	21.5	-	21.7
1.33	4,507	12.00	97.1	26.6	62	-
1.66	3,146	11.94	61.1	18.0	-	16.3
1.82	2,476	11.76	-	35.5	-	-
1.98	2,023	11.66	-	15.9	-	-
2.17	1,693	11.58	29.14	-	38	11.0
2.34	1,495	11.52	-	-	-	-
2.56	1,417	12.14	-	75.4	-	12.3
3.21	734	12.03	16.0	45.3	-	-
3.78	423	-	-	-	-	-
4.48	339	10.76	-	-	-	7.7
5.18	336	10.82	10.5	-	38	-
5.88	308	10.71	9.5	-	-	7.6
6.32	217	9.90	-	-	36	-

The following is the Ames test data printed in a form modified from the program of Hasselblad et al. (1980). The first line of each test usually prefixed SAMPLE: gives a brief description of the test. The second line indicates the Ames test strain used and if S-9 activation was used (+ = yes, - = no). The data then follow; dose, plate count and mean and standard deviation are shown. The dose is normally in grams of medium extracted per plate. The exception is for control tests in which the concentration of known mutagen or S-9 is given as the dose. The average slope and 95 percent confidence limits around it are also shown for some data sets. The values calculated are often shown to more significant figures than have any meaning and many standard deviations are shown for three and fewer samples. This is the result of computer output and not intended to be otherwise interpeted. The following is a list of abreviations and symbols used in the Ames test data output:

( ) = the solvent used in the Ames test is shown in brackets

ETOH = ethanol

MEOH = methanol

BOH = benzene

SAMPLE: COAL, 1:4, XAD, (DMSO) DATE: 9-26-81
STRAIN: TA98 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
600.00	GMS	43 65	54.00	15.56
60.00	GMS	66 92 52	70.00	20.30
6.00	GMS	45 72 65	60.67	14.01
0.00	GMS	57 46 65	56.00	9.54

AVERAGE SLOPE (LINEAR REGR.) = -0.012
95% CONF. LIMITS = (-0.051, 0.028)

SAMPLE: COAL, 1:4, XAD, (DMSO) DATE: 9-26-81
STRAIN: TA98 ACTIVATION:-

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
600.00	GMS	21 35 14	23.33	10.60
60.00	GMS	14 16	15.00	1.41
6.00	GMS	30 9	19.50	14.85
0.00	GMS	20 9 18	15.67	5.86

AVERAGE SLOPE (LINEAR REGR.) = 0.011
95% CONF. LIMITS = (-0.008, 0.031)

SAMPLE: COAL, 1:4, XAD, (DMSO) DATE: 9-26-81
STRAIN: TA100 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
600.00	GMS	194 119 119	144.00	43.30
60.00	GMS	143 118 140	133.67	13.65
6.00	GMS	59 113 99	90.33	28.02
0.00	GMS	122 106 167	131.67	31.63

AVERAGE SLOPE (LINEAR REGR.) = 0.047
95% CONF. LIMITS = (-0.030, 0.123)

SAMPLE: COAL, 1:4, XAD, (DMSO) DATE: 9-26-81
STRAIN: TA100 ACTIVATION:-

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
600.00	GMS	121 109 122	117.33	7.23
60.00	GMS	105 91	98.00	9.90
6.00	GMS	68 80 47	65.00	16.70
0.00	GMS	59 118 68	81.67	31.79

AVERAGE SLOPE (LINEAR REGR.) = 0.067
95% CONF. LIMITS = (0.019, 0.115)

SAMPLE: COAL, 1:4, XAD, (DMSO) DATE: 9-30-81
STRAIN: TA100 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
600.00	GMS	67 56 47 42	53.00	10.98
60.00	GMS	74 55 106 59	73.50	23.16
6.00	GMS	96 84 56 61	74.25	18.95
0.00	GMS	117 72 58 67	78.50	26.31

AVERAGE SLOPE (LINEAR REGR.) = -0.039
95% CONF. LIMITS = (-0.077, -0.001)

SAMPLE: COAL, 1:4, XAD, (DMSO) DATE: 9-26-81
STRAIN: TA1535 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
600.00	GMS	56 30 35	40.33	13.80
60.00	GMS	27 29 28	28.00	1.00
6.00	GMS	32 30 15	25.67	9.29
0.00	GMS	26 22 32	26.67	5.03

AVERAGE SLOPE (LINEAR REGR.) = 0.024
95% CONF. LIMITS = (0.006, 0.041)

SAMPLE: COAL, 1:4, XAD, (DMSO) DATE: 9-26-81
STRAIN: TA1535 ACTIVATION:-

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
600.00	GMS	4 15 19	12.67	7.77
60.00	GMS	6 12	9.00	4.24
6.00	GMS	14 8 14	12.00	3.46
0.00	GMS	19 21 14	18.00	3.61

AVERAGE SLOPE (LINEAR REGR.) = -0.002
95% CONF. LIMITS = (-0.015, 0.011)

SAMPLE: COAL, 1:4, XAD, (DMSO) DATE: 9-26-81
STRAIN: TA1537 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
600.00	GMS	56 56 35	49.00	12.12
60.00	GMS	54 31 70	51.67	19.60
6.00	GMS	58 66 80	68.00	11.14
0.00	GMS	47 41 45	44.33	3.06

AVERAGE SLOPE (LINEAR REGR.) = -0.010
95% CONF. LIMITS = (-0.044, 0.024)

SAMPLE: COAL, 1:4, XAD, (DMSO) DATE: 9-26-81
STRAIN: TA1537 ACTIVATION:-

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
600.00	GMS	17 39	28.00	15.56
60.00	GMS	20 20	20.00	0.00
6.00	GMS	31 19 14	21.33	8.74
0.00	GMS	21 26 9	18.67	8.74

AVERAGE SLOPE (LINEAR REGR.) = 0.014
95% CONF. LIMITS = (-0.009, 0.036)

SAMPLE: COAL, 1:4, XAD, (DMSO) DATE: 9-26-81
STRAIN: TA1538 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
600.00	GMS	153 125 81	119.67	36.30
60.00	GMS	52 68 59	59.67	8.02
6.00	GMS	58 53 52	54.33	3.21
0.00	GMS	109 71 32	70.67	38.50

AVERAGE SLOPE (LINEAR REGR.) = 0.099
95% CONF. LIMITS = (0.041, 0.157)

SAMPLE: COAL, 1:4, CASCADE, (WATER) DATE: 10-22-82
STRAIN: TA98 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
600.00	GMS	48 24 27	33.00	13.08
60.00	GMS	31 23 31	28.33	4.62
6.00	GMS	27 29 17	24.33	6.43
0.00	GMS	14 31 27	24.00	8.89

AVERAGE SLOPE (LINEAR REGR.) = 0.013
95% CONF. LIMITS = (-0.005, 0.032)

SAMPLE: COAL, 1:4, CASCADE, (WATER) DATE: 10-22-82
STRAIN: TA98 ACTIVATION:-

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
600.00	GMS	17 21 19	19.00	2.00
60.00	GMS	18 16 19	17.67	1.53
6.00	GMS	20 23 17	20.00	3.00
0.00	GMS	13 19 19	17.00	3.46

AVERAGE SLOPE (LINEAR REGR.) = 0.001
95% CONF. LIMITS = (-0.005, 0.007)

SAMPLE: COAL, 1:4, CASCADE, (WATER) DATE: 10-22-82
STRAIN: TA100 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
600.00	GMS	112 49	80.50	44.55
60.00	GMS	110 122 131	121.00	10.54
6.00	GMS	79 82 109	90.00	16.52
0.00	GMS	109 113 76	99.33	20.31

AVERAGE SLOPE (LINEAR REGR.) = -0.034
95% CONF. LIMITS = (-0.099, 0.031)

SAMPLE: COAL, 1:4, CASCADE, (WATER) DATE: 10-22-82
STRAIN: TA100 ACTIVATION:-

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
600.00	GMS	54 67 49	56.67	9.29
60.00	GMS	47 39	43.00	5.66
6.00	GMS	66 73 79	72.67	6.51
0.00	GMS	69 101 43	71.00	29.05

AVERAGE SLOPE (LINEAR REGR.) = -0.017
95% CONF. LIMITS = (-0.060, 0.027)

SAMPLE: COAL, 1:4, CASCADE, (WATER) DATE: 10-22-82
STRAIN: TA1535 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
600.00	GMS	17 14 21	17.33	3.51
60.00	GMS	41 27	34.00	9.90
6.00	GMS	32 19 27	26.00	6.56
0.00	GMS	33 18 31	27.33	8.14

AVERAGE SLOPE (LINEAR REGR.) = -0.018
95% CONF. LIMITS = (-0.035, -0.002)

SAMPLE: COAL, 1:4, CASCADE, (WATER) DATE: 10-22-82
STRAIN: TA1535 ACTIVATION:-

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
600.00	GMS	7 9 13	9.67	3.06
60.00	GMS	14 6 4	8.00	5.29
6.00	GMS	8 7 11	8.67	2.08
0.00	GMS	19 7 6	10.67	7.23

AVERAGE SLOPE (LINEAR REGR.) = 0.001
95% CONF. LIMITS = (-0.010, 0.011)

SAMPLE: COAL, 1:4, CASCADE, (WATER) DATE: 10-22-82
STRAIN: TA1537 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
600.00	GMS	31 19	25.00	8.49
60.00	GMS	22 29 14	21.67	7.51
6.00	GMS	21 22 27	23.33	3.21
0.00	GMS	17 28 26	23.67	5.86

AVERAGE SLOPE (LINEAR REGR.) = 0.003
95% CONF. LIMITS = (-0.012, 0.018)

SAMPLE: COAL, 1:4, CASCADE, (WATER) DATE: 10-22-82
STRAIN: TA1537 ACTIVATION:-

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
600.00	GMS	TOXIC		
60.00	GMS	32 12 14	19.33	11.02
6.00	GMS	9 17 7	11.00	5.29
0.00	GMS	14 17 13	14.67	2.08

SAMPLE: COAL, 1:4, CASCADE, (WATER) DATE: 10-22-82
STRAIN: TA1538 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
600.00	GMS	27 19 11	19.00	8.00
60.00	GMS	14 13 21	16.00	4.36
6.00	GMS	16 29	22.50	9.19
0.00	GMS	27 18 16	20.33	5.86

AVERAGE SLOPE (LINEAR REGR.) = -0.001
95% CONF. LIMITS = (-0.016, 0.014)

SAMPLE: COAL, 1:4, CASCADE, (WATER) DATE: 10-22-82
STRAIN: TA1538 ACTIVATION:-

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
600.00	GMS	4 7 9	6.67	2.52
60.00	GMS	6 13 8	9.00	3.61
6.00	GMS	11 12 9	10.67	1.53
0.00	GMS	16 5 7	9.33	5.86

AVERAGE SLOPE (LINEAR REGR.) = -0.005
95% CONF. LIMITS = (-0.013, 0.003)

SAMPLE: COAL, SOXHLET, ETOH, (ETOH) DATE: 9-16-82
STRAIN: TA97 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
10.00	GMS	105 96 112	104.33	8.02
1.00	GMS	121 115 109	115.00	6.00
0.10	GMS	147 95 119	120.33	26.03
0.00	GMS	122 124 68	104.67	31.77

AVERAGE SLOPE (LINEAR REGR.) = -0.892
95% CONF. LIMITS = (-3.638, 1,853)

SAMPLE: COAL, SOXHLET, ETOH, (ETOH) DATE: 9-16-82
STRAIN: TA97 ACTIVATION:-

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
10.00	GMS	85 84 01	56.67	48.21
1.00	GMS	84 01 53	46.00	41.94
0.10	GMS	91 73 69	77.67	11.72
0.00	GMS	73 101 71	81.67	16.77

SAMPLE: COAL, SOXHLET, ETOH, (ETOH) DATE: 9-16-82
STRAIN: TA98 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
10.00	GMS	29 42 17	29.33	12.50
1.00	GMS	23 17 21	20.33	3.06
0.10	GMS	TOXIC		
0.00	GMS	28 18 29	25.00	6.08

SAMPLE: COAL, SOXHLET, ETOH, (ETOH) DATE: 9-16-82
STRAIN: TA98 ACTIVATION:-

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
10.00	GMS	28 31	29.50	2.12
1.00	GMS	41 27 28	32.00	7.81
0.10	GMS	32 17 14	21.00	9.64
0.00	GMS	23 15 21	19.67	4.16

AVERAGE SLOPE (LINEAR REGR.) = 0.687
95% CONF. LIMITS = (-0.625, 1.999)

SAMPLE: COAL, SOXHLET, ETOH, (ETOH) DATE: 9-16-82
STRAIN: TA100 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
10.00	GMS	109 82 114	101.67	17.21
1.00	GMS	72 83 82	79.00	6.08
0.10	GMS	67 65 54	62.00	7.00
0.00	GMS	68 97 70	78.33	16.20

AVERAGE SLOPE (LINEAR REGR.) = 3.007
95% CONF. LIMITS = (1.170, 4,843)

SAMPLE: COAL, SOXHLET, ETOH, (ETOH) DATE: 9-16-82
STRAIN: TA100 ACTIVATION:-

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
10.00	GMS	TOXIC		
1.00	GMS	62 47 68	59.00	10.82
0.10	GMS	42 73 81	65.33	20.60
0.00	GMS	89 49 78	79.00	20.66

SAMPLE: COAL, SOXHLET, ETOH, (ETOH) DATE: 9-16-82
STRAIN: TA1535 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
10.00	GMS	TOXIC		
1.00	GMS	13 12 17	14.00	2.65
0.10	GMS	26 13 22	20.33	6.66
0.00	GMS	22 17 21	20.00	2.65

SAMPLE: COAL, SOXHLET, ETOH, (ETOH) DATE: 9-16-82
STRAIN: TA1535 ACTIVATION:-

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
10.00	GMS	TOXIC		
1.00	GMS	17 14 9	13.33	4.04
0.10	GMS	13 12 21	15.33	4.93
0.00	GMS	12 19 13	14.67	3.79

SAMPLE: COAL, SOXHLET, ETOH, (ETOH) DATE: 9-16-82
STRAIN: TA1537 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
10.00	GMS	TOXIC		
1.00	GMS	9 12 4	8.33	4.04
0.10	GMS	20 19 9	16.00	6.08
0.00	GMS	9 9 11	9.67	1.15

SAMPLE: COAL, SOXHLET, ETOH, (ETOH) DATE: 9-16-82
STRAIN: TA1537 ACTIVATION:-

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
10.00	GMS	4	4.00	0.00
1.00	GMS	5 4 7	5.33	1.53
0.10	GMS	6 4 3	4.33	1.53
0.00	GMS	7 5 2	4.67	2.52

AVERAGE SLOPE (LINEAR REGR.) = -0.061
95% CONF. LIMITS = (-0.434, 0.312)

SAMPLE: COAL, SOXHLET, ETOH, (ETOH) DATE: 9-16-82
STRAIN: TA1538 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
10.00	GMS	TOXIC		
1.00	GMS	TOXIC		
0.10	GMS	42 21 27	30.00	10.82
0.00	GMS	39 42 21	34.00	11.36

SAMPLE: COAL, SOXHLET, ETOH, (ETOH) DATE: 9-16-82
STRAIN: TA1538 ACTIVATION:-

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
10.00	GMS	TOXIC		
1.00	GMS	TOXIC		
0.10	GMS	7 6 11	8.00	2.65
0.00	GMS	4 7 9	6.67	2.52

SAMPLE: RS, 1:4, CASCADE, (WATER) DATE: 10-14-82
STRAIN: TA97 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
15.00	GMS	105 96 112	104.33	8.02
1.50	GMS	121 115 109	115.00	6.00
0.15	GMS	147 95 119	120.33	26.03
0.00	GMS	122 124 68	104.67	31.77

AVERAGE SLOPE (LINEAR REGR.) = -0.595
95% CONF. LIMITS = (-2.425, 1.235)

SAMPLE: RS, 1:4, CASCADE, (WATER) DATE: 10-14-82
STRAIN: TA97 ACTIVATION:-

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
15.00	GMS	85 84 60	76.33	14.15
1.50	GMS	84 60 53	65.67	16.26
0.15	GMS	91 73 69	77.67	11.72
0.00	GMS	73 101 71	81.67	16.77

AVERAGE SLOPE (LINEAR REGR.) = 0.005
95% CONF. LIMITS = (-1.353, 1.364)

SAMPLE: RS, 1:4, CASCADE, (WATER) DATE: 10-9-82
STRAIN: TA98 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
600.00	GMS	58 71 41	56.67	15.04
60.00	GMS	42 21 60	41.00	19.52
6.00	GMS	17 19 34	23.33	9.29
0.00	GMS	51 23 33	35.67	14.19

AVERAGE SLOPE (LINEAR REGR.) = 0.042
95% CONF. LIMITS = (0.008, 0.076)

SAMPLE: RS, 1:4, CASCADE, (WATER) DATE: 10-9-82
STRAIN: TA98 ACTIVATION:-

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
600.00	GMS	27 31	29.00	2.83
60.00	GMS	14 19 31	21.33	8.74
6.00	GMS	17 21 22	20.00	2.65
0.00	GMS	24 29 13	22.00	8.19

AVERAGE SLOPE (LINEAR REGR.) = 0.014
95% CONF. LIMITS = (-0.002, 0.029)

SAMPLE: RS, 1:4, CASCADE, (WATER) DATE: 10-14-82
STRAIN: TA98 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
15.00	GMS	29 42 17	29.33	12.50
1.50	GMS	23 17 21	20.33	3.06
0.15	GMS	TOXIC		
0.00	GMS	28 18 29	25.00	6.08

SAMPLE: RS, 1:4, CASCADE, (WATER) DATE: 10-14-82
STRAIN: TA98 ACTIVATION:-

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
15.00	GMS	28 31	29.50	2.12
1.50	GMS	41 27 28	32.00	7.81
0.15	GMS	32 17 14	21.00	9.64
0.00	GMS	23 15 21	19.67	4.16

AVERAGE SLOPE (LINEAR REGR.) = 0.458
95% CONF. LIMITS = (-0.417, 1.333)

SAMPLE: RS, 1:4, CASCADE, (WATER) DATE: 10-9-82
STRAIN: TA100 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
600.00	GMS	102 43 78	74.33	29.67
60.00	GMS	114 161	137.50	33.23
6.00	GMS	102 98 74	91.33	15.14
0.00	GMS	56 102 119	92.33	32.59

AVERAGE SLOPE (LINEAR REGR.) = -0.044
95% CONF. LIMITS = (-0.117, 0.029)

SAMPLE: RS, 1:4, CASCADE, (WATER) DATE: 10-9-81
STRAIN: TA100 ACTIVATION:-

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
600.00	GMS	66 42 59	55.67	12.34
60.00	GMS	78 101 42	73.67	29.74
6.00	GMS	63 58 73	64.67	7.64
0.00	GMS	99 58 72	76.33	20.84

AVERAGE SLOPE (LINEAR REGR.) = -0.027
95% CONF. LIMITS = (-0.069, 0.015)

SAMPLE: RS, 1:4, CASCADE, (WATER) DATE: 10-14-82
STRAIN: TA100 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
15.00	GMS	109 82 114	101.67	17.21
1.50	GMS	72 83 82	79.00	6.08
0.15	GMS	67 65 54	62.00	7.00
0.00	GMS	68 97 70	78.33	16.20

AVERAGE SLOPE (LINEAR REGR.) = 2.004
95% CONF. LIMITS = (0.780, 3.229)

SAMPLE: RS, 1:4, CASCADE, (WATER) DATE: 10-14-82
STRAIN: TA100 ACTIVATION:-

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
15.00	GMS	TOXIC		
1.50	GMS	62 47 68	59.00	10.82
0.15	GMS	42 73 81	65.33	20.60
0.00	GMS	89 49 78	72.00	20.66

SAMPLE: RS, 1:4, CASCADE, (WATER) DATE: 10-9-82
STRAIN: TA1535 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
600.00	GMS	12 17 19	16.00	3.61
60.00	GMS	21 14 41	25.33	14.01
6.00	GMS	7 21 23	17.00	8.72
0.00	GMS	19 14 31	21.33	8.74

AVERAGE SLOPE (LINEAR REGR.) = -0.008
95% CONF. LIMITS = (-0.029, 0.013)

SAMPLE: RS, 1:4, CASCADE, (WATER) DATE: 10-9-82
STRAIN: TA1535 ACTIVATION:-

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
600.00	GMS	8 11 13	10.67	2.52
60.00	GMS	9 19 21	16.33	6.43
6.00	GMS	27 24 7	19.33	10.79
0.00	GMS	13 17 15	15.00	2.00

AVERAGE SLOPE (LINEAR REGR.) = -0.011
95% CONF. LIMITS = (-0.025, 0.003)

SAMPLE: RS, 1:4, CASCADE, (WATER) DATE: 10-14-82
STRAIN: TA1535 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
15.00	GMS	TOXIC		
1.50	GMS	13 12 17	14.00	2.65
0.15	GMS	26 13 22	20.33	6.66
0.00	GMS	22 17 21	20.00	2.65

SAMPLE: RS, 1:4, CASCADE, (WATER) DATE: 10-14-82
STRAIN: TA1535 ACTIVATION:-

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
15.00	GMS	TOXIC		
1.50	GMS	17 14 9	13.33	4.04
0.15	GMS	13 12 21	15.33	4.93
0.00	GMS	12 19 13	14.67	3.79

SAMPLE: RS, 1:4, CASCADE, (WATER) DATE: 10-9-82
STRAIN: TA1537 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
600.00	GMS	28 31 24	27.67	3.51
60.00	GMS	24 29 26	26.33	2.52
6.00	GMS	21 29 17	22.33	6.11
0.00	GMS	9 26 24	19.67	9.29

AVERAGE SLOPE (LINEAR REGR.) = 0.009
95% CONF. LIMITS = (-0.004, 0.023)

SAMPLE: RS, 1:4, CASCADE, (WATER) DATE: 10-9-82
STRAIN: TA1537 ACTIVATION:-

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
600.00	GMS	12 7 9	9.33	2.52
60.00	GMS	4 11 6	7.00	3.61
6.00	GMS	9 7 3	6.33	3.06
0.00	GMS	3 5 12	6.67	4.73

AVERAGE SLOPE (LINEAR REGR.) = 0.005
95% CONF. LIMITS = (-0.003, 0.012)

SAMPLE: RS, 1:4, CASCADE, (WATER) DATE: 10-14-82
STRAIN: TA1537 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
15.00	GMS	TOXIC		
1.50	GMS	9 12 4	8.33	4.04
0.15	GMS	20 19 9	16.00	6.08
0.00	GMS	9 9 11	9.67	1.15

SAMPLE: RS, 1:4, CASCADE, (WATER) DATE: 10-14-82
STRAIN: TA1537 ACTIVATION:-

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
15.00	GMS	4	4.00	0.00
1.50	GMS	5 4 7	5.33	1.53
0.15	GMS	6 4 3	4.33	1.53
0.00	GMS	7 5 2	4.67	2.52

AVERAGE SLOPE (LINEAR REGR.) = -0.041
95% CONF. LIMITS = (-0.290, 0.208)

SAMPLE: RS, 1:4, CASCADE, (WATER) DATE: 10-9-82
STRAIN: TA1538 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
600.00	GMS	71 43 60	58.00	14.11
60.00	GMS	62 37 31	43.33	16.44
6.00	GMS	47 48 33	42.67	8.39
0.00	GMS	49 61 70	60.00	10.54

AVERAGE SLOPE (LINEAR REGR.) = 0.015
95% CONF. LIMITS = (-0.017, 0.046)

SAMPLE: RS, 1:4, CASCADE, (WATER) DATE: 10-9-82
STRAIN: TA1538 ACTIVATION:-

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
600.00	GMS	19 31 18	22.67	7.23
60.00	GMS	33 37 42	37.33	4.51
6.00	GMS	31 14 27	24.00	8.89
0.00	GMS	13 21 26	20.00	6.56

AVERAGE SLOPE (LINEAR REGR.) = -0.005
95% CONF. LIMITS = (-0.027, 0.017)

SAMPLE: RS, 1:4, CASCADE, (WATER) DATE: 10-14-82
STRAIN: TA1538 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
15.00	GMS	TOXIC		
1.50	GMS	TOXIC		
0.15	GMS	42 21 27	30.00	10.82
0.00	GMS	39 42 21	34.00	11.36

SAMPLE: RS, 1:4, CASCADE, (WATER) DATE: 10-14-82
STRAIN: TA1538 ACTIVATION:-

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
15.00	GMS	TOXIC		
1.50	GMS	TOXIC		
0.15	GMS	7 6 11	8.00	2.65
0.00	GMS	4 7 9	6.67	2.52

SAMPLE: RS, 1:4, XAD, (ACETONE) DATE: 9-30-82
STRAIN: TA98 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
600.00	GMS	20 45 58	41.00	19.31
60.00	GMS	39 52 46	45.67	6.51
6.00	GMS	57 66 52	58.33	7.09
0.00	GMS	45 33 47	41.67	7.57

AVERAGE SLOPE (LINEAR REGR.) = -0.013
95% CONF. LIMITS = (-0.041, 0.015)

SAMPLE: RS, 1:4, XAD, (ACETONE) DATE: 9-30-82

STRAIN: TA98 ACTIVATION:-

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
600.00	GMS	16 16 17	16.33	0.58
60.00	GMS	17 13 14	14.67	2.08
6.00	GMS	8 7	7.50	0.71
0.00	GMS	22 14 14	16.67	4.62

AVERAGE SLOPE (LINEAR REGR.) = 0.005
95% CONF. LIMITS = (-0.005, 0.015)

SAMPLE: RS, 1:4, XAD, (ACETONE) DATE: 9-30-82
STRAIN: TA100 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
600.00	GMS	105 100 86	97.00	9.85
60.00	GMS	85 85 81	83.67	2.31
6.00	GMS	71 80 58	69.67	11.06
0.00	GMS	94 80 73	82.33	10.69

AVERAGE SLOPE (LINEAR REGR.) = 0.033
95% CONF. LIMITS = (0.010, 0.056)

SAMPLE: RS, 1:4, XAD, (ACETONE) DATE: 9-30-82
STRAIN: TA100 ACTIVATION:-

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
600.00	GMS	85 98 60	81.00	19.31
60.00	GMS	47 74 65	62.00	13.75
6.00	GMS	82 73 68	74.33	7.09
0.00	GMS	81 52 53	62.00	16.46

AVERAGE SLOPE (LINEAR REGR.) = 0.025
95% CONF. LIMITS = (-0.009, 0.058)

SAMPLE: RS, 1:4, XAD, (ACETONE) DATE: 9-30-82
STRAIN: TA1535 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
600.00	GMS	40 31 40	37.00	5.20
60.00	GMS	55 39 35	43.00	10.58
6.00	GMS	40 65 46	50.33	13.05
0.00	GMS	30 52 30	37.33	12.70

AVERAGE SLOPE (LINEAR REGR.) = -0.011
95% CONF. LIMITS = (-0.036, 0.014)

SAMPLE: RS, 1:4, XAD, (ACETONE) DATE: 9-30-82
STRAIN: TA1535 ACTIVATION:-

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
600.00	GMS	17 8 22	15.67	7.09
60.00	GMS	14 17 8	13.00	4.58
6.00	GMS	14 16 5	11.67	5.86
0.00	GMS	15 6 21	14.00	7.55

AVERAGE SLOPE (LINEAR REGR.) = 0.005
95% CONF. LIMITS = (-0.009, 0.018)

SAMPLE: RS, 1:4, XAD, (ACETONE) DATE: 9-30-82
STRAIN: TA1537 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
600.00	GMS	66 43 52	53.67	11.59
60.00	GMS	44 42 33	39.67	5.86
6.00	GMS	39 42 43	41.33	2.08
0.00	GMS	53 45 43	47.00	5.29

AVERAGE SLOPE (LINEAR REGR.) = 0.018
95% CONF. LIMITS = (0.002, 0.035)

SAMPLE: RS, 1:4, XAD, (ACETONE) DATE: 9-30-82
STRAIN: TA1537 ACTIVATION:-

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
600.00	GMS	18 42 18	26.00	13.86
60.00	GMS	20 33 28	27.00	6.56
6.00	GMS	31 20 19	23.33	6.66
0.00	GMS	29 31 33	31.00	2.00

AVERAGE SLOPE (LINEAR REGR.) = -0.002
95% CONF. LIMITS = (-0.021, 0.017)

SAMPLE: RS, 1:4, XAD, (ACETONE) DATE: 9-30-82
STRAIN: TA1538 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
600.00	GMS	67 83 72	74.00	8.19
60.00	GMS	42 57	49.50	10.61
6.00	GMS	67 71 78	72.00	5.57
0.00	GMS	82 44	63.00	26.87

AVERAGE SLOPE (LINEAR REGR.) = 0.017
95% CONF. LIMITS = (-0.018, 0.051)

SAMPLE: RS, 1:4, XAD, (ACETONE) DATE: 9-30-82
STRAIN: TA1538 ACTIVATION:-

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
600.00	GMS	7 4	5.50	2.12
60.00	GMS	5 10	7.50	3.54
6.00	GMS	9	9.00	0.00
0.00	GMS	5 3 12	6.67	4.73

AVERAGE SLOPE (LINEAR REGR.) = -0.003
95% CONF. LIMITS = (-0.012, 0.006)

SAMPLE: RS, SOXLET, ETOH, (ETOH) DATE: 9-7-82
STRAIN: TA97 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
10.00	GMS	91 110 58	86.33	26.31
1.00	GMS	142 118 186	148.67	34.49
0.10	GMS	111 107 164	127.33	31.82
0.00	GMS	120 105 106	110.33	8.39

AVERAGE SLOPE (LINEAR REGR.) = -4.087
95% CONF. LIMITS = (-8.146, -0.027)

SAMPLE: RS, SOXLET, ETOH, (ETOH) DATE: 9-7-82
STRAIN: TA97 ACTIVATION:-

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
10.00	GMS	44 30 27	33.67	9.07
1.00	GMS	71 55 19	48.33	26.63
0.10	GMS	86 97 108	97.00	11.00
0.00	GMS	109 26 79	71.33	42.03

AVERAGE SLOPE (LINEAR REGR.) = -4.273
95% CONF. LIMITS = (-8.270, -0.275)

SAMPLE: RS, SOXLET, ETOH, (ETOH) DATE: 9-7-82
STRAIN: TA98 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
10.00	GMS	TOXIC		
1.00	GMS	83 46 54	61.00	19.47
0.10	GMS	21 59 31	37.00	19.70
0.00	GMS	35 27 32	31.33	4.04

SAMPLE: RS, SOXLET, ETOH, (ETOH) DATE: 9-7-82
STRAIN: TA98 ACTIVATION:-

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
10.00	GMS	TOXIC		
1.00	GMS	17 16 16	16.33	0.58
0.10	GMS	17 9 5	10.33	6.11
0.00	GMS	13 16 4	11.00	6.24

SAMPLE: RS, SOXLET, ETOH, (ETOH) DATE: 9-7-82
STRAIN: TA100 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
10.00	GMS	TOXIC		
1.00	GMS	66 62 73	67.00	5.57
0.10	GMS	59 67 56	60.67	5.69
0.00	GMS	80 58 91	76.33	16.80

SAMPLE: RS, SOXLET, ETOH, (ETOH) DATE: 9-7-82
STRAIN: TA100 ACTIVATION:-

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
10.00	GMS	TOXIC		
1.00	GMS	TOXIC		
0.10	GMS	42 72 74	62.67	17.93
0.00	GMS	101 91 93	95.00	5.29

SAMPLE: RS, SOXLET, ETOH, (ETOH) DATE: 9-16-82
STRAIN: TA100 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
10.00	GMS	102 111 64	92.33	24.95
1.00	GMS	146 101	123.50	31.82
0.10	GMS	42 79 89	70.00	24.76
0.00	GMS	68 97 70	78.33	16.20

AVERAGE SLOPE (LINEAR REGR.) = 0.930
95% CONF. LIMITS = (-3.123, 4.983)

SAMPLE: RS, SOXLET, ETOH, (ETOH) DATE: 9-7-82
STRAIN: TA1535 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
10.00	GMS	TOXIC		
1.00	GMS	37 24 32	31.00	6.56
0.10	GMS	21 30 30	27.00	5.20
0.00	GMS	16 26 13	18.33	6.81

SAMPLE: RS, SOXLET, ETOH, (ETOH) DATE: 9-7-82
STRAIN: TA1535 ACTIVATION:-

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
10.00	GMS	TOXIC		
1.00	GMS	15 8 19	14.00	5.57
0.10	GMS	4 8 19	10.33	7.77
0.00	GMS	14 5 5	8.00	5.20

SAMPLE: RS, SOXLET, ETOH, (ETOH) DATE: 9-7-82
STRAIN: TA1537 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
10.00	GMS	TOXIC		
1.00	GMS	19 14 7	13.33	6.03
0.10	GMS	16 14 17	15.67	1.53
0.00	GMS	16 13 13	14.00	1.73

SAMPLE: RS, SOXLET, ETOH, (ETOH) DATE: 9-7-82
STRAIN: TA1537 ACTIVATION:-

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
10.00	GMS	TOXIC		
1.00	GMS	TOXIC		
0.10	GMS	4 9 8	7.00	2.65
0.00	GMS	3 4 7	4.67	2.08

SAMPLE: RS, SOXLET, ETOH, (ETOH) DATE: 9-7-82
STRAIN: TA1538 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
10.00	GMS	TOXIC		
1.00	GMS	TOXIC		
0.10	GMS	TOXIC		
0.00	GMS	28 21 32	27.00	5.57

SAMPLE: RS, SOXLET, ETOH, (ETOH) DATE: 9-7-82
STRAIN: TA1538 ACTIVATION:-

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
10.00	GMS	TOXIC		
1.00	GMS	TOXIC		
0.10	GMS	TOXIC		
0.00	GMS	3 7 9	6.33	3.06

SAMPLE: TSS, 1:4, CASCADE, (WATER) DATE: 5-31-82
STRAIN: TA98 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
600.00	GMS	43 35 32	36.67	5.69
60.00	GMS	42 39 29	36.67	5.81
6.00	GMS	33 36 41	36.67	4.04
0.00	GMS	36 41 42	39.67	3.21

AVERAGE SLOPE (LINEAR REGR.) = -0.002
95% CONF. LIMITS = (-0.013, 0.009)

SAMPLE: TSS, 1:4, CASCADE, (WATER) DATE: 5-31-82
STRAIN: TA98 ACTIVATION:-

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
600.00	GMS	12 25 18	18.33	6.51
60.00	GMS	22 27 32	27.00	5.00
6.00	GMS	29 28 18	25.00	6.08
0.00	GMS	20 25 33	26.00	6.56

AVERAGE SLOPE (LINEAR REGR.) = -0.013
95% CONF. LIMITS = (-0.026, 0.000)

SAMPLE: TSS, 1:4, CASCADE, (WATER) DATE: 5-31-82
STRAIN: TA100 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
600.00	GMS	103 125 102	110.00	13.00
60.00	GMS	114 94 99	102.33	10.41
6.00	GMS	64 98 108	90.00	23.07
0.00	GMS	84 107 123	104.67	19.60

AVERAGE SLOPE (LINEAR REGR.) = 0.019
95% CONF. LIMITS = (-0.019, 0.058)

SAMPLE: TSS, 1:4, CASCADE, (WATER) DATE: 5-31-82
STRAIN: TA100 ACTIVATION:-

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
600.00	GMS	57 68 58	61.00	6.08
60.00	GMS	72 84 66	74.00	9.17
6.00	GMS	70 82 76	76.00	6.00
0.00	GMS	81 83 68	77.33	8.14

AVERAGE SLOPE (LINEAR REGR.) = -0.026
95% CONF. LIMITS = (-0.041, -0.010)

SAMPLE: TSS, 1:4, CASCADE, (WATER) DATE: 5-31-82
STRAIN: TA1535 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
600.00	GMS	28 25 26	26.33	1.53
60.00	GMS	21 20 23	21.33	1.53
6.00	GMS	19 16 26	20.33	5.13
0.00	GMS	25 22 22	23.00	1.73

AVERAGE SLOPE (LINEAR REGR.) = 0.008
95% CONF. LIMITS = (0.002, 0.015)

SAMPLE: TSS, 1:4, CASCADE, (WATER) DATE: 5-31-82
STRAIN: TA1535 ACTIVATION:-

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
600.00	GMS	11 18 14	14.33	3.51
60.00	GMS	12 13 17	14.00	2.65
6.00	GMS	17 10 16	14.33	3.79
0.00	GMS	12 17 18	15.67	3.21

AVERAGE SLOPE (LINEAR REGR.) = -0.001
95% CONF. LIMITS = (-0.008, 0.006)

SAMPLE: TSS, 1:4, CASCADE, (WATER) DATE: 5-31-82
STRAIN: TA15377 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
600.00	GMS	13 19 7	13.00	6.00
60.00	GMS	15 10 11	12.00	2.65
6.00	GMS	18 12 17	15.67	3.21
0.00	GMS	15 19 13	15.67	3.06

AVERAGE SLOPE (LINEAR REGR.) = -0.003
95% CONF. LIMITS = (-0.012, 0.006)

SAMPLE: TSS, 1:4, CASCADE, (WATER) DATE: 5-31-82
STRAIN: TA1537 ACTIVATION:-

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
600.00	GMS	TOXIC		
60.00	GMS	7 10	8.50	2.12
6.00	GMS	8 5 5	6.00	1.73
0.00	GMS	10 8 5	7.67	2.52

SAMPLE: TSS, 1:4, CASCADE, (WATER) DATE: 5-31-82
STRAIN: TA1538 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
600.00	GMS	43	43.00	0.00
60.00	GMS	45 49 60	51.33	7.77
6.00	GMS	52 59 42	51.00	8.54
0.00	GMS	64 56 42	54.00	11.14

AVERAGE SLOPE (LINEAR REGR.) = -0.016
95% CONF. LIMITS = (-0.045, 0.013)

SAMPLE: TSS, 1:4, CASCADE, (WATER) DATE: 5-31-82
STRAIN: TA1538 ACTIVATION:-

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
600.00	GMS	TOXIC		
60.00	GMS	16 21	18.50	3.54
6.00	GMS	23 19 22	21.33	2.08
0.00	GMS	27 25 23	25.00	2.00

SAMPLE: TSS, 1:4, XAD, (DMSO) DATE: 6-17-82
STRAIN: TA98 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
600.00	GMS	41 39 29	36.33	6.43
60.00	GMS	13 39 32	28.00	13.45
6.00	GMS	52 32 65	49.67	16.62
0.00	GMS	53 31 42	42.00	11.00

AVERAGE SLOPE (LINEAR REGR.) = -0.009
95% CONF. LIMITS = (-0.041, 0.023)

SAMPLE: TSS, 1:4, XAD, (DMSO) DATE: 6-17-82
STRAIN: TA98 ACTIVATION:-

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
600.00	GMS	17 21 18	18.67	2.08
60.00	GMS	22 13 14	16.33	4.93
6.00	GMS	13 9 17	13.00	4.00
0.00	GMS	15 14 17	15.33	1.53

AVERAGE SLOPE (LINEAR REGR.) = 0.007
95% CONF. LIMITS = (-0.001, 0.014)

SAMPLE: TSS, 1:4, XAD, (ETOH) DATE: 6-18-82
STRAIN: TA98 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
600.00	GMS	25 80 29	44.67	30.66
60.00	GMS	31 60 49	46.67	14.64
6.00	GMS	30 20 30	26.67	5.77
0.00	GMS	22 20 33	25.00	7.00

AVERAGE SLOPE (LINEAR REGR.) = 0.024
95% CONF. LIMITS = (-0.018, 0.065)

SAMPLE: TSS, 1:4, XAD, (ETOH) DATE: 6-18-82
STRAIN: TA98 ACTIVATION:-

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
600.00	GMS	33 45	39.00	8.49
60.00	GMS	16 21 8	15.00	6.56
6.00	GMS	17 15 17	16.33	1.15
0.00	GMS	27 21 31	26.33	5.03

AVERAGE SLOPE (LINEAR REGR.) = 0.032
95% CONF. LIMITS = (0.012, 0.052)

SAMPLE: TSS, 1:4, XAD, (DMSO) DATE: 6-17-82
STRAIN: TA100 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
600.00	GMS	102	102.00	0.00
60.00	GMS	67 112 104	94.33	24.01
6.00	GMS	97 94 99	96.67	2.52
0.00	GMS	87 109 112	102.67	13.65

AVERAGE SLOPE (LINEAR REGR.) = 0.005
95% CONF. LIMITS = (-0.047, 0.057)

SAMPLE: TSS, 1:4, XAD, (DMSO) DATE: 6-17-82
STRAIN: TA100 ACTIVATION:-

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
600.00	GMS	112 93 58	87.67	27.39
60.00	GMS	103 98 91	97.33	6.03
6.00	GMS	162 78 98	112.67	43.88
0.00	GMS	101 102 71	91.33	17.62

AVERAGE SLOPE (LINEAR REGR.) = -0.022
95% CONF. LIMITS = (-0.082, 0.037)

SAMPLE: TSS, 1:4, XAD, (ETOH) DATE: 6-18-82
STRAIN: TA100 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
600.00	GMS	172	172.00	0.00
60.00	GMS	118 140 92	116.67	24.03
6.00	GMS	81 97 53	77.00	22.27
0.00	GMS	99 92 113	101.33	10.69

AVERAGE SLOPE (LINEAR REGR.) = 0.134
95% CONF. LIMITS = (0.052, 0.216)

SAMPLE: TSS, 1:4, XAD, (ETOH) DATE: 6-18-82
STRAIN: TA100 ACTIVATION:-

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
600.00	GMS	TOXIC		
60.00	GMS	64 43	53.50	14.85
6.00	GMS	82 66 40	62.67	21.20
0.00	GMS	35 95 68	66.00	30.05

SAMPLE: TSS, 1:4, XAD, (DMSO) DATE: 6-17-82
STRAIN: TA1535 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
600.00	GMS	30 40 34	34.67	5.03
60.00	GMS	34 21 21	25.33	7.51
6.00	GMS	52 17 19	29.33	19.66
0.00	GMS	17 30 20	22.33	6.81

AVERAGE SLOPE (LINEAR REGR.) = 0.015
95% CONF. LIMITS = (-0.009, 0.040)

SAMPLE: TSS, 1:4, XAD, (DMSO) DATE: 6-17-82
STRAIN: TA1535 ACTIVATION:-

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
600.00	GMS	28 24 31	27.67	3.51
60.00	GMS	16 15 21	17.33	3.21
6.00	GMS	53 27 31	37.00	14.00
0.00	GMS	31 30 22	27.67	4.93

AVERAGE SLOPE (LINEAR REGR.) = -0.002
95% CONF. LIMITS = (-0.025, 0.022)

SAMPLE: TSS, 1:4, XAD, (ETOH) DATE: 6-18-82
STRAIN: TA1535 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
600.00	GMS	47 67 44	52.67	12.50
60.00	GMS	64 40	52.00	16.97
6.00	GMS	81 29 33	47.67	28.94
0.00	GMS	41 21 17	26.33	12.86

AVERAGE SLOPE (LINEAR REGR.) = 0.022
95% CONF. LIMITS = (-0.024, 0.068)

SAMPLE: TSS, 1:4, XAD, (ETOH) DATE: 6-18-82
STRAIN: TA1535 ACTIVATION:-

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
600.00	GMS	TOXIC		
60.00	GMS	TOXIC		
6.00	GMS	17 21 27	21.67	5.03
0.00	GMS	14 30 17	20.33	8.50

SAMPLE: TSS, 1:4, XAD, (DMSO) DATE: 6-17-82
STRAIN: TA1537 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
600.00	GMS	27 26 13	22.00	7.81
60.00	GMS	40 15 26	27.00	12.53
6.00	GMS	43 42 43	42.67	0.58
0.00	GMS	32 30 21	27.67	5.86

AVERAGE SLOPE (LINEAR REGR.) = -0.019
95% CONF. LIMITS = (-0.041, 0.003)

SAMPLE: TSS, 1:4, XAD, (DMSO) DATE: 6-17-82
STRAIN: TA1537 ACTIVATION:-

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
600.00	GMS	15 7 8	10.00	4.36
60.00	GMS	15 6 15	12.00	5.20
6.00	GMS	3 14 15	10.67	6.66
0.00	GMS	7 16 4	9.00	6.24

AVERAGE SLOPE (LINEAR REGR.) = -0.001
95% CONF. LIMITS = (-0.013, 0.011)

SAMPLE: TSS, 1:4, XAD, (ETOH) DATE: 6-18-82
STRAIN: TA1537 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
600.00	GMS	21	21.00	0.00
60.00	GMS	36 32 17	28.33	10.02
6.00	GMS	19 13 14	15.33	3.21
0.00	GMS	14 26 15	18.33	6.66

AVERAGE SLOPE (LINEAR REGR.) = 0.005
95% CONF. LIMITS = (-0.026, 0.035)

SAMPLE: TSS, 1:4, XAD, (ETOH) DATE: 6-18-82
STRAIN: TA1537 ACTIVATION:-

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
600.00	GMS	TOXIC		
60.00	GMS	29 16 19	21.33	6.81
6.00	GMS	13 11	12.00	1.41
0.00	GMS	14 18 7	13.00	5.57

SAMPLE: TSS, 1:4, XAD, (DMSO) DATE: 6-17-82
STRAIN: TA1538 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
600.00	GMS	57 47 78	60.67	15.82
60.00	GMS	71 26 52	49.67	22.59
6.00	GMS	61 58 73	64.00	7.94
0.00	GMS	46 48 72	55.33	14.47

AVERAGE SLOPE (LINEAR REGR.) = 0.006
95% CONF. LIMITS = (-0.030, 0.042)

SAMPLE: TSS, 1:4, XAD, (DMSO) DATE: 6-17-82
STRAIN: TA1538 ACTIVATION:-

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
600.00	GMS	17 27 15	19.67	6.43
60.00	GMS	43 17 32	30.33	13.01
6.00	GMS	19 16 13	16.00	3.00
0.00	GMS	18 15 14	15.67	2.08

AVERAGE SLOPE (LINEAR REGR.) = 0.000
95% CONF. LIMITS = (-0.021, 0.022)

SAMPLE: TSS, 1:4, XAD, (ETOH) DATE: 6-18-82
STRAIN: TA1538 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
600.00	GMS	TOXIC		
60.00	GMS	29 41 15	28.33	13.01
6.00	GMS	56 45 55	52.00	6.08
0.00	GMS	28 47 35	36.67	9.61

SAMPLE: TSS, 1:4, XAD, (ETOH) DATE: 6-18-82
STRAIN: TA1538 ACTIVATION:-

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
600.00	GMS	13 14	13.50	0.71
60.00	GMS	46 13 8	22.33	20.65
6.00	GMS	19 16 10	15.00	4.58
0.00	GMS	17 14 15	15.33	1.53

AVERAGE SLOPE (LINEAR REGR.) = -0.005
95% CONF. LIMITS = (-0.034, 0.023)

SAMPLE: TSS, XAD CHECK, (DMSO) DATE: 9-23-82
STRAIN: TA97 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
7.50	GMS	207 314 291	270.67	56.32
4.95	GMS	202 277 297	258.67	50.08
1.50	GMS	211 167 142	173.33	34.93
0.50	GMS	101 112	106.50	7.78
0.15	GMS	162 143 151	152.00	9.54
0.00	GMS	102 118 161 112 87 102	113.67	25.48

AVERAGE SLOPE (LINEAR REGR.) = 28.125
95% CONF. LIMITS = (19.523, 36.727)

SAMPLE: TSS, XAD CHECK, (DMSO) DATE: 9-23-82
STRAIN: TA98 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
7.50	GMS	418	418.00	0.00
4.95	GMS	512 403 407	440.67	61.81
1.50	GMS	211 299 173	227.67	64.63
0.50	GMS	64 172 122	119.33	54.05
0.15	GMS	48 32 31	37.00	9.54
0.00	GMS	27 16 19 23 29 31 21 32	24.75	5.87

AVERAGE SLOPE (LINEAR REGR.) = 84.232
95% CONF. LIMITS = (71.774, 96.691)

SAMPLE: TSS, XAD CHECK, (DMSO) DATE: 9-23-82
STRAIN: TA1537 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
7.50	GMS	844 412	628.00	305.47
4.95	GMS	312	312.00	0.00
1.50	GMS	114 67	90.50	33.23
0.50	GMS	52 14 17	27.67	21.13
0.15	GMS	19 23 4	15.33	10.02
0.00	GMS	2 8 3 9 14 2 9 4 6	6.33	4.03

AVERAGE SLOPE (LINEAR REGR.) = 79.728
95% CONF. LIMITS = (65.134, 94.321)

SAMPLE: TSS, 1:4, L/L, (ETOH) DATE: 12-19-82
STRAIN: TA98 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
157.50	GMS	65 30 57	50.76	18.34
78.75	GMS	38 29 29	32.00	5.20
15.75	GMS	26 42 35	34.33	8.02
7.88	GMS	26 21 30	25.67	4.51
1.58	GMS	29 14 40	27.67	13.05
0.16	GMS	32 20 31	27.67	6.66
0.00	GMS	32 27 27 26 32 22	27.67	3.83

AVERAGE SLOPE (LINEAR REGR.) = 0.132
95% CONF. LIMITS = (0.067, 0.198)

SAMPLE: TSS, SOXHLET, ETOH, (ETOH) DATE: 12-19-82
STRAIN: TA98 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
1.00	GMS	65 104 107	92.00	23.43
0.67	GMS	84 125 146	118.33	31.53
0.50	GMS	145 139 144	142.67	3.21
0.10	GMS	56 54 65	58.33	5.86
0.05	GMS	61 47 53	53.67	7.02
0.00	GMS	32 27 27 26 32 22	27.67	3.83

AVERAGE SLOPE (LINEAR REGR.) = 222.191
95% CONF. LIMITS = (201.527, 242.855)

SAMPLE: TSS, SOXHLET, BENZENE, (DMSO) DATE: 2-16-82
STRAIN: TA98 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
10.00	GMS	TOXIC		
1.00	GMS	78 104 80	87.33	14.47
0.10	GMS	17 22 19	19.33	2.52
0.00	GMS	28 39 31	32.67	5.69

AVERAGE SLOPE (LINEAR REGR.) = 48.97

SAMPLE: TSS, SOXHLET, BENZENE, (DMSO) DATE: 2-16-82
STRAIN: TA98 ACTIVATION:-

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
10.00	GMS	TOXIC		
1.00	GMS	19 28	23.50	6.36
0.10	GMS	12 14 21	15.67	4.73
0.00	GMS	18 17 25	20.00	4.36

SAMPLE: TSS, SOXHLET, BENZENE, (DMSO) DATE: 2-16-82
STRAIN: TA100 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
10.00	GMS	TOXIC		
1.00	GMS	44 52 45	47.00	4.36
0.10	GMS	78 82 74	78.00	4.00
0.00	GMS	83 53 70	68.67	15.04

SAMPLE: TSS, SOXHLET, BENZENE, (DMSO) DATE: 2-16-82
STRAIN: TA100 ACTIVATION:-

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
10.00	GMS	TOXIC		
1.00	GMS	TOXIC		
0.10	GMS	55 57 36	49.33	11.59
0.00	GMS	58 72 69	66.33	7.37

SAMPLE: TSS, SOXHLET, BENZENE, (DMSO) DATE: 2-16-82
STRAIN: TA1535 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
10.00	GMS	TOXIC		
1.00	GMS	28	28.00	0.00
0.10	GMS	22 23 17	20.67	3.21
0.00	GMS	27 26 19	24.00	4.36

SAMPLE: TSS, SOXHLET, BENZENE, (DMSO) DATE: 2-16-82
STRAIN: TA1537 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
10.00	GMS	TOXIC		
1.00	GMS	29 34	31.50	3.54
0.10	GMS	12 9 8	9.67	2.08
0.00	GMS	7 10 8	8.33	1.53

AVERAGE SLOPE (LINEAR REGR.) = 23.57

SAMPLE: TSS, SOXHLET, BENZENE, (DMSO) DATE: 2-16-82
STRAIN: TA1537 ACTIVATION:-

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
10.00	GMS	TOXIC		
1.00	GMS	TOXIC		
0.10	GMS	3 5	4.00	1.41
0.00	GMS	4 9 8	7.00	2.65

SAMPLE: TSS, SOXHLET, BENZENE, (DMSO) DATE: 2-16-82
STRAIN: TA1538 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
10.00	GMS	TOXIC		
1.00	GMS	TOXIC		
0.10	GMS	17 21	19.00	2.83
0.00	GMS	42 37 41	40.00	2.65

SAMPLE: TSS, SOXHLET, BENZENE, (DMSO) DATE: 2-16-82
STRAIN: TA1538 ACTIVATION:-

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
10.00	GMS	TOXIC		
1.00	GMS	TOXIC		
0.10	GMS	TOXIC		
0.00	GMS	13 17 11	13.67	3.06

SAMPLE: TSS, SOXHLET, ETOH, (ETOH) DATE: 1-9-82
STRAIN: TA98 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
8.00	GMS	TOXIC		
0.80	GMS	153 513 402	356.00	184.36
0.08	GMS	143 193 82	139.33	55.59
0.00	GMS	65 35 42	47.33	15.70

AVERAGE SLOPE (LINEAR REGR.) = 352.24

SAMPLE: TSS, SOXHLET, ETOH, (ETOH) DATE: 1-9-82
STRAIN: TA98 ACTIVATION:

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
8.00	GMS	TOXIC		
0.80	GMS	TOXIC		
0.08	GMS	TOXIC		
0.00	GMS	21 15 8	14.67	6.51

SAMPLE: TSS, SOXHLET, ETOH, (ETOH) DATE: 2-16-82
STRAIN: TA98 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
2.50	GMS	205 181 236	207.33	27.57
1.00	GMS	481 794 246	507.00	274.92
0.50	GMS	224 181 221	208.67	24.01
0.15	GMS	74 80 86	80.00	6.00
0.08	GMS	47 68 83	66.00	18.08
0.00	GMS	31 30 44	35.00	7.81

AVERAGE SLOPE (LINEAR REGR.) = 466.959
95% CONF. LIMITS = (311.138, 622.781)

SAMPLE: TSS, SOXHLET, ETOH, (ETOH) DATE: 2-20-82
STRAIN: TA98 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
8.00	GMS	48 80	64.00	22.63
0.80	GMS	434 319 349	367.33	59.65
0.08	GMS	95 78 84	85.67	8.62
0.00	GMS	40 62 35	45.67	14.36

AVERAGE SLOPE (LINEAR REGR.) = 397.780
95% CONF. LIMITS = (239.777, 555.814)

SAMPLE: TSS, SOXHLET, ETOH, (ETOH) DATE: 7-28-82
STRAIN: TA98 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
2.00	GMS	191 108 81	126.67	57.33
0.20	GMS	286 266 183	245.00	54.62
0.02	GMS	91 104 70	88.33	17.16
0.00	GMS	95 83 96	91.33	7.23

AVERAGE SLOPE (LINEAR REGR.) = -0.673
95% CONF. LIMITS = (-54.882, 53.536)

SAMPLE: TSS, SOXHLET, ETOH, (ETOH) DATE: 7-28-82
STRAIN: TA98 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
2.00	GMS	702 599	650.50	72.83
0.20	GMS	108 79 144	110.33	32.56
0.02	GMS	37 22 31	30.00	7.55
0.00	GMS	17 28 40	28.33	11.50

AVERAGE SLOPE (LINEAR REGR.) = 309.821
95% CONF. LIMITS = (284.539, 335.104)

SAMPLE: TSS, SOXHLET, ETOH, (ETOH) DATE: 1-9-82
STRAIN: TA100 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
8.00	GMS	TOXIC		
0.80	GMS	221 242 146	203.00	50.47
0.08	GMS	121 183 169	157.67	32.52
0.00	GMS	104 94 155	117.67	32.72

SAMPLE: TSS, SOXHLET, ETOH, (ETOH) DATE: 1-9-82
STRAIN: TA100 ACTIVATION:-

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
8.00	GMS	TOXIC		
0.80	GMS	TOXIC		
0.08	GMS	TOXIC		
0.00	GMS	87 85 97	89.67	6.43

SAMPLE: TSS, SOXHLET, ETOH, (ETOH) DATE: 2-16-82
STRAIN: TA100 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
2.50	GMS	117 97 74	96.00	21.52
1.00	GMS	122 106 124	117.33	9.87
0.50	GMS	123 86 146	118.33	30.27
0.15	GMS	93 108 74	91.67	17.04
0.08	GMS	106 97 79	94.00	13.75
0.00	GMS	118 100 73	97.00	22.65

AVERAGE SLOPE (LINEAR REGR.) = 49.656
95% CONF. LIMITS = (-11.258, 110.569)

SAMPLE: TSS, SOXHLET, ETOH, (ETOH) DATE: 2-20-82
STRAIN: TA100 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
8.00	GMS	84	84.00	0.00
0.80	GMS	169	169.00	0.00
0.08	GMS	151 171 186	169.33	17.56
0.00	GMS	145 147 126	139.33	11.59

AVERAGE SLOPE (LINEAR REGR.) = -8.898
95% CONF. LIMITS = (-14.647, -3.148)

SAMPLE: TSS, SOXHLET, ETOH, (ETOH) DATE: 1-9-82
STRAIN: TA1535 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
8.00	GMS	TOXIC		
0.80	GMS	47 68 44	53.00	13.08
0.08	GMS	61 55 58	58.00	3.00
0.00	GMS	60 52 41	51.00	9.54

SAMPLE: TSS, SOXHLET, ETOH, (ETOH) DATE: 1-9-82
STRAIN: TA1535 ACTIVATION:-

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
8.00	GMS	TOXIC		
0.80	GMS	14	14.00	0.00
0.08	GMS	18 13 12	14.33	3.21
0.00	GMS	17 20 20	19.00	1.73

SAMPLE: TSS, SOXHLET, ETOH, (ETOH) DATE: 1-9-82
STRAIN: TA1537 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
8.00	GMS	TOXIC		
0.80	GMS	81 47 84	70.67	20.55
0.08	GMS	33 57 47	45.67	12.06
0.00	GMS	21 51 32	34.67	15.18

AVERAGE SLOPE (LINEAR REGR.) = 40.93

SAMPLE: TSS, SOXHLET, ETOH, (ETOH) DATE: 1-9-82
STRAIN: TA1537 ACTIVATION:-

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
8.00	GMS	TOXIC		
0.80	GMS	TOXIC		
0.08	GMS	TOXIC		
0.00	GMS	19 16 17	17.33	1.53

SAMPLE: TSS, SOXHLET, ETOH, (ETOH) DATE: 2-16-82
STRAIN: TA1537 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
2.50	GMS	32 30 109	57.00	45.04
1.00	GMS	138 93 153	128.00	31.22
0.50	GMS	55 70 32	52.33	19.14
0.15	GMS	45 53 45	47.67	4.62
0.08	GMS	29 21 19	23.00	5.29
0.00	GMS	11 19 9	13.00	5.29

AVERAGE SLOPE (LINEAR REGR.) = 105.361
95% CONF. LIMITS = (79.215, 131.508)

SAMPLE: TSS, SOXHLET, ETOH, (ETOH) DATE: 2-20-82
STRAIN: TA1537 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
8.00	GMS	245 155	200.00	63.64
0.80	GMS	118 98 105	107.00	10.15
0.08	GMS	84 82 82	82.67	1.15
0.00	GMS	53 45 28	42.00	12.77

AVERAGE SLOPE (LINEAR REGR.) = 16.483
95% CONF. LIMITS = (10.104, 22.861)

SAMPLE: TSS, SOXHLET, ETOH, (ETOH) DATE: 7-28-82
STRAIN: TA1537 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
2.00	GMS	380 475 512	455.67	68.09
0.20	GMS	121 101	111.00	14.14
0.02	GMS	7 9 13	9.67	3.06
0.00	GMS	8 6 8	7.33	1.15

AVERAGE SLOPE (LINEAR REGR.) = 219.110
95% CONF. LIMITS = (190.658, 247.561)

SAMPLE: TSS, SOXHLET, (SECOND), ETOH, (ETOH) DATE: 9-7-82
STRAIN: TA97 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
10.00	GMS	119 105 122	115.33	9.07
1.00	GMS	204 155 183	180.33	24.54
0.10	GMS	113 119 108	113.33	5.51
0.00	GMS	120 105 106	110.33	8.39

AVERAGE SLOPE (LINEAR REGR.) = -1.370
95% CONF. LIMITS = (-6.025, 3.286)

SAMPLE: TSS, SOXHLET, (SECOND), ETOH, (ETOH) DATE: 9-7-82
STRAIN: TA98 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
10.00	GMS	15 28 13	18.67	8.14
1.00	GMS	98 54 59	70.33	24.09
0.10	GMS	30 46 52	42.67	11.37
0.00	GMS	32 26 35	31.00	4.58

AVERAGE SLOPE (LINEAR REGR.) = 34.824
95% CONF. LIMITS = (-3.716, 51.982)

SAMPLE: TSS, SOXHLET, (SECOND), ETOH, (ETOH) DATE: 9-7-82
STRAIN: TA100 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
10.00	GMS	102 111 64	92.33	24.95
1.00	GMS	146 101	123.50	31.82
0.10	GMS	42 79 64	61.67	18.61
0.00	GMS	68 97 70	78.33	16.20

AVERAGE SLOPE (LINEAR REGR.) = 1.272
95% CONF. LIMITS = (-2.877, 5.421)

SAMPLE: TSS, SOXHLET, (SECOND), ETOH, (ETOH) 9-7-82
STRAIN: TA1537 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
10.00	GMS	40 13 18	23.67	14.36
1.00	GMS	21 26 26	24.33	2.89
0.10	GMS	16 16 15	15.67	0.58
0.00	GMS	16 13 13	14.00	1.73

AVERAGE SLOPE (LINEAR REGR.) = 0.670
95% CONF. LIMITS = (-0.396, 1.736)

SAMPLE: TSS, SOXHLET, BENZENE/MEOH, (DMSO), 4-6-82
STRAIN: TA98 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
10.00	GMS	TOXIC		
1.00	GMS	217 233 190	213.33	21.73
0.10	GMS	48 58 42	49.33	8.08
0.00	GMS	34 23 29	28.67	5.51

AVERAGE SLOPE (LINEAR REGR.) = 183.7

SAMPLE: TSS, SOXHLET, BENZENE/MEOH, (DMSO), 4-6-82
STRAIN: TA98 ACTIVATION:-

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
10.00	GMS	TOXIC		
1.00	GMS	TOXIC		
0.10	GMS	14 13 11	12.67	1.53
0.00	GMS	21 17 19	19.00	2.00

SAMPLE: TSS, SOXHLET, BENZENE/MEOH, (DMSO), 4-6-82
STRAIN: TA100 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
10.00	GMS	TOXIC		
1.00	GMS	42 61	51.50	13.44
0.10	GMS	98 97 111	102.00	7.81
0.00	GMS	83 96 95	91.33	7.23

SAMPLE: TSS, SOXHLET, BENZENE/MEOH, (DMSO), 4-6-82
STRAIN: TA100 ACTIVATION:-

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
10.00	GMS	TOXIC		
1.00	GMS	TOXIC		
0.10	GMS	55 66 52	57.67	7.37
0.00	GMS	62 84 73	73.00	11.00

SAMPLE: TSS, SOXHLET, BENZENE/MEOH, (DMSO), 4-6-82
STRAIN: TA1535 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
10.00	GMS	TOXIC		
1.00	GMS	49 11 13	24.33	21.39
0.10	GMS	16 18 20	18.00	2.00
0.00	GMS	22 24 13	19.67	5.86

SAMPLE: TSS, SOXHLET, BENZENE/MEOH, (DMSO), 4-6-82
STRAIN: TA1535 ACTIVATION:-

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
10.00	GMS	TOXIC		
1.00	GMS	TOXIC		
0.10	GMS	9 9 6	8.00	1.73
0.00	GMS	6 3 7	5.33	2.08

SAMPLE: TSS, SOXHLET, BENZENE/MEOH, (DMSO), 4-6-82
STRAIN: TA1537 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
10.00	GMS	TOXIC		
1.00	GMS	37	37.00	0.00
0.10	GMS	16 14 15	15.00	1.00
0.00	GMS	16 13 18	15.67	2.52

SAMPLE: TSS, SOXHLET, BENZENE/MEOH, (DMSO), 4-6-82
STRAIN: TA1537 ACTIVATION:-

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
10.00	GMS	TOXIC		
1.00	GMS	TOXIC		
0.10	GMS	9 4 8	7.00	2.65
0.00	GMS	11 4 9	61.50	74.25

SAMPLE: TSS, SOXHLET, BENZENE/MEOH, (DMSO), 4-6-82
STRAIN: TA1538 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
10.00	GMS	TOXIC		
1.00	GMS	TOXIC		
0.10	GMS	16 31	23.50	10.61
0.00	GMS	43 46 48	45.67	2.52

SAMPLE: TSS, SOXHLET, BENZENE/MEOH, (DMSO), 4-6-82
STRAIN: TA1538 ACTIVATION:-

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
10.00	GMS	TOXIC		
1.00	GMS	TOXIC		
0.10	GMS	TOXIC		
0.00	GMS	22 29 17	22.67	6.03

SAMPLE: TSS, 2.5:1, ACIDIC, (ETOH) DATE: 8-4-82
STRAIN: TA98 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
130.00	GMS	36	36.00	0.00
71.50	GMS	46 33 29	36.00	8.89
13.00	GMS	25 25 32	27.33	4.04
7.15	GMS	27 29 24	26.67	2.52
1.30	GMS	22 26 36	28.00	7.21
0.00	GMS	27 21 25	24.33	3.06

AVERAGE SLOPE (LINEAR REGR.) = 0.142
95% CONF. LIMITS = (0.042, 0.241)

SAMPLE: TSS, 2.5:1, ACIDIC, (DMSO) DATE: 8-4-82
STRAIN: TA98 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
130.00	GMS	29 28 23	26.67	3.21
71.50	GMS	19 17 25	20.33	4.16
13.00	GMS	28 34 20	27.33	7.02
7.15	GMS	36 23 32	30.33	6.66
1.30	GMS	27 25 19	23.67	4.16
0.00	GMS	31 32 21	28.00	6.08

AVERAGE SLOPE (LINEAR REGR.) = -0.020
95% CONF. LIMITS = (-0.076, 0.035)

SAMPLE: TSS, 2.5:1, ACIDIC, (ETOH) DATE: 8-4-82
STRAIN: TA100 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
130.00	GMS	66 70	68.00	2.83
71.50	GMS	96 141 116	117.67	22.55
13.00	GMS	125 75 131	110.33	30.75
7.15	GMS	64 71 106	80.33	22.50
1.30	GMS	141 121 92	118.00	24.64
0.00	GMS	116 87 93	98.67	15.31

AVERAGE SLOPE (LINEAR REGR.) = -0.208
95% CONF. LIMITS = (-3.162, 2.746)

SAMPLE: TSS, 2.5:1, ACIDIC, (DMSO) DATE: 8-4-82
STRAIN: TA100 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
130.00	GMS	66 96 69	77.00	16.52
71.50	GMS	86 61	73.50	17.68
13.00	GMS	95 91 62	82.67	18.01
7.15	GMS	98 87 90	91.67	5.69
1.30	GMS	93 82 89	88.00	5.57
0.00	GMS	78 133 86	99.00	29.72

AVERAGE SLOPE (LINEAR REGR.) = -0.872
95% CONF. LIMITS = (-2.716, 0.971)

SAMPLE: TSS, 2.5:1, ACIDIC, (ETOH) DATE: 8-4-82
STRAIN: TA1537 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
130.00	GMS	TOXIC		
71.50	GMS	9 4	6.50	3.54
13.00	GMS	17 11 8	12.00	4.58
7.15	GMS	8 15 15	12.67	4.04
1.30	GMS	21 16 11	16.00	5.00
0.00	GMS	14 11 7	10.67	3.51

AVERAGE SLOPE (LINEAR REGR.) = -0.074
95% CONF. LIMITS = (-0.566, 0.418)

SAMPLE: TSS, 2.5:1, ACIDIC, (DMSO) DATE: 8-4-82
STRAIN: TA1537 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
130.00	GMS	17 14 16	15.67	1.53
71.50	GMS	16 17 13	15.33	2.08
13.00	GMS	15 8 14	12.33	3.79
7.15	GMS	19 14 11	14.67	4.04
1.30	GMS	13 14 16	14.33	1.53
0.00	GMS	6 8 9	7.67	1.53

AVERAGE SLOPE (LINEAR REGR.) = 0.197
95% CONF. LIMITS = (-0.238, 0.633)

SAMPLE: TSS, 2.5:1, BASIC, (ETOH) DATE: 8-4-82
STRAIN: TA98 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
130.00	GMS	25 20 28	24.33	4.04
71.50	GMS	32 28 32	30.67	2.31
13.00	GMS	37 27 22	28.67	7.64
7.15	GMS	24 26 30	26.67	3.06
1.30	GMS	31 27 35	31.00	4.00
0.00	GMS	27 21 25	24.33	3.06

AVERAGE SLOPE (LINEAR REGR.) = 0.095
95% CONF. LIMITS = (-0.470, 0.660)

SAMPLE: TSS, 2.5:1, BASIC, (DMSO) DATE: 8-4-82
STRAIN: TA98 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
130.00	GMS	31 31 30	30.67	0.58
71.50	GMS	37 49 28	38.00	10.54
13.00	GMS	21 31 26	26.00	5.00
7.15	GMS	48 34 32	38.00	8.72
1.30	GMS	37 23 27	29.00	7.21
0.00	GMS	31 32 21	28.00	6.08

AVERAGE SLOPE (LINEAR REGR.) = 0.113
95% CONF. LIMITS = (-0.043, 0.270)

SAMPLE: TSS, 2.5:1, BASIC, (ETOH) DATE: 8-4-82
STRAIN: TA100 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
130.00	GMS	96 72 139	102.33	33.95
71.50	GMS	72 86 89	82.33	9.07
13.00	GMS	59 93 94	82.00	19.92
7.15	GMS	215 78 97	130.00	74.22
1.30	GMS	104 89 65	86.00	19.67
0.00	GMS	116 87 93	98.67	15.31

AVERAGE SLOPE (LINEAR REGR.) = -0.022
95% CONF. LIMITS = (-0.372, 0.329

SAMPLE: TSS, 2.5:1, BASIC, (ETOH) DATE: 8-4-8
STRAIN: TA1537 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
130.00	GMS	6 8 6	6.67	1.1
71.50	GMS	4 9 3	5.33	3.21
13.00	GMS	51 13 5	23.00	24.5
7.15	GMS	7 14 9	10.00	3.61
1.30	GMS	9 13 14	12.00	2.6
0.00	GMS	14 11 7	10.67	3.51

AVERAGE SLOPE (LINEAR REGR.) = 0.820
95% CONF. LIMITS = (-0.499, 2.139)

SAMPLE: TSS, 2.5:1, BASIC, (DMSO) DATE: 8-4-82
STRAIN: TA1537 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
130.00	GMS	16 11 9	12.00	3.61
71.50	GMS	3 6 13	7.33	5.13
13.00	GMS	10 9 8	9.00	1.00
7.15	GMS	9 6 7	7.33	1.53
1.30	GMS	7 4 8	6.33	2.08
0.00	GMS	6 8 9	7.67	1.53

AVERAGE SLOPE (LINEAR REGR.) = 0.141
95% CONF. LIMITS = (-0.035, 0.316)

SAMPLE: TSS, 2.5:1, NEUTRAL, (ETOH) DATE: 8-4-82
STRAIN: TA98 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
130.00	GMS	35 28	31.50	4.95
71.50	GMS	18 25 21	21.33	3.51
13.00	GMS	30 26 17	24.33	6.66
7.15	GMS	19 25 24	22.67	3.21
1.30	GMS	24 11 15	16.67	6.66
0.00	GMS	27 21 25		

SAMPLE: TSS, 2.5:1, NEUTRAL, (DMSO) DATE: 8-4-82
STRAIN: TA98 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
130.00	GMS	14 36 39	29.67	13.65
71.50	GMS	29 38 31	32.67	4.73
13.00	GMS	25 26 28	26.33	1.53
7.15	GMS	31 37 24	30.67	6.51
1.30	GMS	24 11 26	20.33	8.14
0.00	GMS	32 31 21	28.00	6.08

AVERAGE SLOPE (LINEAR REGR.) = 0.100
95% CONF. LIMITS = (-0.020, 0.221)

SAMPLE: TSS, 2.5:1, NEUTRAL, (ETOH) DATE: 8-4-82
STRAIN: TA100 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
130.00	GMS	61 103 77	80.33	21.20
71.50	GMS	66 83 73	74.00	8.54
13.00	GMS	94 65 62	73.67	17.67
7.15	GMS	90 74 85	83.00	8.19
1.30	GMS	138 106 91	111.67	24.01
0.00	GMS	116 87 93	98.67	15.31

AVERAGE SLOPE (LINEAR REGR.) = -2.545
95% CONF. LIMITS = (-4.431, -0.659)

SAMPLE: TSS, 2.5:1, NEUTRAL, (DMSO) DATE: 8-4-82
STRAIN: TA100 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
130.00	GMS	110 104 89	101.00	10.82
71.50	GMS	97 118 89	101.33	14.98
13.00	GMS	96 66 86	82.67	15.28
7.15	GMS	37 45 37	39.67	4.62
1.30	GMS	121 107 92	106.67	14.50
0.00	GMS	78 133 86	99.00	29.72

AVERAGE SLOPE (LINEAR REGR.) = 0.211
95% CONF. LIMITS = (-0.364, 0.786)

SAMPLE: TSS, 2.5:1, NEUTRAL, (ETOH) DATE: 8-4-82
STRAIN: TA1537 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
130.00	GMS	TOXIC		
71.50	GMS	5 12 9	8.67	3.51
13.00	GMS	13 7 13	11.00	3.46
7.15	GMS	8 6 13	9.00	3.61
1.30	GMS	7 7 5	6.33	1.15
0.00	GMS	14 11 7	10.67	3.51

AVERAGE SLOPE (LINEAR REGR.) = 0.161
95% CONF. LIMITS = (-0.210, 0.531)

SAMPLE: TSS, 2.5:1, NEUTRAL, (DMSO) DATE: 8-4-82
STRAIN: TA1537 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
130.00	GMS	4 9 6	6.33	2.52
71.50	GMS	13 8 9	10.00	2.65
13.00	GMS	7 14 16	12.33	4.73
7.15	GMS	7 8 8	7.67	0.58
1.30	GMS	9 8 4	7.00	2.65
0.00	GMS	6 8 9	7.67	1.53

AVERAGE SLOPE (LINEAR REGR.) = 0.359
95% CONF. LIMITS = (0.048, 0.670)

SAMPLE: TSS, 2.5:1, PAH, (ETOH) DATE: 8-4-82
STRAIN: TA98 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
130.00	GMS	TOXIC		
71.50	GMS	36 25	30.50	7.78
13.00	GMS	24 25 15	21.33	5.51
7.15	GMS	26 26 28	26.67	1.15
1.30	GMS	25 29 31	28.33	3.06
0.00	GMS	27 21 25	24.33	3.06

AVERAGE SLOPE (LINEAR REGR.) = 0.064
95% CONF. LIMITS = (-0.042, 0.169)

SAMPLE: TSS, 2.5:1, PAH, (DMSO) DATE: 8-4-82
STRAIN: TA98 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
130.00	GMS	14 23 22	19.67	4.93
71.50	GMS	18 21 22	20.33	2.08
13.00	GMS	39 27 28	31.33	6.66
7.15	GMS	35 28 24	29.00	5.57
1.30	GMS	26 26 24	25.33	1.15
0.00	GMS	31 32 21	28.00	6.08

AVERAGE SLOPE (LINEAR REGR.) = 0.357
95% CONF. LIMITS = (-0.193, 0.906)

SAMPLE: TSS, 2.5:1, PAH, (ETOH) DATE: 8-4-82
STRAIN: TA100 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
130.00	GMS	TOXIC		
71.50	GMS	TOXIC		
13.00	GMS	46 21	33.50	17.68
7.15	GMS	73 39 53	55.00	17.09
1.30	GMS	84 93 71	82.67	11.06
0.00	GMS	116 87 93	98.67	15.31

AVERAGE SLOPE (LINEAR REGR.) = -4.867
95% CONF. LIMITS = (-6.638, -3.095)

SAMPLE: TSS, 2.5:1, PAH, (DMSO) DATE: 8-4-82
STRAIN: TA100 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
130.00	GMS	82 83 73	79.33	5.51
71.50	GMS	83 89 98	90.00	7.55
13.00	GMS	100 72 75	82.33	15.37
7.15	GMS	73 101 74	82.67	15.89
1.30	GMS	43 132 102	92.33	45.28
0.00	GMS	78 133 86	99.00	29.72

AVERAGE SLOPE (LINEAR REGR.) = -1.212
95% CONF. LIMITS = (-4.160, 1.736)

SAMPLE: TSS, 2.5:1, PAH, (ETOH) DATE: 8-4-82
STRAIN: TA1537 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
130.00	GMS	TOXIC		
71.50	GMS	4 5	4.50	0.71
13.00	GMS	7 13 14	11.33	3.79
7.15	GMS	13 8 4	8.33	4.51
1.30	GMS	12 14 14	13.33	1.15
0.00	GMS	14 11 7	10.67	3.51

AVERAGE SLOPE (LINEAR REGR.) = -0.093
95% CONF. LIMITS = (-0.499, 0.313)

SAMPLE: TSS, 2.5:1, PAH, (DMSO) DATE: 8-4-82
STRAIN: TA1537 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
130.00	GMS	8 14 12	11.33	3.06
71.50	GMS	6 6	6.00	0.00
13.00	GMS	8 14 5	9.00	4.58
7.15	GMS	4 5	4.50	0.71
1.30	GMS	7 3 12	7.33	4.51
0.00	GMS	6 8 9	7.67	1.53

AVERAGE SLOPE (LINEAR REGR.) = 0.026
95% CONF. LIMITS = (-0.007, 0.059)

SAMPLE: TSS, 2.5:1, LYOPHILIZED, (ETOH) DATE: 8-2-82
STRAIN: TA98 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
98.00	GMS	TOXIC		
9.80	GMS	139 166 138	147.67	15.08
0.98	GMS	144 155 107	135.33	25.15
0.00	GMS	146 164 121	143.67	21.59

SAMPLE: TSS, 2.5:1, LYOPHILIZED, (ETOH) DATE: 8-2-82
STRAIN: TA98 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
98.00	GMS	TOXIC		
9.80	GMS	45 45 34	41.33	6.35
0.98	GMS	29 32 34	31.67	2.52
0.00	GMS	33 35 20	29.33	8.14

SAMPLE: TSS, 2.5:1, LYOPHILIZED, (ETOH) DATE: 8-2-82
STRAIN: TA1537 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
98.00	GMS	9	9.00	0.00
9.80	GMS	7 9 4	6.67	2.52
0.98	GMS	2 11 7	6.67	4.51
0.00	GMS	6 4 9	6.33	2.52

AVERAGE SLOPE (LINEAR REGR.) = 0.026
95% CONF. LIMITS = (-0.038, -0.089)

SAMPLE: TSS, SOXHLET, ACIDIC, (ETOH) DATE: 7-30-82
STRAIN: TA97 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
2.00	GMS	34 84 39	52.33	27.54
0.20	GMS	43	43.00	0.00
0.02	GMS	76 82	79.00	4.24
0.00	GMS	96 103	99.50	4.95

AVERAGE SLOPE (LINEAR REGR.) = -15.153
95% CONF. LIMITS = (-33.133, 2.828)

SAMPLE: TSS, SOXHLET, ACIDIC, (ETOH) DATE: 7-21-82
STRAIN: TA98 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
2.00	GMS	34 45	39.50	7.78
0.20	GMS	73 84 68	75.00	8.19
0.02	GMS	85 52 56	64.33	18.01
0.00	GMS	18 17 20	18.33	1.53

AVERAGE SLOPE (LINEAR REGR.) = -4.390
95% CONF. LIMITS = (-25.984, 17.203)

SAMPLE: TSS, SOXHLET, ACIDIC, (ETOH) DATE: 7-28-82
STRAIN: TA98 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
2.00	GMS	111 76 102	96.33	18.18
0.20	GMS	31 42 36	36.33	5.51
0.02	GMS	16 42 1515	22.00	13.34
0.00	GMS	17 28 40	28.33	11.50

AVERAGE SLOPE (LINEAR REGR.) = 35.464
95% CONF. LIMITS = (27.138, 43.790)

SAMPLE: TSS, SOXHLET, ACIDIC, (DMSO) DATE: 7-30-82
STRAIN: TA98 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
2.00	GMS	13 16 21	16.67	4.04
0.20	GMS	15 9 17	13.67	4.16
0.02	GMS	22 14 9	15.00	6.56
0.00	GMS	15 14 16	15.00	1.00

AVERAGE SLOPE (LINEAR REGR.) = 1.026
95% CONF. LIMITS = (-1.730, 3.783)

SAMPLE: TSS, SOXHLET, ACIDIC, (ETOH) DATE: 7-20-82
STRAIN: TA1537 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
2.00	GMS	TOXIC		
0.20	GMS	18 19 31	22.67	7.23
0.02	GMS	29 30 44	34.33	8.39
0.00	GMS	14 27 26	22.33	7.23

SAMPLE: TSS, SOXHLET, BASIC, (ETOH) DATE: 7-30-82
STRAIN: TA97 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
2.00	GMS	TOXIC		
0.20	GMS	TOXIC		
0.02	GMS	TOXIC		
0.00	GMS	96 103	99.50	4.95

SAMPLE: TSS, SOXHLET, BASIC, (ETOH) DATE: 7-21-82
STRAIN: TA98 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
2.00	GMS	167 247 188	200.67	41.48
0.20	GMS	285 248	266.50	26.16
0.02	GMS	46 26 46	39.33	11.55
0.00	GMS	18 17 20	18.33	1.53

AVERAGE SLOPE (LINEAR REGR.) = 65.739
95% CONF. LIMITS = (-0.390, 131.868)

SAMPLE: TSS, SOXHLET, BASIC, (ETOH) DATE: 7-28-82
STRAIN: TA98 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
2.00	GMS	482 311 702	498.33	196.01
0.20	GMS	51 99 83	77.67	24.44
0.02	GMS	17 27 15	19.67	6.43
0.00	GMS	17 28 40	28.33	11.50

AVERAGE SLOPE (LINEAR REGR.) = 237.251
95% CONF. LIMITS = (176.085, 298.418)

SAMPLE: TSS, SOXHLET, BASIC, (DMSO) DATE: 7-28-82
STRAIN: TA98 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
2.00	GMS	15 8 15	12.67	4.04
0.20	GMS	15 15 22	17.33	4.04
0.02	GMS	13 16 16	15.00	1.73
0.00	GMS	15 14 16	15.00	1.00

AVERAGE SLOPE (LINEAR REGR.) = -1.496
95% CONF. LIMITS = (-3.530, 0.539)

SAMPLE: TSS, SOXHLET, BASIC, (ETOH) DATE: 7-20-82
STRAIN: TA1537 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
2.00	GMS	94	94.00	0.00
0.20	GMS	53 52 32	45.67	11.85
0.02	GMS	28 22 40	30.00	9.17
0.00	GMS	14 27 26	22.33	7.23

AVERAGE SLOPE (LINEAR REGR.) = 33.393
95% CONF. LIMITS = (21.406, 45.380)

SAMPLE: TSS, SOXHLET, NEUTRAL, (ETOH) DATE: 7-30-82
STRAIN: TA97 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
2.00	GMS	60 57	58.50	2.12
0.20	GMS	60 67	63.50	4.95
0.02	GMS	54 53 56	54.33	1.53
0.00	GMS	96 103	99.50	4.95

AVERAGE SLOPE (LINEAR REGR.) = -6.412
95% CONF. LIMITS = (-22.255, 9.430)

SAMPLE: TSS, SOXHLET, NEUTRAL, (ETOH) DATE: 7-21-82
STRAIN: TA98 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
2.00	GMS	13 19 24	18.67	5.51
0.20	GMS	23 25	24.00	1.41
0.02	GMS	14 28 14	18.67	8.08
0.00	GMS	18 17 20	18.33	1.53

AVERAGE SLOPE (LINEAR REGR.) = -0.428
95% CONF. LIMITS = (-4.054, 3.199)

SAMPLE: TSS, SOXHLET, NEUTRAL, (ETOH) DATE: 7-20-82
STRAIN: TA1537 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
2.00	GMS	35 29 39	34.33	5.03
0.20	GMS	31 35 33	33.00	2.00
0.02	GMS	29 19 28	25.33	5.51
0.00	GMS	14 27 26	22.33	7.23

AVERAGE SLOPE (LINEAR REGR.) = 4.255
95% CONF. LIMITS = (0.020, 8.490)

SAMPLE: TSS, SOXHLET, PAH, (ETOH) DATE: 7-20-82
STRAIN: TA97 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
2.00	GMS	27 29 27	31.00	5.29
0.20	GMS	42	42.00	0.00
0.02	GMS	30 28	29.00	1.41
0.00	GMS	96 103	99.50	4.95

AVERAGE SLOPE (LINEAR REGR.) = -15.362
95% CONF. LIMITS = (-37.579, 6.855)

SAMPLE: TSS, SOXHLET, PAH, (ETOH) DATE: 7-21-82
STRAIN: TA98 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
2.00	GMS	47	47.00	0.00
0.20	GMS	19	19.00	0.00
0.02	GMS	22 22 18	20.67	2.31
0.00	GMS	18 17 20	18.33	1.53

AVERAGE SLOPE (LINEAR REGR.) = 13.913
95% CONF. LIMITS = (11.481, 16.345)

SAMPLE: TSS, SOXHLET, PAH, (ETOH) DATE: 7-20-82
STRAIN: TA1537 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
2.00	GMS	39 38 46	41.00	4.36
0.20	GMS	20 19 35	24.67	8.96
0.02	GMS	31 26 31	29.33	2.89
0.00	GMS	14 27 26	22.33	7.23

AVERAGE SLOPE (LINEAR REGR.) = 7.976
95% CONF. LIMITS = (3.593, 12.360)

SAMPLE: TSS, SPIKED 2.5:1, L/L, (ETOH) DATE: 7-20-82
STRAIN: TA98 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
2.00	GMS	207 213 231	217.00	12.49
1.00	GMS	312 407 382	367.00	49.24
0.20	GMS	142 166 106	139.00	28.62
0.02	GMS	43 64 51	52.67	10.60
0.00	GMS	18 17 20	18.33	1.53

AVERAGE SLOPE (LINEAR REGR.) = 329.00
95% CONF. LIMITS = (298.020, 359.055)

SAMPLE: TSS, SPIKED 2.5:1, L/L, (ETOH) DATE: 7-20-82
STRAIN: TA97 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
2.00	GMS	312 412 297	340.33	62.52
0.20	GMS	201 114 121	145.33	48.34
0.02	GMS	128 88 91	102.33	22.28
0.00	GMS	96 103	99.50	4.95

AVERAGE SLOPE (LINEAR REGR.) = 121.775
95% CONF. LIMITS = (88.894, 145.214)

SAMPLE: TSS, SPIKED 2.5:1, L/L, (ETOH) DATE: 7-20-82
STRAIN: TA1537 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
2.00	GMS	341 142 178	220.33	106.04
1.00	GMS	282 167 144	197.67	73.93
0.20	GMS	21 17 46	28.00	15.72
0.02	GMS	19 7 21	15.67	7.57
0.00	GMS	14 27 26	22.33	7.23

AVERAGE SLOPE (LINEAR REGR.) = 100.379
95% CONF. LIMITS = (69.005, 135.863)
SAMPLE: TSS, SOXHLET, (ETOH) DATE:7-20-82
STRAIN: TA98 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
2.00	GMS	298 366 172	278.67	98.43
1.00	GMS	441 502 493	478.67	32.93
0.20	GMS	113 119 78	103.33	22.14
0.02	GMS	35 29 63	42.33	18.15
0.00	GMS	18 17 20	18.33	1.53

AVERAGE SLOPE (LINEAR REGR.) = 455.135
95% CONF. LIMITS = (425.104, 485.165)

SAMPLE: TSS, SOXHLET, (ETOH) DATE:7-20-82
STRAIN: TA1537 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
2.00	GMS	211 198 267	225.33	36.67
1.00	GMS	209 232 241	227.33	16.50
0.20	GMS	71 62 66	66.33	4.51
0.02	GMS	32 17 19	22.67	8.14
0.00	GMS	14 27 26	22.33	7.23

AVERAGE SLOPE (LINEAR REGR.) = 206.135
95% CONF. LIMITS = (192.815, 219.454)

BATCH, ISOTHERM, 2.5:1, SHALE 0.00 GRAMS, (ETOH), 8-20-82
STRAIN: TA97 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
1.25	GMS	312 129 276	239.00	96.95
0.82	GMS	204 212 177	197.67	18.34
0.25	GMS	162 138 139	146.33	13.58
0.08	GMS	111 172 132	138.33	30.99
0.03	GMS	98 101 71	90.00	16.52
0.00	GMS	92 102 118	104.00	13.11

AVERAGE SLOPE (LINEAR REGR.) = 106.249
95% CONF. LIMITS = (66.017, 146.480)

BATCH, ISOTHERM, 2.5:1, SHALE 0.00 GRAMS, (ETOH), 8-20-82
STRAIN: TA98 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
1.25	GMS	204 191 384	259.67	107.87
0.82	GMS	110 94 77	93.67	16.50
0.25	GMS	50 44 47	47.00	3.00
0.08	GMS	30 52 42	41.33	11.02
0.03	GMS	48 20 35	34.33	14.01
0.00	GMS	27 22 40	29.67	9.29

AVERAGE SLOPE (LINEAR REGR.) = 160.430
95% CONF. LIMITS = (109.765, 211.094)

BATCH, ISOTHERM, 2.5:1, SHALE 0.00 GRAMS, (ETOH), 8-20-82
STRAIN: TA1537 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
1.25	GMS	399	399.00	0.00
0.82	GMS	295 252 114	220.33	94.56
0.25	GMS	182 157 139	159.33	21.59
0.08	GMS	22 83 38	47.67	31.63
0.03	GMS	21 12 26	19.67	7.09
0.00	GMS	27 18 19	21.33	4.93

AVERAGE SLOPE (LINEAR REGR.) = 267.684
95% CONF. LIMITS = (200.364, 335.004)

BATCH, ISOTHERM, 2.5:1, SHALE 1.00 GRAMS, (ETOH), 8-20-82
STRAIN: TA97 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
1.25	GMS	176 194 248	206.00	37.47
0.82	GMS	181 201 152	178.00	24.64
0.25	GMS	162 109 78	116.33	42.48
0.08	GMS	78 94 93	88.33	8.96
0.03	GMS	111 126 97	111.33	14.50
0.00	GMS	92 102 118	104.00	13.11

AVERAGE SLOPE (LINEAR REGR.) = 88.591
95% CONF. LIMITS = (63.394, 113.787)

BATCH, ISOTHERM, 2.5:1, SHALE 1.00 GRAMS, (ETOH), 8-20-82
STRAIN: TA98 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
1.25	GMS	131 97 102	110.00	18.36
0.82	GMS	40 51 47	46.00	5.57
0.25	GMS	32 27 31	30.00	2.65
0.08	GMS	31 17 19	22.33	7.57
0.03	GMS	17 31 23	23.67	7.02
0.00	GMS	27 22 40	29.67	9.29

AVERAGE SLOPE (LINEAR REGR.) = 59.764
95% CONF. LIMITS = (44.218, 75.311)

BATCH, ISOTHERM, 2.5:1, SHALE 1.00 GRAMS, (ETOH), 8-20-82
STRAIN: TA1537 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
1.25	GMS	270	270.00	0.00
0.82	GMS	243 153	198.00	63.64
0.25	GMS	90 63 67	73.33	14.57
0.08	GMS	67 30 14	37.00	27.18
0.03	GMS	26 16 12	18.00	7.21
0.00	GMS	27 18 19	21.33	4.93

AVERAGE SLOPE (LINEAR REGR.) = 208.705
95% CONF. LIMITS = (177.841, 239.569)

BATCH, ISOTHERM, 2.5:1, SHALE 10.0 GRAMS, (ETOH), 8-20-82
STRAIN: TA97 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
1.25	GMS	113 97 120	110.00	11.79
0.82	GMS	105 141 109	118.33	19.73
0.25	GMS	142 111 112	121.67	17.62
0.08	GMS	109 94 111	104.67	9.29
0.03	GMS	103 117 97	105.67	10.26
0.00	GMS	92 102 118	104.00	13.11

AVERAGE SLOPE (LINEAR REGR.) = 71.771
95% CONF. LIMITS = (1.487, 142.055)

BATCH, ISOTHERM, 2.5:1, SHALE 10.0 GRAMS, (ETOH), 8-20-82
STRAIN: TA98 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
1.25	GMS	48 51 37	45.33	7.37
0.82	GMS	21 29 38	29.33	8.50
0.25	GMS	18 21 28	22.33	5.13
0.08	GMS	36 27 31	31.33	4.51
0.03	GMS	46 32 42	40.00	7.21
0.00	GMS	27 22 40	29.67	9.29

AVERAGE SLOPE (LINEAR REGR.) = 7.357
95% CONF. LIMITS = (-2.134, 16.849)

BATCH, ISOTHERM, 2.5:1, SHALE 10.0 GRAMS, (ETOH), 8-20-82
STRAIN: TA1537 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
1.25	GMS	16 19	17.50	2.12
0.82	GMS	62 23 13	32.67	25.89
0.25	GMS	15 13 15	14.33	1.15
0.08	GMS	13 33 8	18.00	13.23
0.03	GMS	22 10 26	19.33	8.33
0.00	GMS	27 18 19	21.33	4.93

AVERAGE SLOPE (LINEAR REGR.) = 15.952
95% CONF. LIMITS = (-5.426, 37.330)

BATCH, ISOTHERM, 2.5:1, SHALE 50.0 GRAMS, (ETOH), 8-20-82
STRAIN: TA97 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
1.25	GMS	78 59 68	68.33	9.50
0.82	GMS	102 112 76	96.67	18.58
0.25	GMS	43 65 102	70.00	29.82
0.08	GMS	99 94 89	94.00	5.00
0.03	GMS	73 42 76	63.67	18.82
0.00	GMS	92 102 118	104.00	13.11

AVERAGE SLOPE (LINEAR REGR.) = -73.619
95% CONF. LIMITS = (-213.549, 66.310)

BATCH, ISOTHERM, 2.5:1, SHALE 50.0 GRAMS, (ETOH), 8-20-82
STRAIN: TA98 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
1.25	GMS	18 22 30	23.33	6.11
0.82	GMS	20 22 21	21.00	1.00
0.25	GMS	14 22 27	21.00	6.56
0.08	GMS	28 19 8	18.33	10.02
0.03	GMS	33 43 24	33.33	9.50
0.00	GMS	27 22 40	29.67	9.29

BATCH, ISOTHERM, 2.5:1, SHALE 50.0 GRAMS, (ETOH), 8-20-82
STRAIN: TA1537 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
1.25	GMS	29 42 15	28.67	13.50
0.82	GMS	17 26 18	20.33	4.93
0.25	GMS	20 25 24	23.00	2.65
0.08	GMS	24 18 4	15.33	10.26
0.03	GMS	12 15 27	18.00	7.94
0.00	GMS	27 18 19	21.33	4.93

AVERAGE SLOPE (LINEAR REGR.) = 6.572
95% CONF. LIMITS = (-1.168, 14.312)

TSS, SOXHLET, (ETOH) DATE:8-20-82
STRAIN: TA97 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
1.25	GMS	105 97 95	99.00	5.29
0.82	GMS	222 309 317	282.67	52.69
0.25	GMS	117 111 94	107.33	11.93
0.08	GMS	104 116 93	104.33	11.50
0.03	GMS	102 101 123	108.67	12.42
0.00	GMS	92 102 118	104.00	13.11

TSS, SOXHLET, (ETOH) DATE:8-20-82
STRAIN: TA98 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
1.25	GMS	118 192 117	142.33	43.02
0.82	GMS	204 306 271	260.33	51.83
0.25	GMS	144 142 88	124.67	31.77
0.08	GMS	58 74 54	62.00	10.58
0.03	GMS	32 38 41	37.00	4.58
0.00	GMS	27 22 40	29.67	9.29

AVERAGE SLOPE (LINEAR REGR.) = 277.131
95% CONF. LIMITS = (232.253, 322.008)

TSS, SOXHLET, (ETOH) DATE:8-20-82
STRAIN: TA1537 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
1.25	GMS	356 355 197	302.67	91.51
0.82	GMS	98 62 57	72.33	22.37
0.25	GMS	66 21 51	46.00	22.91
0.08	GMS	64 23 18	35.00	25.24
0.03	GMS	24 17 25	22.00	4.36
0.00	GMS	27 18 19	21.33	4.93

AVERAGE SLOPE (LINEAR REGR.) = 188.795
95% CONF. LIMITS = (128.980, 248.609)

BATCH, ISOTHERM, 2.5:1, SHALE 100, GRAMS, (ETOH), 8-20-82
STRAIN: TA97 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
1.25	GMS	66 113 101	93.33	24.42
0.82	GMS	99 83 92	91.33	8.02
0.25	GMS	62 87 144	97.67	42.03
0.08	GMS	49 77 113	79.67	32.08
0.03	GMS	85 96 43	74.67	27.97
0.00	GMS	92 102 118	104.00	13.11

AVERAGE SLOPE (LINEAR REGR.) = 27.339
95% CONF. LIMITS = (-152.307, 206.986)

BATCH, ISOTHERM, 2.5:1, SHALE 100, GRAMS, (ETOH), 8-20-82
STRAIN: TA98 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
1.25	GMS	29 22 21	24.00	4.36
0.82	GMS	31 29 18	26.00	7.00
0.25	GMS	7 31 28	22.00	13.08
0.08	GMS	32 28 27	29.00	2.65
0.03	GMS	40 18 16	24.67	13.32
0.00	GMS	27 22 40	29.67	9.29

AVERAGE SLOPE (LINEAR REGR.) = -23.829
95% CONF. LIMITS = (-81.212, 33.555)

BATCH, ISOTHERM, 2.5:1 (NO SPIKE), (ETOH) DATE:8-20-82
STRAIN: TA97 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
1.25	GMS	99 74 104	92.33	16.07
0.82	GMS	39 112 137	96.00	50.92
0.25	GMS	114 139 142	131.67	15.37
0.08	GMS	107 119 82	102.67	18.88
0.03	GMS	64 109 113	95.33	27.21
0.00	GMS	92 102 118	104.00	13.11

AVERAGE SLOPE (LINEAR REGR.) = 131.940
95% CONF. LIMITS = (23.989, 239.891)

BATCH, ISOTHERM, 2.5:1 (NO SPIKE), (ETOH) DATE: 8-20-82
STRAIN: TA98 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
1.25	GMS	33 20 32	28.33	7.23
0.82	GMS	31 21 42	31.33	10.50
0.25	GMS	26 16 19	20.33	5.13
0.08	GMS	31 21 42	31.33	10.50
0.03	GMS	31 34 22	29.00	6.24
0.00	GMS	27 22 40	29.67	9.29

BATCH, ISOTHERM, 2.5:1 (NO SPIKE), (ETOH) DATE: 8-20-82
STRAIN: TA1537 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
1.25	GMS	22 6 23	17.00	9.54
0.82	GMS	17 37 33	29.00	10.58
0.25	GMS	26 25 39	30.00	7.81
0.08	GMS	16 13 19	16.00	3.00
0.03	GMS	26 25 17	22.67	4.93
0.00	GMS	27 18 19	21.33	4.93

AVERAGE SLOPE (LINEAR REGR.) = 35.359
95% CONF. LIMITS = (-1.858, 72.576)

BATCH, ISOTHERM, DDW, SHALE 0 GMS DATE:9-10-82
STRAIN: TA98 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
1.25	GMS	692 471 409	524.00	148.76
0.83	GMS	318 271 342	310.33	36.12
0.25	GMS	136 111 109	118.67	15.04
0.08	GMS	80 72 81	77.67	4.93
0.03	GMS	48 52 53	51.00	2.65
0.00	GMS	42 66 35 40 47 54 45 34	45.38	10.56

AVERAGE SLOPE (LINEAR REGR.) = 368.
95% CONF. LIMITS = (321, 415)

BATCH, ISOTHERM, DDW, SHALE .25 GMS DATE:9-10-82
STRAIN: TA98 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
1.25	GMS	617 309 598	508.00	172.60
0.83	GMS	405 352 511	422.67	80.96
0.25	GMS	97 104 97	99.33	4.04
0.08	GMS	38 59 45	47.33	10.69
0.03	GMS	28 58 47	44.33	15.18
0.00	GMS	42 66 35 40 47 54 45 34	45.38	10.56

AVERAGE SLOPE (LINEAR REGR.) = 402
95% CONF. LIMITS = (340, 463)

BATCH, ISOTHERM, DDW, SHALE .50 GMS DATE:9-10-82
STRAIN: TA98 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
1.25	GMS	459 311 498	422.67	98.65
0.83	GMS	311 209 378	299.33	85.10
0.25	GMS	118 102 101	107.00	9.54
0.08	GMS	22 45 41	36.00	12.29
0.03	GMS	54 58 22	44.67	19.73
0.00	GMS	42 66 35 40 47 54 45 34	45.38	10.56

AVERAGE SLOPE (LINEAR REGR.) = 311
95% CONF. LIMITS = (271, 351)

BATCH, ISOTHERM, DDW, SHALE 1.0 GMS DATE:9-10-82
STRAIN: TA98 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
1.25	GMS	141 167 102	136.67	32.72
0.82	GMS	91 108 74	91.00	17.00
0.25	GMS	64 37 42	47.67	14.36
0.08	GMS	56 47 57	53.33	5.51
0.03	GMS	40 41 43	41.33	1.53
0.00	GMS	42 66 35 40 47 54 45 34	45.38	10.56

AVERAGE SLOPE (LINEAR REGR.) = 69.730
95% CONF. LIMITS = (55.406, 84.054)

BATCH, ISOTHERM, DDW, SHALE 2.5 GMS DATE:9-10-82
STRAIN: TA98 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
1.25	GMS	62 89 76	75.67	13.50
0.82	GMS	62 41 47	50.00	10.82
0.25	GMS	43 21 27	30.33	11.37
0.08	GMS	66 31 33	43.33	19.66
0.03	GMS	17 49 24	30.00	16.82
0.00	GMS	42 66 35 40 47 54 45 34	45.38	10.56

AVERAGE SLOPE (LINEAR REGR.) = 23.738
95% CONF. LIMITS = (9.994, 37.482)

BATCH, ISOTHERM, DDW, SHALE 5.0 GMS DATE:9-10-82
STRAIN: TA98 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
1.25	GMS	31 21 42	31.33	10.50
0.82	GMS	26 41 33	33.33	7.51
0.25	GMS	17 42 14	24.33	15.37
0.08	GMS	63 41 39	47.67	13.32
0.03	GMS	43 44 52	46.33	4.93
0.00	GMS	42 66 35 40 47 54 45 34	45.38	10.56

AVERAGE SLOPE (LINEAR REGR.) = -82.845
95% CONF. LIMITS = (-141.912, -23.778)

BATCH, ISOTHERM, DDW, SHALE 10.0 GMS DATE:9-10-82
STRAIN: TA98 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
1.25	GMS	46 40 28	38.00	9.17
0.82	GMS	39 47 51	45.67	6.11
0.25	GMS	55 32 57	48.00	13.89
0.08	GMS	21 39 39	33.00	10.39
0.03	GMS	69 34 41	48.00	18.52
0.00	GMS	42 66 35 40 47 54 45 34	45.38	10.56

AVERAGE SLOPE (LINEAR REGR.) = 3.175
95% CONF. LIMITS = (-65.862, 72.213)

BATCH, ISOTHERM, 2.5:1, FLASK WASH DATE:9-18-82
STRAIN: TA98 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
1.25	GMS	96 43 39	80.00	32.14
0.82	GMS	72 81 39	64.00	22.11
0.25	GMS	42 44 57	47.67	8.14
0.08	GMS	26 32 33	30.33	3.79
0.03	GMS	61 63 74	66.00	7.00
0.00	GMS	85 56 55 42	59.50	18.16

BATCH, ISOTHERM, 2.5:1, SHALE 0 GMS DATE:9-18-82
STRAIN: TA98 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
1.25	GMS	497 513 591	533.67	50.29
0.82	GMS	342 298 409	349.67	55.90
0.25	GMS	201 173 129	167.67	36.30
0.08	GMS	102 107 111	106.67	4.51
0.03	GMS	60 82 66	69.33	11.37
0.00	GMS	85 56 55 42	59.50	18.16

AVERAGE SLOPE (LINEAR REGR.) = 367.153
95% CONF. LIMITS = (335.828, 398.479)

BATCH, ISOTHERM, 2.5:1, SHALE .25 GMS DATE:9-18-82
STRAIN: TA98 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
1.25	GMS	661 521 477	553.00	96.08
0.82	GMS	377 204 311	297.33	87.31
0.25	GMS	199 108 136	147.67	46.61
0.08	GMS	113 117 124	118.00	5.57
0.03	GMS	60 82	71.00	15.56
0.00	GMS	85 56 55 42	59.50	18.16

AVERAGE SLOPE (LINEAR REGR.) = 361.894
95% CONF. LIMITS = (301.982, 421.806)

BATCH, ISOTHERM, 2.5:1, SHALE .5 GMS DATE:9-18-82
STRAIN: TA98 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
1.25	GMS	423 612 407	480.67	114.02
0.82	GMS	222 301 241	254.67	41.24
0.25	GMS	109 152 143	134.67	22.68
0.08	GMS	78 47 72	65.67	16.44
0.03	GMS	55 68 61	61.33	6.51
0.00	GMS	85 56 55 42	59.50	18.16

AVERAGE SLOPE (LINEAR REGR.) = 317.892
95% CONF. LIMITS = (267.009, 368.776)

BATCH, ISOTHERM, 2.5:1, SHALE 1.0 GMS DATE:9-18-82
STRAIN: TA98 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
1.25	GMS	321 324 271	305.33	29.77
0.82	GMS	131 171 144	148.67	20.40
0.25	GMS	102 91 117	103.33	13.05
0.08	GMS	94 101 98	97.67	3.51
0.03	GMS	83 83	83.00	0.00
0.00	GMS	85 56 55 42 29 66 65	53.63	19.00

AVERAGE SLOPE (LINEAR REGR.) = 170.365
95% CONF. LIMITS = (141.097, 199.633)

BATCH, ISOTHERM, 2.5:1, SHALE 2.5 GMS DATE:9-18-82
STRAIN: TA98 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
1.25	GMS	191 107 132	143.33	43.13
0.82	GMS	101 93 81	91.67	10.07
0.25	GMS	57 86 108	83.67	25.58
0.08	GMS	86 72 97	85.00	12.53
0.03	GMS	57 46 42	48.33	7.77
0.00	GMS	85 56 55 42 29 66 65	53.63	19.00

AVERAGE SLOPE (LINEAR REGR.) = 63.344
95% CONF. LIMITS = (40.675, 86.013)

BATCH, ISOTHERM, 2.5:1, SHALE 5.0 GMS DATE:9-18-82
STRAIN: TA98 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
1.25	GMS	102 109 76	95.67	17.39
0.82	GMS	91 96 94	93.67	2.52
0.25	GMS	86 85 83	84.67	1.53
0.08	GMS	106 73 83	87.33	16.92
0.03	GMS	65 68 60	64.33	4.04
0.00	GMS	85 56 55 42 29 66 65	53.63	19.00

AVERAGE SLOPE (LINEAR REGR.) = 43.188
95% CONF. LIMITS = (15.555, 70.821)

BATCH, ISOTHERM, 2.5:1, SHALE 10.0 GMS DATE:9-18-82
STRAIN: TA98 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
1.25	GMS	54 61 57	57.33	3.51
0.82	GMS	69 74 52	65.00	11.53
0.25	GMS	40 62 56	52.67	11.37
0.08	GMS	35 63 55	51.00	14.42
0.03	GMS	72 42 60	58.00	15.10
0.00	GMS	85 56 55 42 29 66 85 31	56.13	21.82

AVERAGE SLOPE (LINEAR REGR.) = 10.534
95% CONF. LIMITS = (-15.396, 36.464)

TSS, SOXHLET DATE:9-18-82
STRAIN: TA98 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
1.25	GMS	654 473	563.50	127.99
0.82	GMS	401 332 331	354.67	40.13
0.25	GMS	309 199 207	238.33	61.33
0.08	GMS	114 147 151	137.33	20.31
0.03	GMS	67 74 72	71.00	3.61
0.00	GMS	85 56 55 42 29 66 65	53.63	19.00

AVERAGE SLOPE (LINEAR REGR.) = 381.247
95% CONF. LIMITS = (326.640, 435.854)

SAMPLE: COLUMN, SORPTION, SAMPLE 1, (ETOH) DATE: 9-29-82
STRAIN: TA98 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
0.64	GMS	38 26 15	26.33	11.50
0.42	GMS	27 22 29	26.00	3.61
0.13	GMS	32 24 37	31.00	6.56
0.04	GMS	21 35 31	29.00	7.21
0.01	GMS	38 36 25	33.00	7.00
0.00	GMS	28 34 27 41 32 19	30.17	7.41

AVERAGE SLOPE (LINEAR REGR.) = 1.844

SAMPLE: COLUMN, SORPTION, SAMPLE 2, (ETOH) DATE: 9-29-82
STRAIN: TA98 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
0.67	GMS	21 21 30	24.00	5.20
0.44	GMS	22 36 35	31.00	7.81
0.13	GMS	58 23 29	36.67	18.72
0.04	GMS	29 27 38	31.33	5.86
0.01	GMS	37 33 29	33.00	4.00
0.00	GMS	28 34 27 41 32 19	30.17	7.41

AVERAGE SLOPE (LINEAR REGR.) = .552
95% CONF. LIMITS = (-14.348, 15.451)

SAMPLE: COLUMN, SORPTION, SAMPLE 3, (ETOH) DATE: 9-29-82
STRAIN: TA98 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
0.64	GMS	59 76 46	60.33	15.04
0.42	GMS	29 28 36	31.00	4.36
0.13	GMS	17 34 38	29.67	11.15
0.04	GMS	24 28 33	28.33	4.51
0.01	GMS	48 30 29	35.67	10.69
0.00	GMS	28 34 27 41 32 19	30.17	7.41

AVERAGE SLOPE (LINEAR REGR.) = 35.246
95% CONF. LIMITS = (25.205, 45.288)

SAMPLE: COLUMN, SORPTION, SAMPLE 4, (ETOH) DATE: 9-29-82
STRAIN: TA98 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
0.66	GMS	24 30 45	33.00	10.82
0.43	GMS	39 24 33	32.00	7.55
0.13	GMS	48 44 25	39.00	12.29
0.04	GMS	35 31 34	33.33	2.08
0.01	GMS	33 21 24	26.00	6.24
0.00	GMS	28 34 27 41 32 19	30.17	7.41

AVERAGE SLOPE (LINEAR REGR.) = 77.949
95% CONF. LIMITS = (38.800, 117.094)

SAMPLE: COLUMN, SORPTION, SAMPLE 5, (ETOH) DATE: 9-29-82
STRAIN: TA98 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
0.54	GMS	72 60 42	58.00	15.10
0.36	GMS	28 31 37	32.00	4.58
0.11	GMS	29 36 33	32.67	3.51
0.04	GMS	45 28 37	36.67	8.50
0.01	GMS	50 26 27	34.33	13.58
0.00	GMS	28 34 27 41 32 19	30.17	7.41

AVERAGE SLOPE (LINEAR REGR.) = 34.378
95% CONF. LIMITS = (24.419, 47.058)

SAMPLE: COLUMN, SORPTION, SAMPLE 6, (ETOH) DATE: 9-29-82
STRAIN: TA98 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
0.64	GMS	201 176 232	203.00	28.05
0.42	GMS	107 161 97	121.67	34.43
0.13	GMS	47 56 51	51.33	4.51
0.04	GMS	29 41 32	34.00	6.24
0.01	GMS	34 19 20	24.33	8.39
0.00	GMS	28 34 27 41 32 19	30.17	7.41

AVERAGE SLOPE (LINEAR REGR.) = 268.024
95% CONF. LIMITS = (250.343, 281.383)

SAMPLE: COLUMN, SORPTION, SAMPLE 7, (ETOH) DATE: 9-29-82
STRAIN: TA98 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
0.53	GMS	222 192 198	204.00	15.87
0.35	GMS	88 109 93	96.67	10.97
0.11	GMS	68 39 41	49.33	16.20
0.04	GMS	26 35 42	34.33	8.02
0.01	GMS	19 26 30	25.00	5.57
0.00	GMS	28 34 27 41 32 19	30.17	7.41

AVERAGE SLOPE (LINEAR REGR.) = 311.252
95% CONF. LIMITS = (284.969, 326.498)

SAMPLE: COLUMN, SORPTION, CONTROL, 4, SPIKE, (ETOH) 9-29-82
STRAIN: TA98 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
5.00	GMS	TOXIC		
3.30	GMS	601	601.00	0.00
1.00	GMS	491 452 519	487.33	33.65
0.33	GMS	151 162 163	158.67	6.66
0.10	GMS	49 38 44	43.67	5.51
0.00	GMS	28 34 27 41 32 19	30.17	7.41

AVERAGE SLOPE (LINEAR REGR.) = 464.221
95% CONF. LIMITS = (436.265, 492.177)

SAMPLE: S-9 CHECK, 5 UGS BAP, (DMSO) DATE: 8-26-81
STRAIN: TA100 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
0.00	MGS	47	47.00	0.00
50.00	MGS	441 428 398	422.33	22.05
100.00	MGS	971 895 841	902.33	65.31
150.00	MGS	1061 1147 1152	1120.00	51.16

SAMPLE: S-9 CHECK, 10 UGS BAP, (DMSO) DATE: 8-26-81
STRAIN: TA100 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
0.00	MGS	81 78 72	77.00	4.58
50.00	MGS	335 415 423	391.00	48.66
100.00	MGS	383 431 361	391.67	35.80
150.00	MGS	565 509 484	519.33	41.48

SAMPLE: S-9 CHECK, .5 GMS MUTAGEN, (ETOH) DATE: 7-13-82
STRAIN: TA98 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
12.50	MGS	137 191 213	180.33	39.11
25.00	MGS	204 212 258	224.67	29.14
37.50	MGS	211 187 243	213.67	28.09
50.00	MGS	301 219 231	250.33	44.29
75.00	MGS	267 241 218	242.00	24.52

SAMPLE: S-9 CHECK, .5 GMS MUTAGEN, (ETOH) DATE: 7-13-82
STRAIN: TA1537 ACTIVATION:+

DOSE	UNITS	PLATE COUNTS	MEAN	S.D.
12.50	MGS	64 42	53.00	15.56
25.00	MGS	70 53 62	61.67	8.50
37.50	MGS	83 91 71	81.67	10.07
50.00	MGS	62 69 78	69.67	8.02
75.00	MGS	102 67 81	83.33	17.62

Appendix B

Computer Program Listing

Computer program used to solve Equation 16 (modified from van Genucthen and Alves 1982).

```
 100          PROGRAM SORPT
 200          IMPLICIT REAL*8 (A-H,O-Z)
 300          DIMENSION TITLE(20)
 400          OPEN(UNIT=4,STATUS='OLD',FILE='SORIN.DAT')
 500          OPEN(UNIT=6,STATUS='NEW',FILE='SOROUT.DAT')
 600          READ(4,*)NC
 700          DO 4 K=1,NC
 800          READ(4,1001) TITLE
 900          WRITE(6,1002) TITLE
1000    C
1100    C     READ AND WRITE INPUT PARAMETERS
1200    C
1300          READ(4,*)V,D,R,TO,CI,CO
1400          READ(4,*)XI,DX,XM,TI,DT,TM
1500          WRITE(6,1004)V,D,R,TO,CI,CO
1600    C
1700          D=D/R
1800          V=V/R
1900          IF(DX.EQ.0.)DX=1.0
2000          IF(DT.EQ.0.)DT=1.0
2100          IMAX=(XM+DX-XI)/DX
2200          JMAX=(TM+DT-TI)/DT
2300          E=0.0
2400          DO 4 J=1,JMAX
2500          IF(IMAX.GE.J)WRITE(6,1005)
2600          TIME=TI+(J-1)*DT
2700          DO 4 I=1,IMAX
2800          X=XI+(I-1)*DX
2900          VVO=0.0
3000          IF(X.EQ.0)GOTO 1
3100          VVO=V*R*TIME/X
3200     1    DO 2 M=1,2
3300          A1=0.0
3400          A2=0.0
3500          T=TIME+(1-M)*TO
3600          IF(T.LE.0.)GOTO 2
3700          CM=(X-V*T)/DSQRT(4.*D*T)
3800          CP=(X+V*T)/DSQRT(4.*D*T)
3900          Q=V*X/D
4000          A1=0.5*(EXF(E,CM)+EXF(Q,CP))
4100          A2=0.5*EXF(E,CM)+V*DSQRT(.3183099*T/D)*EXF(-CM*CM,E)-0.5*(1.+Q+V*V
4200         #*T/D)*EXF(Q,CP)
4300          IF(M.EQ.2)GOTO 3
4400          CONC1=CI+(CO-CI)*A1
4500          CONC2=CI+(CO-CI)*A2
4600     2    CONTINUE
4700     3    CONC1=CONC1-CO*A1
4800          CONC2=CONC2-CO*A2
4860          XTIME=0.0
4900     4    WRITE(6,1006)X,XTIME,VVO,CONC1,CONC2
5000    C
5100    C
5200     1001 FORMAT(20A4)
5300     1002 FORMAT(1H1,10X,82(1H*)/11X,1H*,80X,1H*/11X,1H*,9X,'ONE-DIMENSIONAL
5400         # CONVECTIVE-DISPERSIVE EQUATION',25X,1H*/11X,1H*,80X,1H*/11X,1H*,
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```
5500        #9X,'SEMI-INFINITE PROFILE',50X,1H*/11X,1H*,9X,'NO PRODUCTION AND D
5600        #ECAY',48X,1H*/11X,1H*,9X,'LINEAR ADSORPTION (R)',50X,1H*/11X,1H*,9
5700        #X,'CONSTANT INITIAL CONCENTRATION  (CI)',36X,1H*/11X,1H*,9X,'INPUT
5800        # CONCENTRATION = CO (T.LE.TO)',37X,1H*/11X,1H*,29X,'= 0 (T.GT.TO)'
5900        #,37X,1H*/11X,1H*,80X,1H*/11X,1H*,20A4,1H*/11X,1H*,80X,1H*/11X,82(1
6000        #H*))
6100     1005 FORMAT(///11X,'DISTANCE',11X,'TIME',7X,'PORE VOLUME',12X,'CONCENTR
6200        #ATION'/14X,'(X)',13X,'(T)',11X,'(VVO)',6X,'FIRST-TYPE BC',4X,'THIR
6300        #D-TYPE BC')
6400     1004 FORMAT(//11X,'INPUT PARAMETERS'/11X,16(1H=)//11X,'V =',F12.4,15X,'
6500        #D =',F12.4/11X,'R =',F12.4,15X,'TO =',F11.4/11X,'CI =',F11.4,15X,'
6600        #CO =',F11.4)
6700     1006 FORMAT(4X,3(5X,F10.4),3X,F12.4,5X,F12.4)
6800          STOP
6900          END
7000          FUNCTION EXF(A,B)
7100    C
7200    C     TO CALCULATE EXP(A) ERFC(B)
7300    C
7400          IMPLICIT REAL*8 (A-H,O-Z)
7500          EXF=0.0
7600          IF((DABS(A).GT.170.).AND.(B.LE.0.))RETURN
7700          IF(B.NE.0.0)GOTO 110
7800          EXF=DEXP(A)
7900          RETURN
8000     110  C=A-B*B
8100          IF((DABS(C).GT.170.).AND.(B.GT.0.))RETURN
8200          IF(C.LT.-170.)GOTO 440
8300          X=DABS(B)
8400          IF(X.GT.3.0)GOTO 220
8500          T=1./(1.+.3275911*X)
8600          Y=T*(.2548296-T*(.2844967-T*(1.421414-T*(1.453152-1.061405*T))))
8700          GOTO 330
8800     220  Y=.5641896/(X+.5/(X+1./(X+1.5/(X+2./(X+2.5/(X+1.))))))
8900     330  EXF=Y*DEXP(C)
9000     440  IF(B.LT.0.0)EXF=2.*DEXP(A)-EXF
9100          RETURN
9200          END
```