Genotoxicity of 22 Pesticides in Microtitration SOS Chromotest

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ABSTRACT

The authors adapted microtitration techniques to the SOS Chromotest, and thereby established a simple and reliable modified genotoxicity test: the Microtitration SOS Chromotest. This test employs microtitration techniques and photometric analysis with numerical readout. These data are integrated into the computerized data processing program Statistical Analysis System to obtain genotoxicity and toxicity results of a chemical or chemical mixture within 16 h.

In order to test the applicability of the new system, the genotoxicity and toxicity of 22 pesticides were studied. A two-sample t test was employed to determine the level of significance ($p < 0.05$). Nine of the pesticides were found to induce the SOS response in *Escherichia coli* cells. Their relative degrees of induction based on SOS inducing potency values are as follows: captafol > captan > folpet > dinoseb > ferbam > linuron > alachlor > phosmet > cacodylic acid. The addition of rat liver S9 mix does not transform the nongenotoxic pesticides tested into genotoxic forms but significantly decreases the inducing ability of most positive pesticides.

The Microtitration SOS Chromotest is rapid and efficient in providing evidence for decision-making related to cleanup of chemical spills and discharges where time is a critical factor. This method maintains its high accuracy and sensitivity compared with conventionally used assays. The studies of pesticide genotoxicity using this test confirm the applicability, reliability, and sensitivity of the Microtitration SOS Chromotest as a simple, efficient, and rapid genotoxicity prescreening system as well as an excellent research tool for genotoxic mechanism studies.

INTRODUCTION

Industrialization and technology have increased the standard of living for people around the world. However, at the same time, a large amount of chemical waste is being generated and released into the environment as the result of human activity. The chemical pollutants have various adverse effects on human health. The most significant effects are muta-

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genesis and carcinogenesis of certain pollutants. The inappropriate use, treatment, and disposal of these pollutants pose serious threats to local communities as well as to all citizens in the long run.

The system that has received the widest use in genetic toxicology is the Ames/*Salmonella* assay (Ames *et al.*, 1975), and it is regarded as a standard for bacterial mutagenicity tests. Recently, scientists have discovered a series of genes in bacteria called the SOS regulatory network (taken from SOS, "Save our Ship" of Morse code, the telegraph alphabet; Elespuru, 1987). The SOS network is the first regulatory network affected by DNA damage to be recognized and is the only one whose regulation is understood in some detail at the molecular level (Walker, 1984). This SOS system has been the basis for the development of a series of alternative genotoxicity tests (Hofnung and Quillardet, 1986) that can be used for the screening of chemical compounds and complex mixtures, including environmental samples.

One of these SOS type tests, the SOS Chromotest, was developed by Quillardet *et al.* (1982). They developed an operon fusion that associates β-galactosidase gene (*lac* Z) with the sfiA gene, an SOS function involved in cell division inhibition. Whenever the SOS function is activated, because of bacterial DNA damage, the β-galactosidase biosynthesis is turned on. The addition of the enzyme substrate, *o*-nitrophenyl-β-D-galactosidase results in the production of yellow-colored nitrophenol, which can be easily measured colorimetrically. The chemicals tested may inhibit protein synthesis at specific concentrations, which would result in an underestimation of β-galactosidase induction. This is corrected by measuring general protein synthesis during the incubation period. Alkaline phosphatase enzyme is assayed in parallel with β-galactosidase. The ratio of the two activities (β-galactosidase to alkaline phosphatase) is a measure of the specific activity of β-galactosidase. The SOS Chromotest has advantages over the conventional bacterial mutagenicity tests such as the Ames/*Salmonella* test. The SOS Chromotest results can be obtained within a few hours instead of a few days for other tests. Testing can be accomplished in conditions incompatible with further cell division. Compounds toxic to the bacteria such as antineoplastic genotoxicants can be detected despite their toxicity. Genotoxic compounds that require metabolic activation in liquid medium do not require extra handling.

The SOS Chromotest has been used to evaluate genotoxicity of chemicals, complex mixtures, and environmental samples. Several authors have made direct comparisons between the SOS Chromotest and the Ames test to evaluate the usefulness of SOS Chromotest in screening for chemical mutagenic activity. Quillardet *et al.* (1985) tested 83 compounds using the SOS Chromotest and compared the results to that

of the Ames test. They found a 90% overla
in these two systems. They also found th
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However, overlap was only 50%, and there
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Environmental samples have been test
by Xu *et al.* (1987) and Dutka *et al.* (1986, 1
6 sediment samples from ponds in Prince
sample showed a weak SOS response and
the response in the presence of S9 fractio
51 inshore sediment samples from Lake
positive. Ten of 40 sediment samples fro
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and 1 showed a considerable increase aft
1987).

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Environmental samples have been tested with the SOS Chromotest by Xu *et al.* (1987) and Dutka *et al.* (1986, 1987). Xu *et al.* (1987) tested 6 sediment samples from ponds in Prince Edward Island. All but one sample showed a weak SOS response and all showed an increase in the response in the presence of S9 fraction. Dutka *et al* (1986) tested 51 inshore sediment samples from Lake Ontario, of which 3 were positive. Ten of 40 sediment samples from the Detroit and Niagara rivers and inshore Lake Erie were found to be significantly positive, and 1 showed a considerable increase after activation (Dutka *et al.*, 1987).

The original SOS Chromotest procedures employ test tubes exclusively (Quillardet and Hofnung, 1985), thus limiting the efficiency of the test. We have introduced microtitration techniques into the SOS Chromotest to establish a faster, more simple, and accurate system—the Microtitration SOS Chromotest. A Statistical Analysis Sys-

tem (SAS) program has been employed to process the automatically generated data. Results can be obtained within 6 h.

MATERIALS AND METHODS

Twenty-two pesticides were studied for their activation of the SOS function in *E. coli* using the Microtitration SOS Chromotest. Pesticide standards were obtained from the U.S. Environmental Protection Agency (EPA) Pesticides & Industrial Chemical Repository. All pesticides were dissolved and diluted in filter-sterilized DMSO. Seven different concentrations of each pesticide were selected to cover concentrations from nontoxic to those that are toxic to the cells. The test consists of colorimetric assays of enzymatic activities after incubating the tester strain in the presence of various concentrations of compounds tested. A detailed description of procedures has been published elsewhere (Xu *et al.*, 1989). Briefly, an exponential-phase culture grown overnight in lactose broth (LB) medium (amended with ampicillin) at 37°C is diluted 1 : 50 into fresh LB medium and incubated at the same temperature for 2 h. This culture is combined with both S9 mixture and phosphate buffer (3 volumes culture +1 volume S9 mix and phosphate buffer). Fractions of the above mixtures (0.6 mL) are distributed into glass test tubes containing 20 μL of the compound to be tested. After 2 h interaction between the compound and the growing bacterial cells at 37°C with shaking, 50 μL of the mixture is dispensed into each well of a sterile 96-well microplate, one row of wells for each dilution of compound tested. For each compound, two microplates are prepared; one for normal testing, the other for testing under exoteric activation of S-9. Among 12 wells of any row, 6 wells are amended with 200 μL of *o*-nitrophenyl-β-D-galactoside solution (2 mg/mL in B buffer) for β-galactosidase assay, the other half with 200 μL of *p*-nitrophenyl phosphate solution (2 mg/mL in P buffer) for alkaline phosphatase assay. The solutions are added by an 8-channel pipetter. They are measured at a 420 nm wavelength on an automatic microplate reader (Dynetech MR-600) immediately after addition of substrates. A second reading is made in the same way after 45 min of enzyme reaction at room temperature. The data from these two readings are submitted into a SAS program for computation of means for enzyme activities and responses (for detailed calculations, see Xu *et al.*, 1989).

RESULTS

The sensitivity of the SOS Chromotest is affected by a number of factors: temperature, bacterial metabolic state, and cell density. Figure 1A

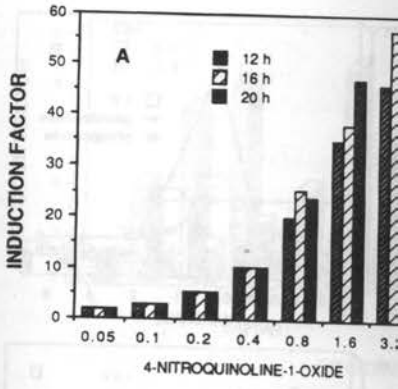

Fig. 1 Effects of bacterial incubation p
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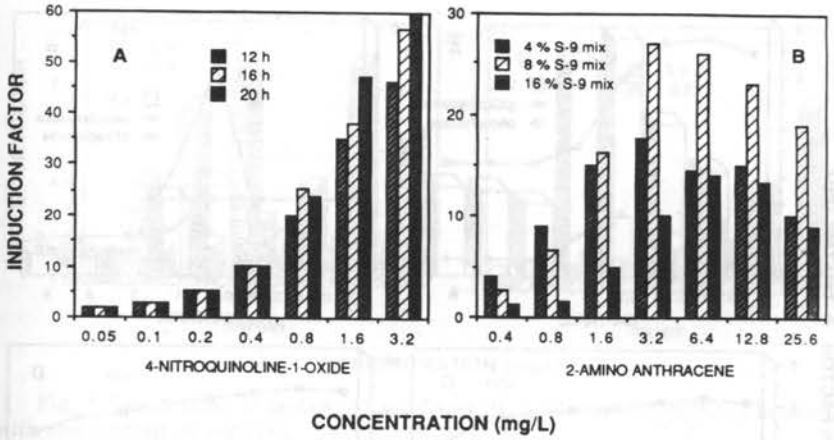

Fig. 1 Effects of bacterial incubation periods (A) and the concentration of S9 mix (B) on the sensitivity of Microtitration SOS Chromotest. The 4-nitroquinoline 1-oxide was assayed without the addition of S9 mix; 2-amino anthracene was tested with the addition of S9 mix.

shows the effect of different bacterial incubation periods on sensitivity of the Microtitration SOS Chromotest using 4-nitroquinoline 1-oxide (4NQO) as an inducer of the SOS reaction. It can be seen that, except at higher concentrations of 4NQO in which the 20-h incubation time slightly increases the response from the chemical, none of the incubation periods shows significant advantage over others in enhancing the sensitivity of the system. Therefore, a 16-h incubation was employed as the standard bacterial incubation period. For testing with exoteric metabolic activation (plus rat liver S9 mix), the concentration of S9 may also influence the sensitivity of the test. Three different concentrations of S9 were added to systems containing the same concentration of 2-amino anthracene, an indirect mutagen (Fig. 1B). Results show that 8% S9 mix gives the best activation for 2-amino anthracene. Thus, 8% S9 mix was used as a routine concentration to test metabolic activation.

Figures 2A and 2B show the enzyme activities and relative induction factor (IF) of captafol at various concentrations without, and with, S9 addition, respectively. In both cases, alkaline phosphatase activity was high in the negative control and at lower concentrations of captafol. As concentrations increase to a specific point (0.25 mg/L without S9; 0.5 mg/L with S9), alkaline phosphatase activity started to decline due to the inhibitory effect on cell metabolism, including protein biosynthesis. At about 2 mg/L captafol, further decrease in activity of this enzyme

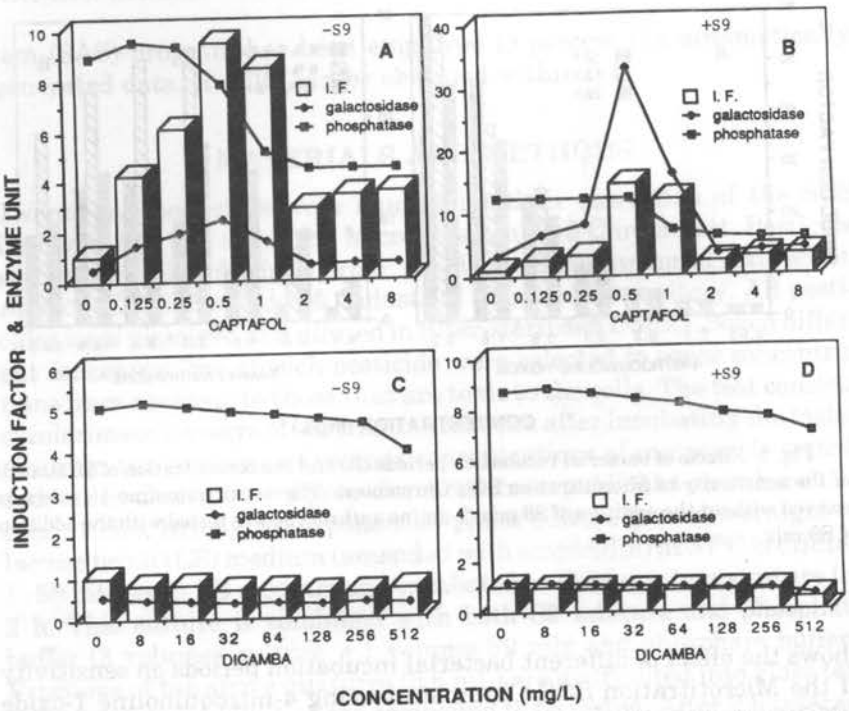

Fig. 2 Relationship between enzyme activities of β-galactosidase, alkaline phosphatase, and the induction factor calculated for captafol without S9 addition (A) and with S9 (B); for dicamba without S9 addition (C) and with S9 addition (D). Induction factor values are shown in vertical bars. Enzyme units are shown as curves.

was not evident. On the other hand, β-galactosidase activity was already significantly higher at the lowest concentration than the negative control and it continued increasing as concentration increased. Beyond 0.5 mg/L captafol, β-galactosidase activity began to decrease due to the toxicity of captafol, which could not be totally corrected. The average activity of both enzymes was enhanced by the addition of S9 mix, indicating a stimulatory effect of S9 mix on bacterial growth and on the SOS responses (*e.g.*, at 0.5 mg/L, β-galactosidase activity with S9 was ten times higher than that without S9). Nevertheless, the difference in calculated induction factors was not as great (14.5 vs. 9.5 at 0.5 mg/L captafol). This indicates that S9 mix removed part of the toxic effect of captafol (see alkaline phosphatase activity).

In contrast, dicamba exhibited no significant effect on *E. coli* cells based on the activities of the two enzymes and the calculated induction factors (Fig. 2C and Fig. 2D). It can be seen that alkaline phosphatase

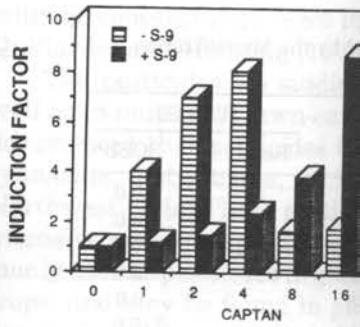

Fig. 3 Genotoxicity of captan (A) with and without S9 addition.

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Captan showed strong ind
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3B). In case of captafol, the situ
Fig. 2B). The inducing peaks w
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called for. S9 is an extract from
gism of the genotoxicity from
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more than 2.0 mg/L captan.

Table I summarizes the gen
Microtitration SOS Chromotest
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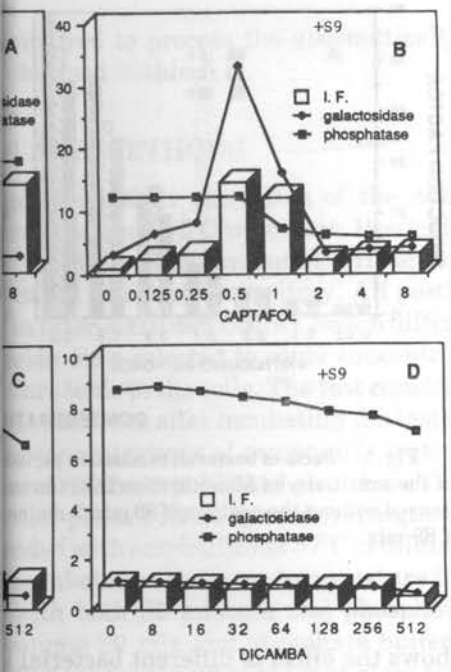
grees of induction based on SOS



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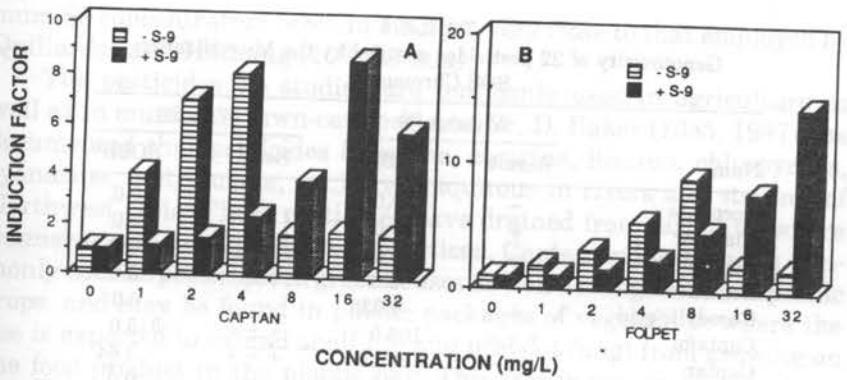

Fig. 3 Genotoxicity of captan (A) and folpet (B) in Microtitration SOS Chromotest with and without S9 addition.

activity slightly decreased in both cases as dicamba concentration increased. The β-galactosidase activity remained relatively constant throughout the tested dosage range. No significant increase in resultant induction factors was observed. The stimulatory effect of S9 fraction on bacterial growth is also evident.

Captan showed strong inducing ability in the Microtitration SOS Chromotest both with and without S9 addition (Fig. 3A). Due to increasing toxic effects, at higher concentrations there is a decline of the induction factor. The addition of S9 significantly ameliorated the genotoxicity of captan. A clear graphical illustration of this is the shifting of the induction peak to the higher concentration range for captan (*i.e.*, from 4 to 16 mg/L). Similar phenomena were observed for folpet (Fig. 3B). In case of captafol, the situation is more complicated (Fig. 2A and Fig. 2B). The inducing peaks with and without S9 addition appeared at the same concentration, *i.e.*, no evidence of peak shift. At low concentrations (0.125 and 0.25 mg/L), the addition of S9 fraction significantly decreased the genotoxicity; at higher concentrations (0.5 and 1.0 mg/L), the addition increased captafol's genotoxicity. More experimentation using varied dosages of S9 and the higher concentrations of captan are called for. S9 is an extract from rat liver of complex nature and synergism of the genotoxicity from captan seems to occur at high captan concentrations. No significant effect was observed at concentrations of more than 2.0 mg/L captan.

Table I summarizes the genotoxicity results of 22 pesticides in the Microtitration SOS Chromotest. Of the 22 pesticides, 9 show statistically significant induction over control ($p < 0.05$). Their relative degrees of induction based on SOS induction values area as follows: cap-

TABLE I
Genotoxicity of 22 pesticides assayed by the Microtitration SOS Chromotest

Name	Without S9		With S9	
	Result[a]	SOSIP[b]	Result	SOSIP
Acephate	−	0.0	−	0.0
Alachlor	+	0.047	−	0.0
Atrazine	−	0.0	−	0.0
Bentazon	−	0.0	−	0.0
Cacodylic acid	+	0.035	−	0.0
Captafol	+ + +	106.0	+ + +	315.0
Captan	+ + +	18.10	+ + +	3.64
Carbaryl	−	0.0	−	0.0
Chlorpyrifos	−	0.0	−	0.0
Cyanazine	−	0.0	−	0.0
Dicamba	−	0.0	−	0.0
Dimethoate	−	0.0	−	0.0
Dinoseb	+ + +	0.72	+ +	0.72
Ditalimphos	−	0.0	−	0.0
Ferbam	+ + +	0.38	+ + +	0.08
Folpet	+ + +	6.71	+ + +	3.19
Linuron	+ +	0.12	−	0.0
Metribuzine	−	0.0	−	0.0
Monocrotophos	−	0.0	−	0.0
Nitrofen	−	0.0	−	0.0
Phosmet	+	0.045	+	0.026
Trichlorfon	−	0.0	−	0.0

[a] IF: <1.5 (−, negative), = 1.5–2.0 (+, weak positive), = 2.0–4.0 (+ +, positive), >4.0 (+ + +, strong positive).

[b] SOSIP: SOS inducing potency/nmol (See Xu *et al.*, 1989, for calculations).

tafol > captan > folpet > dinoseb > ferbam > linuron > alachlor > phosmet > cacodylic acid. Also, the addition of S9 mix decreases the genotoxicity of these pesticides except captafol, shown in the decline of SOS induction factor values.

DISCUSSION

Apart from the use of the 96-well microplates, the automatic colorometric assay with numerical readout, and the computerized statistical analysis of the results, the Microtitration SOS Chromotest is similar to the original procedures (Quillardet and Hofnung, 1985). We incubate bacteria for 16 h, the original incubation time is overnight. The optimum S9 concentration is 8% i
Quillardet and Hofnung (10%

The pesticides we studie
well as in municipal lawn-car
documented that pesticides (a
cyanazine, metribuzine, etc.)
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intensive row-crop agriclture i
monly used as pesticides in gre
crops, and may be found in pl
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under U.S. regulations, becau
Hoar (1986) has found a relat
and human cancer.

Our study identified 40%
genotoxic. These pesticides ha
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where inadequate "day interva
keting of the pesticide-treated
of the pesticide residues. Recen
cide dispersal on dust particles
In the United States, human e

Further studies are requi
and other possible injurious ef
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De Flora *et al.* (1984) noti
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captan activity in the Ames t
activity in the *E. coli* DNA-re
and Fig. 3B) agree: Captan and
Metabolic deactivation can occu
explain the reported lack of ca
assay in mice (Innes *et al.*, 196
reported to only be weakly car
(National Cancer Institute, 197

All of the 9 pesticides fou
Chromotest are direct-acting in
the rat liver S9 mix, which sim
the human body, usually decr

LE I
assayed by the Microtitration
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S9	With S9	
SOSIP[b]	Result	SOSIP
0.0	–	0.0
0.047	–	0.0
0.0	–	0.0
0.0	–	0.0
0.035	–	0.0
106.0	+ + +	315.0
18.10	+ + +	3.64
0.0	–	0.0
0.0	–	0.0
0.0	–	0.0
0.0	–	0.0
0.0	–	0.0
0.72	+ +	0.72
0.0	–	0.0
0.38	+ + +	0.08
6.71	+ + +	3.19
0.12	–	0.0
0.0	–	0.0
0.0	–	0.0
0.0	–	0.0
0.045	+	0.026
0.0	–	0.0

..5–2.0 (+, weak positive), = 2.0–4.0
rong positive).
ency/nmol (See Xu *et al.*, 1989, for

eb > ferbam > linuron > alachlor >
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CUSSION

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ncubation time is overnight. The opti-

mum S9 concentration is 8% in our test, very close to that employed by Quillardet and Hofnung (10% S9 mix).

The pesticides we studied are commonly used in agriculture as well as in municipal lawn-care projects. Dr. D. Baker (1985, 1987) has documented that pesticides (alachlor, atrazine, linuron, chlorpyrifos, cyanazine, metribuzine, etc.) are ubiquitous in rivers and streams of Northwest Ohio. These pesticides have drained from farmland where intensive row-crop agriclture is practiced. Captan and ferbam are commonly used as pesticides in greenhouses as well as fungicides for specific crops, and may be found in plastic packages of vegetables, where the use is expected to extend shelf life and prevent fungi from growing on the food product in the plastic bag. This last is not an approved use under U.S. regulations, because human exposure can be extensive. Hoar (1986) has found a relationship between agricultural chemicals and human cancer.

Our study identified 40% of 22 pesticides, in general, use to be genotoxic. These pesticides have broad distribution in water resources and potable water supplies. In addition, those applying the genotoxic pesticides will receive dosages beyond those encountered in drinking water. Some of these pesticides will enter the human diet in situations where inadequate "day intervals" (time between application and marketing of the pesticide-treated products) prevent adequate breakdown of the pesticide residues. Recently, there has been recognition of pesticide dispersal on dust particles from wind erosion of agricultural land. In the United States, human exposure is far from trivial.

Further studies are required to demonstrate the carcinogenicity, and other possible injurious effects on the human body, of these pesticides (especially captafol, which is generally considered innocuous).

De Flora *et al.* (1984) noticed that captan and folpet were direct-acting genotoxicants of rather high potency in both the Ames test and the *E. coli* DBA-repair test. They also found that S9 addition decreased captan activity in the Ames test, as well as both captan and folpet activity in the *E. coli* DNA-repair test. Our results (Table I; Fig. 3A and Fig. 3B) agree: Captan and folpet have very high genotoxic potency. Metabolic deactivation can occur with both captan and folpet. This may explain the reported lack of carcinogenicity of both pesticides in an assay in mice (Innes *et al.*, 1969). In a more recent study, captan was reported to only be weakly carcinogenic in mice and negative in rats (National Cancer Institute, 1977).

All of the 9 pesticides found positive in the Microtitration SOS Chromotest are direct-acting inducers (Table I). A mediating effect is the rat liver S9 mix, which simulates the liver metabolic systems of the human body, usually decreasing genotoxicity of most genotoxic

TABLE II
Comparison of genotoxic potencies of selected positive pesticides

Compound	Mutagenic potency[a] (Revertants/nmol)	SOSIP (nmol)
Captan	93.67	18.10
Folpet	15.00	6.71
Captafol	3.11	106.0
Ferbam	1.33	0.38
Phosmet	0.34	0.045

[a] From Shirasu *et al.* (1982).

pesticides tested, except captafol (Table I). This suggests that a defensive system exists in liver enzymes to ameliorate the genotoxic effects of these pesticides. This may be due to nonspecific enzymes whose natural site is on liver smooth endoplasmic reticulum. The postulated function of such nonspecific enzymes is to gradually break down toxic chemicals not generally in the evolutionary history of living cells.

Table 2 presents a comparison of genotoxic potencies of selected positive pesticides measured by two short-term genotoxic assays: Ames test (Shirasu *et al.*, 1982) and Microtitration SOS Chromotest. Except for captafol, the pesticides are constant in relative genotoxic potencies: captan > folpet > ferbam > phosmet. Captafol, which is the third genotoxic pesticide of the five by mutagenic potency, becomes the strongest positive pesticide based on SOS inducing potency. This is probably due to the different principles of these two systems. The Ames test primarily detects the base-substitution or base-shift in the DNA molecule caused by an agent, whereas the Microtitration SOS Chromotest detects DNA cross-links, deletions, and insertions caused by an agent. The results suggest that captafol induces DNA cross-links or deletions/insertions more than it induces base substitution/shift.

The 22 pesticides tested are diverse in molecular structure and in practical use. Results of these pesticides from the Microtitration SOS Chromotest are compared to those obtained using the Ames/*Salmonella* reversion test (Table III). The Ames test data for ditalimphos is not available from the literature. Of the 21 pesticides, 12 give the same response in both the Microtitration SOS Chromotest and Ames test; the remaining 9 produce conflicting results in these two tests—an overlap of 57%. Among 12 pesticides with similar results, captafol, captan, ferbam, folpet, and phosmet are positive in both assays; atrazine, bentazon, carbaryl, chlorpyrifos, cyanazine, dicamba, and metribuzin are negative in both systems. Within the 9 conflicting ones, 5

Comparison of Micro
Salmonella Muta

Name	C
Acephate	
Alachlor	
Atrazine	
Bentazon	
Cacodylic acid	
Captafol	
Captan	
Carbaryl	
Chlorpyrifos	
Cyanazine	
Dicamba	
Dimethoate	
Dinoseb	
Ditalimphos	
Ferbam	
Folpet	
Linuron	
Metribuzine	
Monocrotophos	
Nitrofen	
Phosmet	
Trichlorfon	

[a] −: negative; +
(1983).
[b] From IARC, (19
[c] From Waters *et*

pesticides (acephate, dimetho
chlorfon) are positive in the A
Microtitration SOS Chromote
acid, dinoseb, and linuron) are
positive responses in the SOS
(Ohta *et al.*, 1984; Arnaise *et a*
between these two assays were
because they tested similar che
As the diversity of chemicals t
are expected (von der Hude *e*
Chromotest and the Ames tes
therefore 100% correlation sh

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potencies of selected
esticides

ic potency[a] ants/nmol)	SOSIP (nmol)
3.67	18.10
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3.11	106.0
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liverse in molecular structure and in sticides from the Microtitration SOS obtained using the Ames/*Salmonella* mes test data for ditalimphos is not the 21 pesticides, 12 give the same ion SOS Chromotest and Ames test; ting results in these two tests—an icides with similar results, captafol, net are positive in both assays; atrarifos, cyanazine, dicamba, and metrims. Within the 9 conflicting ones, 5

TABLE III
Comparison of Microtitration SOS Chromotest with *Salmonella* Mutagenicity test for 22 pesticides[a]

Name	SOS Chromotest	*Salmonella* mutagenicity	
		Source A	Others
Acephate	–	+	
Alachlor	+	–	
Atrazine	–		—[b]
Bentazon	–	–	
Cacodylic acid	+		—[c]
Captafol	+	+	
Captan	+	+	
Carbaryl	–	–	
Chlorpyrifos	–	–	
Cyanazine	–	–	
Dicamba	–	–	
Dimethoate	–	+	
Dinoseb	+		—[c]
Ditalimphos	–		
Ferbam	+	+	
Folpet	+	+	
Linuron	+	–	
Metribuzine	–	–	
Monocrotophos	–	+	
Nitrofen	–	+	
Phosmet	+	+	
Trichlorfon	–	+	

[a] –: negative; +: positive. Source A: Moriya *et al.* (1983).
[b] From IARC, (1979).
[c] From Waters *et al.* (1982).

pesticides (acephate, dimethoate, monocrotophos, nitrofen, and trichlorfon) are positive in the Ames test but give negative results in the Microtitration SOS Chromotest; the remaining 4 (alachlor, cacodylic acid, dinoseb, and linuron) are negative in the Ames test and produce positive responses in the SOS system. In studies by some investigators (Ohta *et al.*, 1984; Arnaise *et al.*, 1986; Dayan *et al.*, 1987), the overlap between these two assays were surprisingly high (93–100%), probably because they tested similar chemicals within the same structural class. As the diversity of chemicals tested increases, more conflicting results are expected (von der Hude *et al.*, 1988, and our results). The SOS Chromotest and the Ames test assay operate on different principles; therefore 100% correlation should not be expected between these two

systems for a large variety of chemicals. Without doubt, strong positive chemicals (i.e., captan, folpet, captafol) usually give similar responses to both systems, although one might claim that some of them induce more DNA crossing-over or deletions than cause base shifts (such as captafol, Table 2), or vice versa. For relatively weak genotoxic compounds, the tendency of a pesticide toward one type of DNA injury will lead to a difference in results of these two assays.

In addition to the advantages of rapidness, testing mixtures of toxic compounds, assay in liquid medium, etc., the Microtitration SOS Chromotest is shown to be at least as sensitive as the Ames test and as useful in carcinogenicity prediction as the Ames test. These two bioassays can complement each other in revealing genotoxicity of a greater number of chemicals, complex mixtures, and environmental pollutants. For instance, captafol has been generally considered a non-carcinogen and a mutagen of much less hazard than captan and folpet. In *Handbook of Toxic and Hazardous Chemicals* (Sittig, 1981), captafol was not even considered a hazardous substance, and no criteria were set for its permissible concentration in water. The neglect of this strong genotoxin was apparently due to the limitation of the Ames test and other assay systems. Captafol has been shown to be a much more potent inducer than captan and folpet in the Microtitration SOS Chromotest (SOSIP: 106 for captafol vs. 18.1 and 6.71 for captan and folpet). Further investigations should also be done to study the carcinogenicity and other adverse effects of those pesticides found positive in Microtitration SOS Chromotest, but inactive in the Ames assay.

Published opinions as to the usefulness of the SOS Chromotest as a general screening test for chemical mutagens are mixed. Ohta (1984), Quillardet *et al.* (1985), and Hofnung and Quillardet (1986) claimed that, with very few exceptions, most mutagenic genotoxins are also SOS inducers. Mamber *et al.* (1986) stated that the "Chromotest appeared to be less effective than the Ames/*Salmonella* mutagenicity test in the detection of diverse classes of chemical carcinogens." DeFlora *et al.* (1985) concluded that "from a technical point of view, the SOS Chromotest was found to be quite efficient in detecting the activity of both direct-acting genotoxicants and procarcinogens." The results of the present study support the effectiveness, sensitivity, and predictability of the SOS Chromotest. Our study also indicates that the SOS Chromotest and Ames test are complementary to each other. Both should be included when evaluating genotoxicity of chemicals and samples.

At least 18 laboratories have published results with 245 chemicals using this test. The overlap with the Ames test for 180 chemicals is 90%. In a comparison with animal carcinogenicity data from a small subset of chemicals (56) in U.S. EPA Gene-Tox Data Bank, the SOS

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Chromotest and the Ames test gave very similar predictions of animal carcinogenicity. Thus the SOS Chromotest compares quite well with the Ames/*Salmonella* assay as a general screen for potential mutagens and appears to have an advantage in the testing of compounds that might inhibit cell division.

CONCLUSION

The Microtitration SOS Chromotest is confirmed to be a rapid, simple, and sensitive genotoxicity screening test. It is as effective as the Ames test in genotoxicity testing and carcinogenicity prediction. The Microtitration SOS Chromotest and the Ames test have very good correlation when detecting strongly positive genotoxins, whereas they complement each other in testing weak genotoxins. A relatively high percentage of pesticides tested were genotoxins based on the Microtitration SOS Chromotest; excepting captafol, these pesticides can be deactivated to a variable extent by the addition of rat liver extract.

References

Ames, B.N., J. McCann, and E. Yamasaki. 1975. Methods for detecting carcinogens and mutagens with the Salmonella/mammalian-microsome mutagenicity test. Mutation Res. *31*:347–364.

Arnaise, S., H. Boeuf, J.P. Buisson, N. Cantat, P. Demerseman, J. Einhorn, G. Lamotte, M. Lemelin, P.A. Brimer, S.W. Perdue, A.W. Hsie, R. Royer, F. Kelly, and M. Hofnung. 1986. Genotoxic activities of 2-nitronaphthofurans and related molecules. Mutagenesis *1*:217–229.

Baker, D. 1985. Regional water quality impacts of intensive row-crop agriculture: A Lake Erie Basin case study. J. Soil Water Conserv. *40*(1):125–132.

Baker, D. 1987. Lake Erie Agro-ecosystem program: Sediment, nutrient, and pesticide export studies. Report to U.S. EPA February.

Dayan, J., S. Deguingand, C. Truzman, and M. Chevron. 1987. Application of the SOS Chromotest to 10 pharmaceutical agents. Mutation Res. *187*:55–66.

DeFlora, S., G.P. De Renzi, A. Camoirano, M. Astengo, C. Basso, P. Zanacchi, and C. Bennicelli. 1985. Genotoxicity assay of oil dispersants in bacteria (mutation, differential lethality, SOS DNA-repair) and yeast (mitotic crossing-over). Mutation Res. *158*:19–30.

DeFlora, S., P. Zanacchi, A. Camoirano, C. Bennicelli, and G.S. Badolati. 1984. Genotoxic activity and potency of 135 compounds in the Ames reversion test and in a bacterial DNA-repair test. Mutation Res. *133*:161–198.

Dutka, B.J., K. Jones, H. Xu, K.K. Kwan, and R. McInnis. 1987. Priority site selection for degraded areas in the aquatic environment. Water Pollut. Res. J. Can. *22*(2):326–339.

Dutka, B.J., K. Walsh, K.K. Kwan, A. El Shaarawi, D.L. Liu, and K. Thompson. 1986. Priority site selection for degraded areas based on microbial and toxicant screening tests. Water Pollut. Res. J. Can. *21*:267–281.

Elespuru, R.K. 1987. Inducible responses to DNA damage in bacteria and mammalian cells. Environ. Mol. Mutagen. *10*:97–116.

Hoar, S. 1986. Report to National Cancer Research Institute. August.
Hofnung, M., and P. Quillardet. 1986. Recent developments in bacterial short-term tests for the detection of genotoxic agents. Mutagenesis *1*:319–330.
Innes, J.R.M., B.M. Ulland, M.G. Valerio, L. Petrucelli, L. Fishbein, E.R. Hart, A.J. Pallotta, R.R. Bates, H.L. Falk, J.J. Gart, M. Klein, I. Mitchell, and J. Peters. 1969. Bioassay of pesticides and industrial chemicals for tumorigenicity in mice: A preliminary note. J. Natl. Cancer Inst. *42*:1101–1114.
Lecointe, P. 1984. Induction of the sfiA SOS repair function by psoralens in the dark. Mutation Res. *131*:111–113.
Mamber, S.W., W.G. Okasinski, C.D. Pinter, and J.B. Tunac. 1986. The *Escherichia coli* K-12 SOS Chromotest agar spot test for simple, rapid detection of genotoxic agents. Mutation Res. *171*:83–90.
Menon, M.M., E. Sainz, D.Y. Jones, P.R. Taylor, and P.P. Nair. 1986. Isolation, fractionation, and determination of fecal mutagens from man: Abstract 5442. Fed. Proceed. *45*:1090.
National Cancer Institute. 1977. Bioassay of captan for possible carcinogenicity (NCI-CG-TR-15). DHEW Publ. No. (NIH) 77-815.
Ohta, T., N. Nakamura, M. Moriya, Y. Shirasu, and T. Kada. 1984. The SOS-function inducing activity of chemical mutagens in *Escherichia coli*. Mutation Res. *131*:101–109.
Quillardet, P. and M. Hofnung, 1985. The SOS Chromotest, a colorimetric bacterial assay for genotoxins: Procedures. Mutation Res. *147*:65–78.
Quillardet, P., C. de Bellecombe, and M. Hofnung. 1985. The SOS Chromotest, a colormetric bacterial assay for genotoxins: Validation study with 83 compounds. Mutation Res. *147*:79–95.
Quillardet, P., O. Huisman, R. D'Ari, and M. Hofnung. 1982. SOS Chromotest, a direct assay of induction of an SOS function in Escherichia coli K-12 to measure genotoxicity. Proc. Natl. Acad. Sci. USA. *79*:5971–5975.
Reisenfeld, G., I. Kirsch, and S.H. Weissman. 1985. Detection and quantitation of aflatoxin B1 in orange juice by SOS-Chromotest. Food Add. Contam. *2*:253–257.
Rueff, J., A. Laires, H. Borba, T. Chaveca, I. Gomes, and M. Halpern. 1986. Genetic flavanoids: The role of metabolic conditions in the induction of reverse mutation, SOS functions, and sister-chromotid exchanges. Mutagenesis *1*:179–183.
Shirasu, Y., M. Mariya, H. Tezuka, S. Teramoto,T. Ohta, and T. Inoue. 1982. Mutagenicity screening studies on pesticides, P. 331–335. *In* T. Sugimura, S. Kondo, and H. Takebe (eds.), Environmental Mutagens and Carcinogens, Alan R. Liss, Inc., New York.
Sittig, M. 1981. Handbook of Toxic and Hazardous Chemicals, Noyes Publications, Park Ridge, New Jersey.
Thybaud-Lambay, V., F. Decloitre, and Y.A. Courtois. 1986. Role of the *uvr* gene in the SOS function inducing activity of two tryptophan pyrolysis products, Trp-P-1 and Trp-P-2, in Escherichia coli K12. Mutation Res. *173*:177–180.
von der Hude, W., M. Scheutwinkel-Reich, and R. Braun. 1986. Bacterial mutagenicity of the tranquilizing constituents of Vaerianaceae roots. Mutation Res. *169*:23–27.
von der Hude, W., C. Behm, R. Gurtler, and A. Basler. 1988. Evaluation of SOS Chromotest. Mutation Res. *203*:81–94.
Walker, G.C. 1984. Mutagenesis and inducible responses to deoxyribonucleic acid damage in *Escherichia coli*. Microbiol. Rev. *48*:69–93.
Xu, H., B.J. Dutka, and K.K. Kwan. 1987. Genotoxicity studies on sediments using a modified SOS Chromotest. Toxic. Assess. *2*:79–87.
Xu, H., B.J. Dutka, and K. Schurr. 1989. Microtitration SOS Chromotest—A new approach in genotoxicity testing. Toxic. Assess. (in press).

Kinetics and Effec
Preincubation o
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The kinetics and the influence of soil
labeled linuron were investigated at
100% of the water-holding capacity (
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