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Comparative investigation on the mutagenicities of organophosphate, phthalimide, pyrethroid and carbamate insecticides by the Ames and lactam tests

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The *Salmonella* lactam test is a newly developed method for detecting genotoxins. This technique is based on the ability of DNA damaging agents to reverse expression of the β-lactamase gene, an important gene that enables microbes to resist β-lactam antibiotics. A construct p-SELECT™ Control DNA plasmid containing a β-lactamase gene site was constructed in many mutant forms, including point and frameshift mutants. These mutant constructs were introduced into *Salmonella* tester strains whose mutagenicity is based on their ability to reverse expression of the β-lactamase gene. Fourteen pesticides were evaluated for genotoxicity using our newly developed *Salmonella typhimurium* strains JK947 and JK3, which are useful for detecting base substitution mutations. Six pesticides, namely allethrin, captan, folpet, monocrotophos, acephate and carbofuran, proved highly mutagenic in strain JK947, while the first four pesticides were more weakly mutagenic in strain JK3. In comparison, results from the Ames test show strain JK947 to be more sensitive to these pesticides than strains TA100 and TA1535. Strains TA98 and JK1 proved insensitive to allethrin, captan, folpet, acephate, carbofuran and monocrotophos. Among the many advantages of the lactam test are: large numbers of cells can treated and the test is operationally simple and inexpensive; revertant colonies form faster in the lactam test (16 h) than in the Ames test (48 h); the lactam test can detect mutagens present in biological specimens contaminated by histidine and biotin, samples that may give false positive results in the Ames test.

Introduction

Pesticides are high volume, widely used environmental chemicals and there is continuous debate concerning their possible role in many chronic human health effects (Cantelli-Forti *et al.*, 1993; Alavanjia *et al.*, 1996; Hodgson and Levi, 1996). Chronic effects thought to involve pesticides include carcinogenesis (Brown *et al.*, 1990; Blair *et al.*, 1993), neurotoxicity (Tanner and Langston, 1990) and reproductive (Gordon and Shy, 1981; Gray *et al.*, 1994) and developmental effects (Gray *et al.*, 1994; Kelce *et al.*, 1995).

Among the potential hazardous effects of agricultural chemicals, carcinogenesis is of special concern. The mutagenicities of a number of pesticides have been the objects of extensive investigation. These activities have great predictive value for the carcinogenicities of pesticides (Innes *et al.*, 1969; Ames *et al.*, 1975; Mahr and Miltenburger, 1976; Simmon, 1978; Moriya *et al.*, 1983; Rosenkranz *et al.*, 1984). Therefore, it is important to study the relationships between the mutagenic properties and chemical structures of pesticides.

The genetic toxicities of pesticides are determined by several factors, such as: (i) their biological accumulation or degradation in the environment; (ii) their metabolism in humans; (iii) their reactivities with cellular components such as DNA, RNA and proteins (Shirasu *et al.*, 1976). It seems essential that we determine the genotoxic potentials of these pesticides before they are used in agriculture.

Heretofore, pattern recognition (Jurs *et al.*, 1983) and branching analysis (Mager, 1981) have been performed in attempts to correlate chemical structures and genotoxic activities of pesticides. Recently Jurs *et al.* (1983) reported quantitative and qualitative research on the mutagenic potencies of some pesticides and other compounds in a short-term test system. To compare the abilities of selected short-term assays to detect and confirm genotoxic activities of pesticides, Waters *et al.* (1982) extensively studied the genotoxic activities displayed by a number of pesticides presenting diverse chemical structures in different test systems. The short-term bioassays can identify three types of genetic DNA damage: (i) gene or point mutations; (ii) primary DNA damage and repair; (iii) chromosomal alterations.

The *Salmonella* mutagenicity test for mutagens and environmental compounds is the most widely used for short-term testing (Ames *et al.*, 1975). These *Salmonella* strains contain different types of mutations in the *his* operon (Maron and Ames, 1983). The assay can determine whether mutations were frameshifts or base pair substitutions by reverse mutation of the *his* operon. Such mutations result in colony formation on his^- agar plates. Yet the Ames test shows the frequency of spontaneous revertant colonies to be high, if rather slow (48 h), and histidine-contaminated samples are not suitable for assay. Given these considerations, the newly developed lactam test may prove to be complementary to the Ames test.

The β-lactamase gene is an important gene that enables microbes to resist antibiotics of the β-lactam class. It hydrolyzes amide bonds in the β-lactam rings of penicillins and cephalosporins, producing carboxylic derivatives devoid of antimicrobial activity. The four classes of β-lactamase are differentiated according to their amino acid compositions and sequence homologies (Ambler, 1980; Bergstorm *et al.*, 1982).

Site-specific mutagenesis is employed to induce mutations at active β-lactamase gene sites (Lee *et al.*, 1994; Hour *et al.*, 1995). Based on these mutations, we previously developed a series of *Salmonella typhimurium* strains, JK1, JK2, JK3 and JK947, to detect environmental toxicant mutagenicity. Strains JK1 and JK2 were useful for detecting frameshift mutagens (Hour *et al.*, 1995), while strains JK947 and JK3 were useful for detecting alkylating agents (Lee *et al.*, 1994). We tested 14 pesticides for mutagenicity using strains JK947 and JK3 in our developing assay system, called the lactam test. Six pesticides were tested with both strains JK947 and JK3 to

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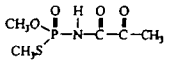
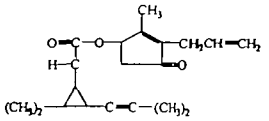
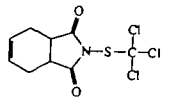
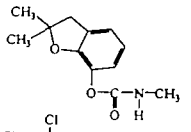
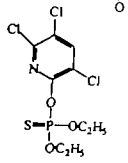
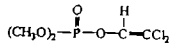
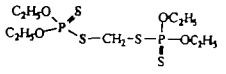
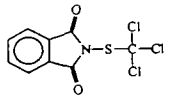
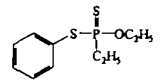
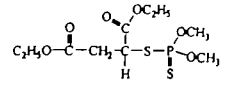
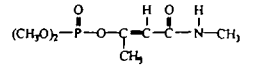
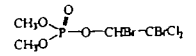
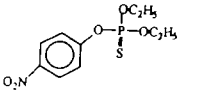
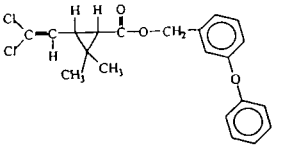
Table I. Common names, chemical names and chemical structures of the pesticides

Compounds	Chemical names	Structures
Acephate	*O, S*-Dimethyl *N*-acetylphosphoramidothioate	
Allethrin	3-Allyl-2-methyl-4-oxo-2-cyclopentenyl[ ]-*cis, trans*-chrysanthemate	
Captan	*N*-(Trichloromethylthio)-4-cyclohexene-1,2-dicarboximide	
Carbofuran	2, 3-Dihydro- 2, 2- dimethylbenzofuran-7-yl methylcarbamate	
Chlorpyrifos	Diethyl 3, 5, 6-trichloro-2-pyridyl phosphorothioate	
Dichlorvos	2, 2-Dichlorovinyl dimethyl phosphate	
Ethion	*O, O, O', O'*-Tetraethyl-*S, S'*-methylene biphosphorodithioate	
Folpet	2-((Trichloromethyl)thio)-1 *H*-isoindole-1, 3(2*H*)-dione	
Fonofos	*O*-Ethyl-*S*-phenyl ethyldithiophosphonate	
Malathion	*S*-(1, 2-bis(ethoxycarbonyl)ethyl)*O, O*-dimethyl phosphorothioate	
Monocrotophos	*cis*-1-Methyl-2-methyl carbamoyl vinyl phosphate	
NALED	*O, O* -Dimethyl-*O*-(1, 2-dibromo-2, 2-dichloro-ethyl)phosphate	
Parathion	*O, O*-Diethyl-*O*-(*p*-nitrophenyl)phosphorothioate	
Permethrin	3-Phenoxybenzyl-2, 2-dimethyl-3-(2, 2- dichlorovinyl)cyclopropanecarboxylate	

show a dose–response relationship and the results obtained indicate that strain JK947 was more sensitive to the pesticides than strain JK3. Generally, the pesticide mutagenicities detected by the lactam test concur with those detected by the Ames test. The differences between the two methods are described and discussed.

Materials and methods

Chemicals

The test chemicals were obtained from the following sources: histidine, D(+)-biotin and agar, Merck (Darmstadt, Germany); NADP, glucose 6-phosphate and phenobarbital, Sigma (St Louis, MO); ampicillin and tetracycline, Boehringer Mannheim (Mannheim, Germany).

Pesticides

Most of the pesticides samples were kindly provided by Dr K.C.Lee (Taiwan Agricultural Chemicals and Toxic Substance Research Institute). All were pure chemicals (Table I).

Bacterial strains

Four strains (TA100, TA1535, JK3 and JK947) of *S.typhimurium* were used. These strains were cultured for 16 h in liquid nutrient broth and stored at −80°C. Their genetic markers and other characteristics, such as responses to positive controls and the numbers of spontaneous revertants, were checked. *Salmonella typhimurium* strains TA100 and TA1535 were kindly provided by Dr Bruce N.Ames (Berkeley, CA), while JK3 and JK947 were developed in our laboratory (Lee *et al.*, 1994).

Reverse mutagenicity assay using the lactam test

The experimental procedures were performed according to the methods described by Ames *et al* (1975). In short, we mixed bacterial suspension, pesticide solution and top agar, then poured the mixture onto the surface of a minimal agar plate treated with modified Vogel-Bonner (VB) E medium (Moriya *et al.*, 1978).

Strain JK-3 was obtained from *S.typhimurium* tester strain TA1535, which was transformed with a mutant DNA plasmid; a dA was replaced by a dG in the 166 nt β-lactamase gene. The lactam test method has been published in detail previously (Hour *et al.*, 1995) and is therefore given in brief here. The bacterial suspension, pesticide solution and top agar were mixed together and later poured onto LB plates containing 50 μg/ml ampicillin. After 16 h the number of revertant colonies can be counted.

Reverse mutagenicity assay using the Ames test

The Ames test was carried out as described by Maron and Ames (1983), with certain modifications. As described in detailing the lactam test, the test mixture contained 0.5 mM histidine/biotin solution added to top agar and was poured onto minimal glucose plates (1.5% agar, 0.4% glucose and 1× VB salts). The revertant colonies were counted after incubation at 37°C for 48 h.

Assay of β-lactamase activity

We used an improved iodometric color reaction based on the ability of the ampicillin resistance gene product, β-lactamase, to hydrolyze penicillin G derivatives into reducing products that can be visualized by discoloration of dark blue iodine–starch complexes (Kuo *et al.*, 1989). The β-lactamase activity assay strains, JK3, TA1535 or CJ236, were added to 5% starch, 5% penicillin G and I_2 (0.1 M)–KI (0.8 M) solution with mixing. The mixed solutions were then incubated at 20°C and their absorbance measured at 620 nm at 5 min intervals.

Tolerance of the JK3 strain to ampicillin

The tester strains were incubated at 37°C overnight. The cultures were treated with increasing doses of ampicillin and cell viabilities were measured with a spectrophotometer at a wavelength of 550 nm.

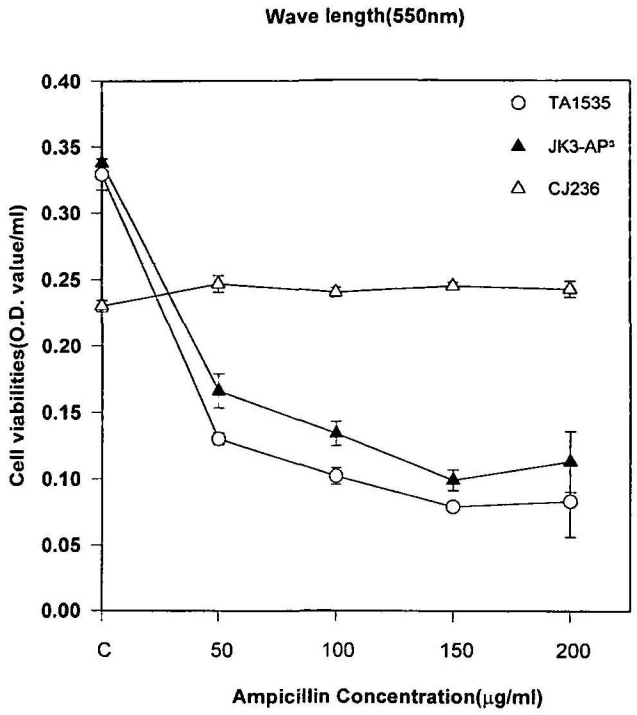


Fig. 1. Tolerance of strain JK3 to ampicillin. Strains TA1535, JK3-AP^s and CJ236 were treated with increasing dose of ampicillin and cell viabilities spectrophotometrically measured at a wavelength of 550 nm, as described in the text.

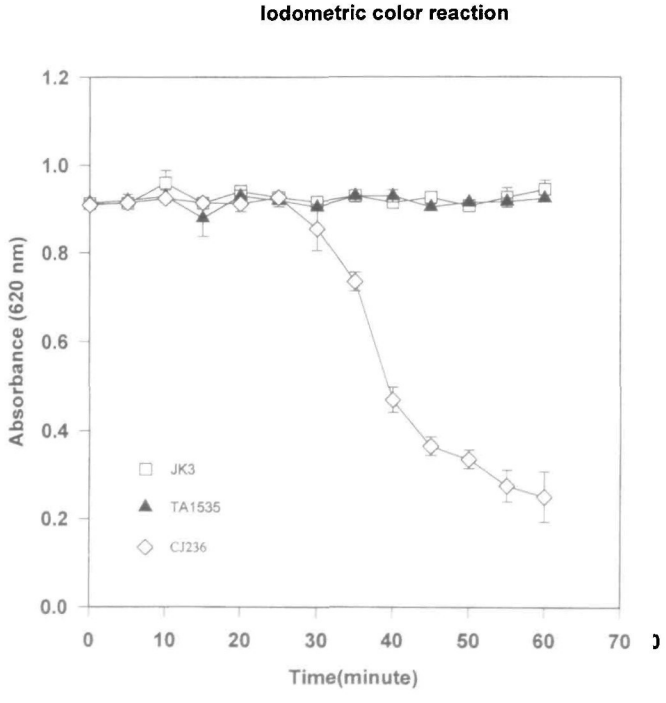


Fig. 2. Assay of β-lactamase activity. Strains JK3, TA1535 and CJ236 were added to 5% starch solution, 5% penicillin G and I_2 (0.1 M)–KI (0.8 M) solution with mixing. The mixed solutions were incubated at 20°C and absorbances measured at 620 nm at 5 min intervals.

Results

In the tolerance test strain JK3-AP^s (ampicillin-sensitive) was very sensitive to ampicillin (50 μg/ml); the negative control strain TA1535 was also very sensitive to ampicillin. Thus the viabilities of these two strains were dramatically decreased

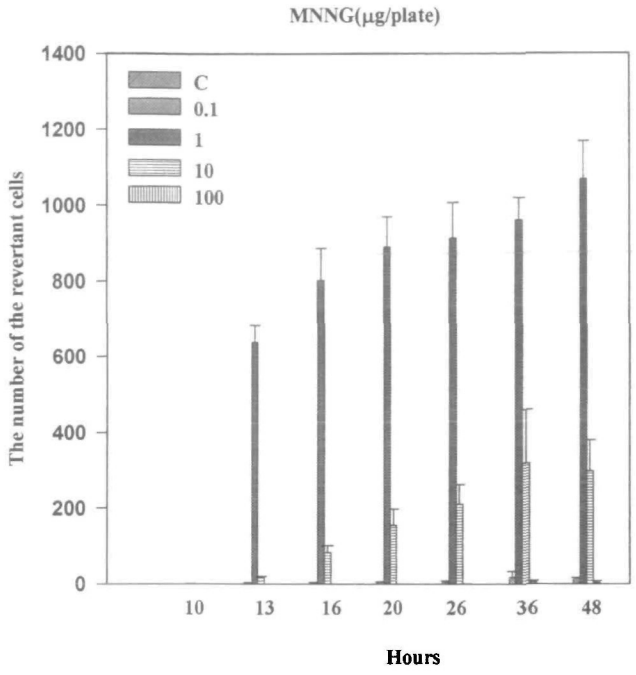


Fig. 3. Effect of incubation period on formation of revertant colonies. The JK3 strain was treated with MNNG (0.1–100 μg/plate) and the number of revertant colonies scored at 10, 13, 16, 20, 26, 36 and 48 h respectively.

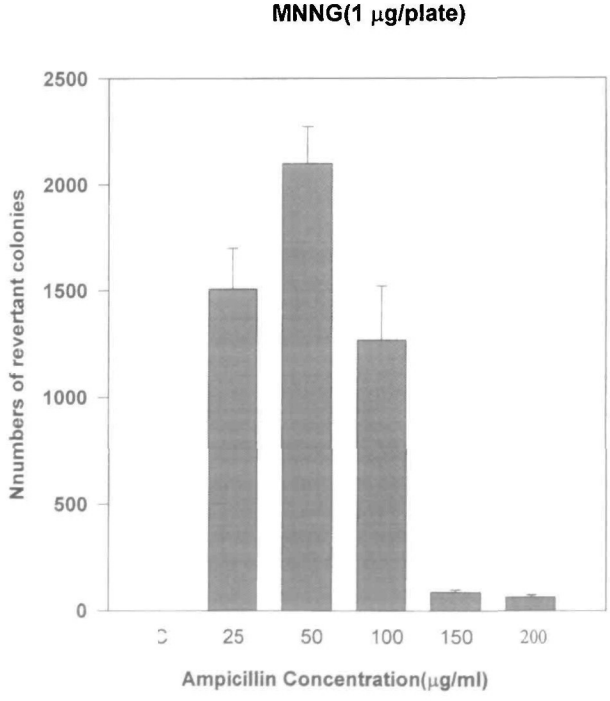


Fig. 4. The number of revertant colonies of JK3 strain induced by MNNG (1 μg/plate) at different ampicillin doses on an LB plate.

when the ampicillin dose was increased. Conversely, the positive strain CJ236, transformed with a β-lactamase gene plasmid, resisted ampicillin toxicity and cell viability was unaffected by increasing doses of ampicillin (Figure 1). In the assay for β-lactamase activity strains JK3-AP^s and TA1535 lost β-lactamase activity, while the control strain CJ236 retained its enzyme activity, as indicated by the iodometric color reaction (Figure 2). According to these results we can confirm that the designed mutant plasmid, with an A→G mutation in

Table II. Numbers of revertant colonies induced by various pesticides in strains JK3 and JK947 in the lactam test[a]

Mutagens	μg/plate (Dose)	JK3 [b]No. of revertant colonies	JK947 [b]No. of revertant colonies
Acephate	Control	0	67±7
	0.1	0	160±33
	1.0	0	205±3
	10	0	469±17
	100	0	201±13
Allethrin	Control	0	20±3
	0.1	0	117±33
	1.0	47±5	210±4
	10	9±4	43±12
	100	4±2	2±1
Captan	Control	2±1	0
	0.1	4±1	14±5
	1.0	6±1	138±22
	10	30±7	11±1
	100	0	0
[c]Carbofuran	Control	0	0
	0.1	3±3	5±4
	1.0	3±3	56±16
	10	5±4	5±2
	100	5±5	0
Folpet	Control	2±1	17±10
	0.1	3±2	21±8
	1.0	6±2	106±36
	10	94±15	1±1
	100	0	0
[c]Monocrotophos			
	Control	7±3	17±8
	0.1	13±2	51±3
	1.0	5±4	17±4
	10	0	2±2
	100	0	0

[a]Number of revertant colonies averaged over three experiments.
[b]Average value = mean ± SD.
[c]Number of revertant colonies averaged over six experiments.

the 166 nt β-lactamase gene, was transformed into strain TA1535; the new strain was called JK3. The lactam test was conducted under the following conditions: revertant JK3 colonies formed after 16 h (Figure 3) and the best reaction time of the strain with mutagens was 30 min. An ampicillin concentration of 50 μg/ml was used in the lactam test (Figure 4). The same experimental conditions were also suitable for strain JK947 in the lactam test (Lee *et al.*, 1994).

In this paper the mutagenicities of 14 pesticides were tested using the Ames and lactam tests. The structures and names of these pesticides are shown in Table I. The mutagenicities of six pesticides, acephate, allethrin, captan, carbofuran, folpet and monocrotophos, as estimated by the lactam test are shown in Table II. Strain JK947 proved more sensitive to acephate and allethrin than strain JK3: the minimum effective dose was 0.1 μg/plate and the numbers of revertant cells were 160 ± 33 and 117 ± 33 respectively. In contrast, strain JK3 showed a mutagenic response during the lactam test when tested with acephate and allethrin was less mutagenic to strain JK3. The minimum effective doses of captan and folpet were 1 μg/plate and the numbers of revertant cells were 138 ± 22 and 106 ± 36 respectively. Captan and folpet seemed to be less mutagenic than acephate and allethrin. Finally, carbofurn and monocrotophos were least mutagenic to strain JK947. It seemed that strain JK947 was more sensitive to pesticides than strain JK3 in the lactam test. The mutagenicities of these six pesticides concurred with previous results obtained using the Ames test (Klopman *et al.*, 1985; Garrett *et al.*, 1986). The other eight pesticides showed no obvious dose–response relationship in the dose range 0.1–100 μg/plate and the numbers of revertant cells were very low (Table III).

Among the six pesticides that yielded positive results in the lactam test; acephate, an organophosphate pesticide, was most mutagenic to strain JK947. The other organophosphate, monocrotophos, was less mutagenic. The two phthalimide fungicides, captan and folpet, were both mutagenically active in strain JK947 in the lactam test. However, captan was more mutagenic to strain JK947 than folpet, since at the same low dose (1 μg/plate) captan induced more revertant cells than folpet. It appeared that captan was more genotoxic than folpet in the lactam test. Allethrin, a pyrethroid insecticide, was also highly mutagenic to strain JK947.

Monocrotophos, allethrin, captan and folpet were weakly mutagenic to strain JK3 in the lactam test. These pesticides were also tested on strains TA1535 and TA100 using the Ames test (results shown in Table IV). Results suggest that strain

Table III. Numbers of revertant colonies induced by various pesticides in strains JK3 and JK947 in the lactam test[a]

Mutagens	μg/plate (Dose)	JK3 [b]No. of revertant colonies	JK947 [b]No. of revertant colonies
Chlor-pyrifos	Control	2±1	0
	0.1	0	2±1
	1.0	0	0
	10	4±1	0
	100	0	0
Dichlorvos	Control	0	0
	0.1	1±1	0
	1.0	0	0
	10	0	0
	100	0	0
[c]Ethion	Control	0	0
	0.1	0	3±3
	1.0	0	0
	10	0	0
	100	0	0
[c]Fonofos	Control	0	0
	0.1	0	1±1
	1.0	2±2	3±2
	10	3±3	0
	100	0	0
[c]Malathion	Control	3±3	0
	0.1	6±3	4±1
	1.0	1±1	1±1
	10	1±1	0
	100	0	0
[d]NALED	C	1±1	2±2
	0.1	1±1	0
	1.0	0	0
	10	0	0
	100	0	0
Parathion	Control	0	3±1
	0.1	2±2	1±1
	1.0	0	0
	10	0	0
	100	0	4±3
Permethrin	Control	0	0
	0.1	2±1	1±1
	1.0	0	2±1
	10	2±2	0
	100	0	0

[a]Number of revertant colonies averaged over three experiments.
[b]Average value = mean ± SD.
[c]Number of revertant colonies averaged over six experiments.
[d]*O,O*-Dimethyl-*O*-(1,2-dibromo-2,2-dichloroethyl)phosphate.

JK947 was more sensitive than strains TA1535 and TA100 to these pesticides under comparable experimental procedures and conditions. Moreover, the six pesticides allethrin, captan, folpet, acephate, carbofuran and monocrotophos were also tested on strains TA98 and JK1 and the results indicate that they were not sensitive to these pesticides (Table V).

Klopman *et al.* (1985) reported that among the most revelant fragments a methoxyphosphinyl and a chlorovinyl group appeared to be common structural subunits responsible for activities detected in the battery of the *S.typhimurium* histidine reversion assay. Our results seem to support these findings. Table VI shows the minimum dosage levels identified in the lactam and Ames tests for allethrin, captan, folpet, acephate, carbofuran and monocrotophos. The minimum dosage level is defined as the lowest concentration of a chemical (μg/plate) that induces a 3-fold increase in revertant colonies over background level. The results appear to show differences in sensitivity between the Ames test strains and the JK strains to these six pesticides. Figure 5a illustrates those pesticides that are contaminated with histidine; the JK947 strain is useful for detection of these pesticides (Figure 5aC and aD). Unlike strain JK947, strain TA100 is unable to accurately detect pesticides contaminated with histidine in the Ames test. This is due to the blurry morphorgy of the revertant colonies and the many pin colonies that are formed when compared with the control group (Figure 5aA and aB). The same results as previously are obtained when these two strains are treated with a fermented milk product in the Ames and lactam tests (Figure 5b).

In summary, it seems that the lactam test was more sensitive

Table IV. Numbers of revertant colonies induced by various pesticides in strains TA1535 and TA100 in the Ames test[a]

Mutagens	μg/plate (Dose)	TA1535 [b]No. of revertant colonies	AT100 [b]No. of revertant colonies
Acephate	Control	9±3	118±25
	0.1	13±4	120±14
	1.0	12±5	121±16
	10	16±3	123±23
	100	17±6	123±19
Allethrin	Control	10±5	102±19
	0.1	13±1	119±28
	1.0	13±5	108±27
	10	14±1	121±24
	100	15±8	119±18
Captan	Control	14±5	106±15
	0.1	14±4	130±10
	1.0	23±5	145±5
	10	114±9	376±59
	100	263±14	619±17
[c]Carbofuran	Control	10±2	101±25
	0.1	13±2	103±15
	1.0	12±3	100±10
	10	11±2	103±17
	100	3±1	106±25
[c]Folpet	Control	8±2	120±13
	0.1	7±3	118±25
	1.0	24±3	126±17
	10	155±2	245±49
	100	110±9	553±43
Monocrotophos	Control	4±3	110±35
	0.1	4±1	117±16
	1.0	8±1	118±13
	10	8±2	106±27
	100	11±3	116±29

[a]Number of revertant colonies averaged over three experiments.
[b]Average value = mean ± SD.
[c]Number of revertant colonies averaged over six experiments.

in detecting these 16 pesticides than the Ames test. The revertant colonies formed after 16 h during the lactam test, faster than the 48 h required by the Ames test. So the two new *S.typhimurium* strains, JK947 and JK3, may be useful for detection of pesticides in contaminated agricultural products.

Discussion

In our previous reports we presented a series of bacterial tester strains for detection of genotoxins using the lactam test (Lee *et al.*, 1994; Hour *et al.*, 1995). Each tester strain contained a different type of mutation in the β-lactamase gene. Strain JK947 was useful for detecting alkylating agents, such as MNNG and MNU; the JK1 and JK2 strains were developed to test frameshift mutagens, for example AAF, 9AA and 2NF. We now present another new tester strain, JK3, that is useful for detecting alkylating agents. Strain JK3 was developed by creating a A→G mutation in the 166 nt β-lactamase gene.

Klopman *et al.* (1985) made use of the Computer-Automated Structure Evaluation (CASE) program to analyze the genotoxic activity of 54 pesticides in five different short-term test systems for measuring gene mutation and DNA damage. The database contained compounds presenting diverse structures, including carbamates, thiocarbamates, organophosphates, halo-aromatics among others. The program automatically selected a methoxyphosphinyl and a chlorovinyl group as the most common structural subunits responsible for activities detected in the battery of the *S.typhimurium* histidine reversion assay.

Strains JK947 and JK3 are useful for detecting point mutation mutagens. In this paper six of the 14 pesticides, captan, folpet, acephate, allethrin, carbofuran and monocrotophos, were tested at doses of 0.1–100 μg/plate. Dose–response relationships were obtained for these pesticides. The fungicides, captan and folpet, proved to be carcinogenic compounds that could cause tumors of the lymphatic system and gastrointestinal tract in an animal model (Hasegawa *et al.*, 1993; Quest *et al.*, 1993). Recently the cytotoxic and cell transforming activities of these two fungicides have been studied in an *in vitro* model by exposing BALB/c 3T3 cells to the chemicals with or without S9 mix-induced bioactivation (Perocco *et al.*, 1995). Hayes (1982) suggested that captan might undergo rapid hydrolysis at the N-S bond to tetrahydrophthalamide and derivatives of the trichloromethylthio sidechain in *in vivo* systems and then the side chain can be converted to form a highly reactive thiophosgene intermediate

Table V. Numbers of revertant colonies induced by various pesticides in strains TA98 and JK1 in the Ames and lactam tests[a]

Mutagens	μg/plate (Dose)	TA98(Ames test) [b]No. of revertant colonies	JK1(Lactam test) [b]No. of revertant colonies
Acephate	*Control	25±6	0
	0.1	21±9	1±1
	1.0	24±16	2±1
	10	24±3	0
	100	25±3	0
Allethrin	Control	27±4	0
	0.1	32±12	0
	1.0	31±6	0
	10	38±4	0
	100	33±10	0
Captan	Control	30±15	0
	0.1	32±6	0
	1.0	28±4	3±1
	10	28±7	0
	100	29±7	0
[c]Carbofuran	Control	22±2	0
	0.1	32±9	0
	1.0	28±6	1±1
	10	33±7	2±1
	100	27±4	0
Folpet	Control	29±9	0
	0.1	25±4	0
	1.0	28±3	1±1
	10	30±4	2±1
	100	27±7	0
Monoc-rotophos			
	Control	25±7	0
	0.1	23±7	0
	1.0	28±3	0
	10	29±5	1±1
	100	21±3	0

[a]Number of revertant colonies averaged over three experiments.
[b]Average value = mean ± SD.
[c]Number of revertant colonies averaged over six experiments.

Table VI. Sensitivity of the Ames *Salmonella* test compared with the lactam test

Chemical	[a]Minimum dosage level (μg/plate)			
		Ames test	Lactam test	
	Strain TA100 [b](Ref.)	TA98	JK947	JK3
Acephate	5000 (Moriya et al., 1983)	[c] –	1.0	–
Allethrin	100 (Herrera et al.,1988)	–	0.1	1.0
Captan	5.0 (Barrueco et al., 1988)	–	0.1	10
Carbofurn	–	1000 (Moriya et al., 1983)	1.0	–
Dichlorvos	100 (Braun, et al., 1982)	–	–	–
Folpet	10 (Barrueco et al., 1988)	–	1.0	10
Monocrotophos	600 (Moriya et al., 1983)	–	0.1	0.1
[d]NALED	17.5 (Braun, et al., 1983)	–	–	–

[a]See text for definition of minimum dose level.
[b]Cited references.
[c]–, no mutagenic activity.
[d]*O,O*-Dimethyl-*O*-(1,2-dibromo-2,2-dichloroethyl)phosphate.

that would directly attack DNA and induce mutagenesis. Captan closely resembles folpet in structure, in that both have trichloromethylthio sidechains, therefore, the mutagenic mechanism of folpet should be similar to that of captan (Barrueco *et al.*, 1988). Moreover, in the lactam test strain JK947 was more sensitive to captan and folpet than strain JK3

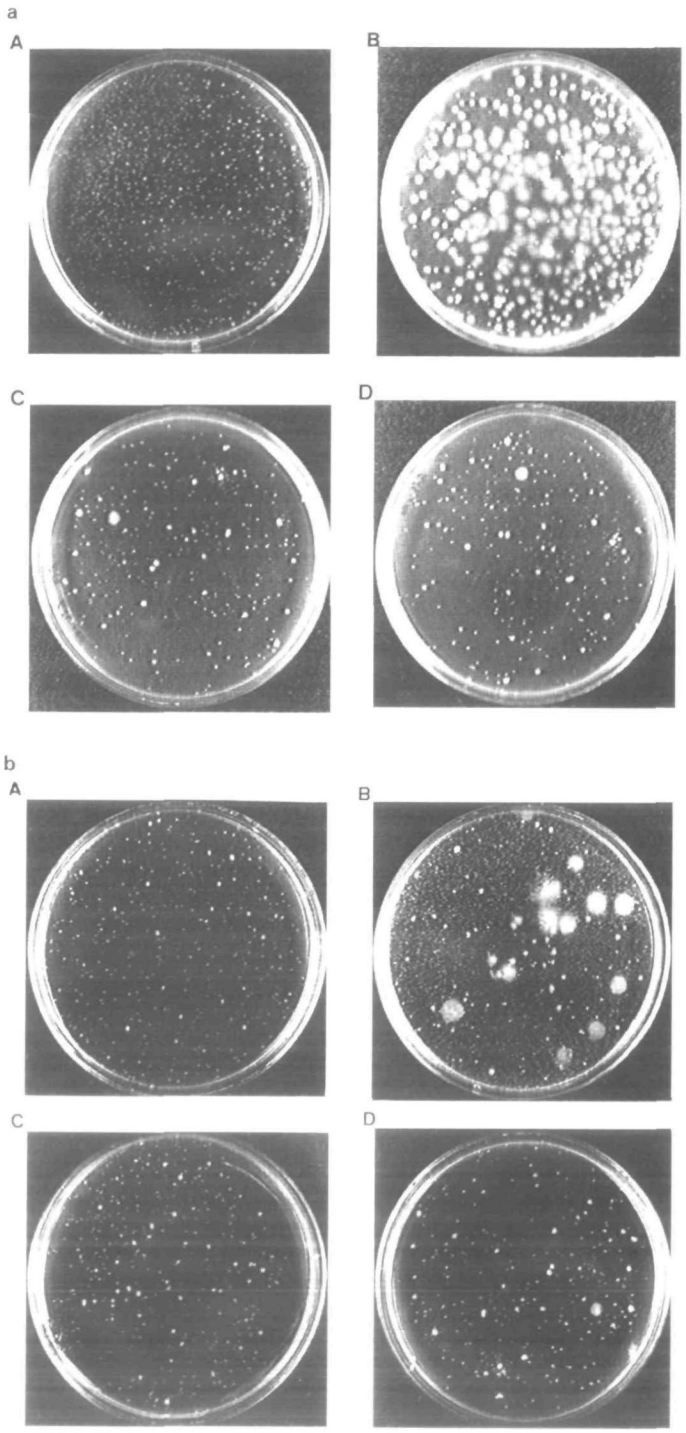


Fig. 5. (a) Analysis of histidine-containing pesticides by the Ames and lactam tests. Ames test: (**A**) control group, TA100 strain + acephate (100 μg/plate) + 1× PBS; (**B**) TA100 strain + acephate (100 μg/plate) + histidine (100 μg/plate). Lactam test: (**C**) JK947 strain + acephate (10 μg/plate) + 1× PBS; (**D**) JK947 strain + acephate (10 μg/plate) + histidine (100 μg/plate). The procedures are described in Materials and Methods. (**b**) Analysis of pesticides in contaminated agricultural products by the Ames and lactam tests. Ames test: (**A**) control group, TA100 strain + acephate (100 μg/plate) + 1× PBS: (**B**) TA100 strain + acephate (100 μg/plate) + yogurt (1 mg/plate). Lactam test: (**C**) JK947 strain + acephate (10 μg/plate) + 1× PBS; (**D**) JK947 strain + acephate 10 μg/plate) + yogurt (a fermented and active cultured milk product) (1 mg/plate). The procedures are described in Materials and methods.

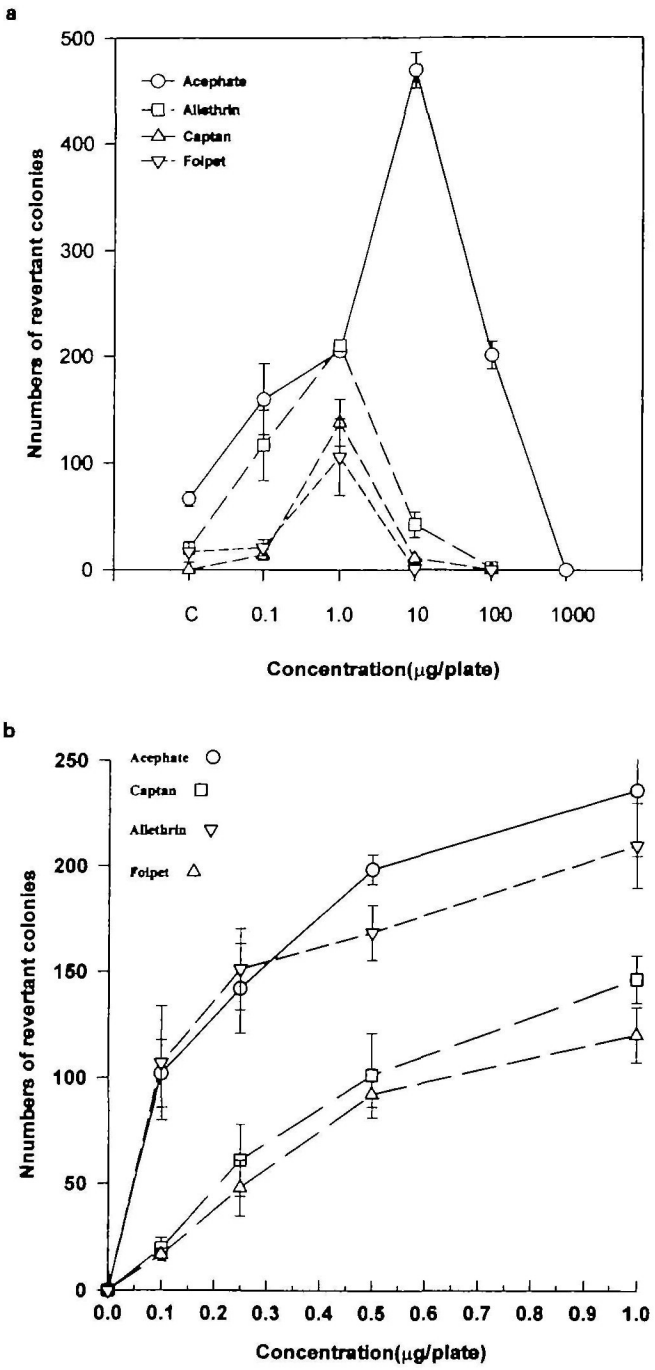


Fig. 6. (a) Formation of bell-shape dose–response curves. JK947 was treated with one of four pesticides, acephate, allethrin, captan or folpet, in the dose ranges 0.1–100 μg/plate. (b) Dose–mutagenicity relationship curves. The curves were obtained using one of four pesticides, acephate, allethrin, captan or folpet, and strain JK947 in the lactam test at doses ranging from 0.1 to 0.5 μg/plate.

(Table II). Acephate and monocrotophos are both organophosphorus pesticides with a methoxyphosphinyl-type group, found mainly in insecticides (Klopman *et al.*, 1985). This is consistent with the observation of Wild (1975) that the phosphorus moieties in organophosphates appear to be good substrates for nucleophilic attack. This might cause phosphorylation of DNA and lead to DNA damage. It appears that the oxygen atom in the P=O bond is required; substituting a sulfur atom was found to suppress activity (Klopman *et al.*,

1985). The P=S form is intrinsically more stable and many pesticides are manufactured in this form, which may be converted subsequently *in vivo* to the biologically active oxon (Karalliedde and Senanayake, 1989). Our results suggest that acephate and monocrotophos might induce a dG→ dA mutation. Allethrin is a pyrethroid insecticide belonging to an important group of chemicals that are constantly increasing in popularity. Allethrin was found to be mutagenic to strain TA100 and non-mutagenic to all other strains (Shirasu *et al.*, 1982, 1984; Waters *et al.*, 1982; Moriya *et al.*, 1983; Wildemauwe *et al.*, 1983). Klopman *et al.* (1985) and Herrera *et al.* (1988) reported that allethrin directly induces frameshift mutations. Our results obtained for allethrin in the lactam test confirm previous reports (Gentile *et al.*, 1982). Carbofuran is a carbamate insecticide that was mutagenic to strain JK947 in the lactam test, yet some investigators have reported that carbofuran is devoid of mutagenic activity in certain *Salmonella* tester strains (Anderson *et al.*, 1972; Waters *et al.*, 1980; Gentile *et al.*, 1982; Klopman *et al.*, 1985). However, Moriya *et al.* (1983) reported results showing that carbofuran is a mutagenic compound in the *Salmonella* test. This result was confirmed by our data (Table II). Finally, the other eight pesticides, including chlorpyrifos, dichlorvos, ethion, fonofos, malathion, *O,O*-dimethyl-*O*-(1,2-dibromo-2,2-dichloroethyl)-phosphate (NALED), parathion and permethrin, were non-mutagenic to strains JK947 and JK3 in the lactam test. Still other pesticides, allethrin, captan, folpet and monocrotophos, were weakly mutagenic to strain JK3 (Table IV). Strain JK3 was more sensitive to nitroso compound derivatives, such as MNNG and MNU, than strain JK947.

In comparing the results of the Ames and lactam tests for the same pesticide dose ranges strain JK947 was more sensitive to these pesticides than strains TA1535 and TA100. For example, when strain JK947 was treated with acephate or allethrin the resulting numbers of revertant colonies yielded obvious dose–response curves. Under the same conditions these pesticides had no significant mutagenic effects on strains TA1535 and TA100, except for captan and folpet. Table VI shows the minimum dosages identified by the lactam and Ames tests for six pesticides, allethrin, captan, folpet, acephate, carbofuran and monocrotophos. The results seem to indicate differences in sensitivity between the Ames test strains and the JK strains to these six pesticides. Strain JK947 was more sensitive to these six pesticides with respect to mutagenesis than strains TA100 and TA98. However, strain TA100 was more sensitive to NALED and dichlorvos with respect to mutagenesis than strains JK947 and JK3 (Braun *et al.*, 1982, 1983). NALED, which is structurally related to dichlorvos (Kappas *et al.*, 1990), is chemically similar to trichlorfon. The breakdown of NALED via hydrolysis generates dichloroacetaldehyde, which is a weakly mutagenic compound; this compound is substituted by an additional bromine to form bromoalkyl derivatives. Bromoalkyl derivatives of halogenated alkanols are significantly more mutagenic to *S.typhimurium* than their corresponding chloroalkyl compounds (Braun *et al.*, 1983). It appears that this type of organophosphorus insecticide was not mutagenic to the JK strains in the lactam test.

In the lactam test a bell-shaped dose–response curve in the 0.1–100 μg/plate dose range was occasionally observed, especially when tester strain JK947 was used (Figure 6a). It is possible that the combined toxic effects of ampicillin and the pesticide may have caused these responses. In order to solve this bell-shaped problem, it is strongly suggested that preliminary testing be carried out using a wide range of tested compounds to determine the linear portion of the dose–response curve (Figure 6a). With JK947 as the tester strain a nearly linear pesticides dose–response relationship was found from 0.1 and 0.5 μg/plate (Figure 6b).

In summary, we have characterized the mutagenic activities of 14 pesticides (Table I) using our newly developed *Salmonella* strains JK947 and JK3. Six of the 14 pesticides were genotoxic to strain JK947 in the lactam test. In comparison with results of the Ames test under the same experimental conditions, results indicate that strain JK947 was more sensitive to pesticides in the lactam test. Furthermore, formation of revertant colonies in the lactam test was faster (16 h) than in the Ames test (48 h) and the lactam test was found to be more useful for detection of pesticides in contaminated agricultural products than the Ames test (Figure 5). Therefore, the lactam test may provide a valuable technique for detecting certain contaminant pesticides and agrochemicals in agricultural foodstuffs.

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