Ames Test: Chemicals To Cancer

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Background, Purpose, Hypothesis

Cancer is a genetic disease accounting for one quarter of all deaths in North America[1]. A possible solution to this cancer toll is to reduce our exposure to mutagens, chemicals capable of inducing genomic mutations and disrupting cellular functions and potentially leading to cancer. Many chemicals in our environment can cause genetic mutations and are potentially responsible for millions of cancer-related deaths. Many of us are unaware of their danger and relation to cancer.

The purpose of this research is to examine environmental pollutants for mutagenic activity. Literary reviews found correlations between environmental pollutants and increased cancer incidence. For example, car exhaust contains polycyclic aromatic hydrocarbons (PAHs) produced during incomplete combustion of carbon. These same chemicals are produced during hydrocracking, a process used in the petroleum industry. Pollution from the petroleum industry in the Athabasca River, Alberta, has travelled downstream, where reports have stated there being a higher cancer incidence[2]. PAHs are known mutagens. A sample from a bus exhaust pipe was tested during this research. Cancer epidemiology also linked exposure to mutagens to factors like diet, culture and geography.

I hypothesized that chemicals present in our environment are mutagens under certain conditions (dosage, type of mutation).

Procedure

The Salmonella-*his* reversion-test (Ames test), a standard reverse mutation assay, was used to test chemicals for mutagenicity. This experiment employed six strains of *Salmonella typhimurium* histidine auxotroph mutants, deficient in the synthesis of histidine, an amino acid

necessary for bacterial growth. The histidine auxotrophs will only grow in a medium containing sufficient histidine supplement. To revert to histidine production (prototrophy), or become *his+*, a reverse mutation must occur in the original *his-* mutation (found in one of the genes involving histidine biosynthesis). When plated onto an agar media containing a trace (1/1000 dilution) of histidine, only *his+* revertants will grow to form a visible colony. The presence of visible colonies signifies a reverse mutation. Each of the six bacterial strains carries a different type of mutation (Table 1), making it possible to assess the type of mutation caused by the chemical under examination. When a chemical mutagen is introduced into the bacterial population on a filter disc, a higher number of revertants will appear, signalling the chemical causes genetic mutations. The Ames test includes using liver extract to simulate mammalian metabolic activity which may alter non-mutagenic chemicals to become mutagenic.

A culture of each strain grown in a histidine medium was diluted to a concentration of 10^8 cells/mL. Then 100 microlitres (µL) of the culture suspension was spread on an agar plate containing a trace of histidine. Five µL of the chemical under analysis was placed on a filter disc in the centre of the plate. These plates were incubated upside down for 48 hours at 37°C. Tests were conducted with the target compound and the target compound plus a bovine liver extract.

Positive and negative controls were conducted using ethidium bromide, N-methyl-N'-nitro-N-nitrosoguanidine (MNNG), and N-ethyl-N'-nitro-N-nitrosoguanidine (ENNG). Initial tests were conducted with gasoline, car exhaust, commonly used pesticides: carbaryl, allethrin, tetramethrin, and piperonyl butoxide, and fish liver extract to test for potential bioaccumulation in food chains. Subsequent tests analyzed trichloroethylene, tetrachloroethylene, barbeque scrapings, chimney soot and bus exhaust accumulation found on the inside of an exhaust pipe.

Responses (increased colony growth) that were deemed worthy of repetition or assessed as a potential response were repeated.

Safety precautions were taken when working with chemicals at a University laboratory. Appropriate eyewear, gloves and masks were worn during lab procedure and collection of mutagens. Aseptic techniques were used, and appropriate disposal of hazardous material was exercised.

Result, Conclusions, Applications

The results of the tests conducted are summarized in Table 2. Negative controls showed sparse, spontaneous revertants of random distribution on the agar plates. Positive controls showed a clear zone of inhibition surrounded by induced revertants of a higher density (Figure 1). The zone of inhibition is where the concentration of the chemical on the filter disc is strong enough to kill the bacteria. The ring of growth is where bacteria have reverted to histidine prototrophy. Responses were assessed using colony density (colonies/cm^2) around the filter disc compared to the zone inoculated with bacteria. Initial experimental results did not show mutagenic activity in the environmental samples tested suggesting that environmental mutagens may be present in our environment, but at levels too low to give a significant positive response in the Ames test. However, statistical evidence suggests correlations between many environmental pollutants and cancer incidence and we should consider accumulative effects of these chemicals on living organisms and be aware of their presence in our environment.

As a result of the insensitivity of the Ames test to low concentrations of chemicals in our environment, additional testing were carried out to examine other concentrated environmental pollutants. Positive responses were observed in barbeque scraping, chimney soot and bus exhaust accumulation. Repetitions confirmed mutagenic activity in bus exhaust, yet did not

confirm a positive response in barbeque scrapings and chimney soot. Further research is needed to assess the risk our chemically-contaminated environment plays in cancer. Analyses of minute traces of pollutants could be detected by gas chromatography mass spectrometry (GC/MS), then pure compounds could be tested with the Ames test. The Ames test does not, however, test carcinogenesis. An appropriate carcinogicity test would be animal experimentation or manipulation of cell cultures.

Acknowledgements

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References

1. American Cancer Society. Cancer Facts & Figures 2007. Atlanta: American Cancer Society; 2007.
2. Wikipedia. Tar sands. Jan. 14, 07. http://en.wikipedia.org/wiki/Tarsands.

Table 1: Ames Test Strains

Strain #	***S. typhimurium* Strain Name**	**Type of Mutation Detected**
1	TA98	detect frame-shift mutations
2	TA100	detect base pair substitutions
3	TA102	detect excism repair
4	TA104	detect base-pair substitutions
5	TA1534	detect frame-shift mutation
6	TA1530	detect base pair substitutions

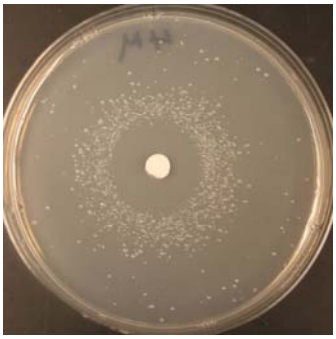

Figure 1: Positive Control with MNNG Strain 4

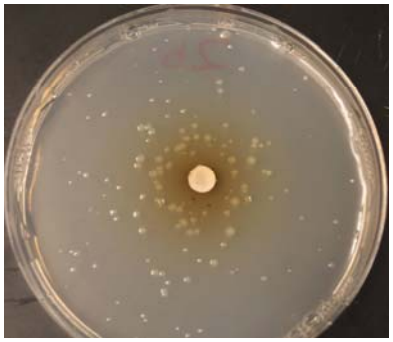

Figure 2: Bus Exhaust with Strain 2

Table 2: Ames Test Results

Strain of *S. typhimurium*	1		2		3		4		5		6	
Liver Extract Added	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes
Chemicals Added												
ENNG	P	nt	P	nt	P	nt	P	nt	P	nt	N	nt
MNNG	P	nt	P	nt	P	nt	P	nt	P	nt	N	nt
ethidium bromide	N	nt	N	nt	N	nt	N	nt	N	nt	N	nt
negative controls	N	nt	N	nt	N	nt	N	nt	N	nt	N	nt
gasoline	N	N	N	N	N	N	N	N	N	N	N	N
car exhaust filter	N	N	N	N	N	N	N	N	N	N	N	N
carbaryl	N	N	N	N	N	N	N	N	N	N	N	N
allethrin	N	N	N	N	N	N	N	N	N	N	N	N
tetramethrin	N	N	N	N	N	N	N	N	N	N	N	N
piperonyl butoxide	N	N	N	N	N	N	N	N	N	N	N	N
fish liver extract	N	N	N	N	N	N	N	N	N	N	N	N
trichloroethylene	N	N	N	N	N	N	N	N	N	N	N	N
tetrachloroethylene	N	N	N	N	N	N	N	N	N	N	N	N
BBQ scrapings 1	N	N	N	N	N	N	N	5.1	N	N	N	N
BBQ scrapings 2	nt	nt	nt	nt	nt	nt	N	N	nt	nt	nt	nt
BBQ scrapings 3	nt	nt	nt	nt	nt	nt	N	N	nt	nt	nt	nt
chimney soot 1	N	N	4.3	3.1	N	N	N	N	N	N	N	N
chimney soot 2	N	N	N	N	N	N	N	N	N	N	N	N
negative liver controls	nt	N	nt	N	nt	N	nt	N	nt	N	nt	N
burnt meat	N	N	N	N	N	N	N	?	N	N	N	N
bus exhaust scrapings 1	25	11	3.3	4.4	N	N	2.3	2.5	N	N	N	N
bus exhaust scrapings 2	8.3	20	4.1	4.7	nt	nt	4.3	4.4	nt	nt	nt	nt
bus exhaust scrapings 3	P	P	7.9	5.2	nt	nt	6.7	3.5	nt	nt	nt	nt

N - negative response
? - possible response (2 to 5 x)
P - positive response (5x)

ENNG - N-ethyl-N'-nitro-N-nitrosoguanidine
MNNG - N-methyl-N'-nitro-N-nitrosoguanidine
nt - not tested

numbers represent ratio of colony density in zone influenced by chemicals relative to backgound