In vitro mutagenicity testing of a potent, new, benzoyl urea insect growth regulator

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Summary. Bacterial mutagenicity assay to detect potential chronic toxicity of a potent, new, benzoyl urea insect growth regulator (CGA-112913 or IKI-7899, formerly UC-62644) was conducted using 5 histidine auxotrophs of *Salmonella typhimurium*. Tests within the concentration range of 0.9–500 µg (saturating)/plate of the compound with and without the 5–9 mammalian metabolic activation system showed no mutagenic effects clearing the way for long-term chronic toxicology studies.
Key words. Benzoyl urea; insect growth regulator; Ames test; histidine auxotrophs, *Salmonella typhimurium*.

A new class of insecticides of the benzoyl urea type that are insect-specific in their mode of action, with extremely low mammalian toxicity, are being developed as environmentally acceptable alternatives to broad-spectrum insecticides[2,3]. The greatest concern for these compounds is whether or not they are carcinogenic as revealed by long-term, chronic toxicology tests using rats and mice that can last up to two years and cost over 20 million US dollars[4,5]. It is important therefore to screen these compounds for mutagenic and carcinogenic effects in other-inexpensive screening systems and choose only the most promising compounds for the long-term chronic toxicology studies.

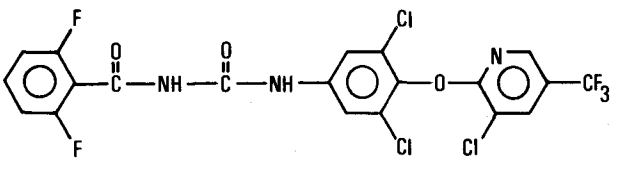
Ames and his co-workers[6,7] developed a sensitive inexpensive bacterial test for detecting chemical mutagens. Since about 85% of the carcinogens tested have proved to be mutagens, those compounds that are positive in Ames' assay can be deemed to be potential carcinogens. The strains of *Salmonella typhimurium* used have been selected for specific sensitivity to a variety of mutagens, namely, the reversion from histidine dependency for growth (auxotrophy) to an independence (prototrophy). The number of revertant colonies produced is a measure of the mutagenicity of the test compound.

In this report we present the results of screening CGA-112913, a potent insect growth regulator (fig.), for mutagenicity in the Ames' system using five different histidine auxotrophs of *Salmonella typhimurium*.

Materials and methods. The tester strains, TA98, TA100, TA1535, TA1537 and TA1538 were kindly provided by Dr Bruce Ames, University of California at Berkeley. TA100 and TA1535 were used for detecting mutagens causing base pair substitutions (point mutations) and the rest of the strains were used for detecting various kinds of frame shift mutations[5]. The strains had a) histidine requirement, b) deep rough character (rfa), c) R-factors and d) *uvrB* deletion which were all tested according to the prescribed procedures[6–8].

A permanent frozen culture was maintained in a deep freezer at −80 °C from which a surface scraping was taken to start the overnight working culture and master plates.

The Vogel-Bonner E medium, S-9 metabolic activation mixture, top agar, nutrient broth (Oxoid) and nutrient agar (Oxoid and Difco) were all prepared according to the prescribed methods[6–9]. The tester strains were checked for the characteristic genetic markers and spontaneous reversion rate and were found to be true to the diagnostic properties[6–8]. 2 ml of molten top agar containing excess of biotin and a trace of histidine was poured into a sterile tube containing 0.1 ml of an overnight nutrient broth culture of the tester strain. To this mixture, a 100-µl solution in dimethyl sulfoxide of the compound to be tested for mutagenicity was added. Aroclor in corn oil was injected into male Sprague-Dawley rats and the liver was obtained on the 5th day, washed in cold KCl solution and homogenized in KCl solution, centrifuged and the supernatant isolated as the S-9 fraction[6,7]. A 0.5-ml aliquot of the S-9 metabolic activation mixture was mixed with the medium, where required. The contents of the tube were poured onto minimal agar plates containing the Vogel-Bonner E medium, incubated at 37 °C in the dark for 48 h and the number of revertant colonies counted as prescribed[6–8]. The material was tested on the five strains at five different concentrations with three plates for each concentration, with and without the S-9 mixture.



The chemical structure of the molt inhibiting insect growth regulator, CGA-112913 (also IKI-7899 and formerly UC-62644). The material is being developed by Ciba-Geigy Ltd., Switzerland, and Ishihara Sangyo Kaisha Ltd., Japan.

Results and discussion. All the 5 strains were found to have a histidine requirement, deep rough character, R-factors, and uvrB deletion and their spontaneous reversion rates are given in table 1. Except for TA1535 which had a slightly lower reversion rate, all others were within the expected range. The positive controls and the dosages used are shown in table 2.

The results of the test with the Insect Growth Regulator, CGA-112913 are summarized in table 3. At concentrations above 500 µg/plate toxicity to the bacterium was observed[6]; also being relatively insoluble tended to precipitate on the surface. The results indicate that in this bacterial system there is no evidence for mutation caused either by base pair substitution (TA100 and TA1535) or by frame-shift (TA98, TA1537, TA1538)[7]. The TA1535 plate with the S-9 mixture at 2 µg/plate had a marginally elevated colony count but this is neither dose-related nor re-

Table 1. Spontaneous reversion rates of the strains of *Salmonella typhimurium* used in this study

Strain	Spontaneous reversion rate (colonies/plate)	
	Expected	Observed
TA98	30–50	30–36
TA100	120–200	150–170
TA1535	10–35	6–7
TA1537	3–15	7–9
TA1538	15–35	9–22

Table 2. Compounds used as positive control agents in the Ames' *Salmonella typhimurium* mutagenicity assay

Strain	± S-9 mixture	Compound	Optimal concentration in µg/plate to produce maximum revertants (5, 10, 50 and 100 tested)
TA98	(−) S-9	2-Nitrofluorene	10
	(+) S-9	2-Aminoanthracene	100
TA100	(−) S-9	N-Methyl-N-nitro-N-nitrosoguanidine (MNNG)	10
	(+) S-9	2-Aminoanthracene	50
TA1535	(−) S-9	MNNG	10
	(+) S-9	2-Aminoanthracene	100
TA1537	(−) S-9	9-Aminoacridine	50
	(+) S-9	2-Aminoanthracene	100
TA1538	(−) S-9	2-Nitrofluorene	10
	(+) S-9	2-Aminoanthracene	100

Table 3. Effects of CGA-112913 (IKI-7899 or UC-62644) on the reversion rate of several histidine auxotrophs of *Salmonella typhimurium*

Concentration	Average number of revertants per plate either with or without the S-9 metabolic activation system in the following strains									
	TA98		TA100		TA1535		TA1537		TA1538	
(µg/plate)	(−) S-9	(+) S-9	(−) S-9	(+) S-9	(−) S-9	(+) S-9	(−) S-9	(+) S-9	(−) S-9	(+) S-9
0.9	56	24	143	110	4	5	4	14	20	10
2.0	70	30	132	132	5	12	5	14	17	10
20.0	44	24	115	127	7	6	3	13	16	12
200.0	43	23	122	119	6	5	3	9	16	15
500.0	29	26	117	111	4	5	4	9	21	10
− control	45	23	118	114	7	4	4	10	24	14
+ control	254	3318	303	268	40	23	66	46	217	97

flected in the rest of the data and can be attributed to experimental variation.

CGA-112913 has been tested in the laboratory, greenhouse and field against the spruce budworm, *Choristoneura fumiferana*, the major pest of Canadian forests, and found to be very effective at extremely low dosages[10,11]. Its acute toxicity in mammals is very low with an LD_{50} of > 4000 mg/kg[12]. Our tests indicate that this material is non-mutagenic in the Ames system and therefore warrants further tests including long-term chronic toxicology studies.

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Genetic studies on gynandromorphism *(sgm, gm)* in *Culex pipiens fatigans*[1]

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Summary. Individuals showing anterior-posterior gynandromorphism or somatic mosaicism of palpi respectively have been found in a laboratory strain of *Culex pipiens fatigans*. The origin of the gynanders on the basis of binucleated eggs bearing an *m* factor and independently fertilized by male gametes of *M* and *m* genotype respectively has been suggested.
Key words. Mosquito; *Culex pipiens fatigans;* gynandromorphism.

Gynandromorphism and intersexuality are frequent in mosquitoes and have been reported in *Culex pipiens fatigans*[2,3], *Culex pipiens*[4,5], *Aedes aegypti*[6,7], *Aedes albopictus*[8] and in *Aedes nigripes*[9]. Laven[4] described an autosomal recessive intersex gene in *Culex pipiens* which feminizes genetic males and McCleland[6] showed that intersexuality is under genetic control in *Aedes aegypti* too. He also isolated a strain where males show sex conversion under certain temperature condition but appear normal when reared at lower temperatures. Craig[10] isolated a different temperature sensitive intersex producing strain. Amir Skanian[11] was successful in inducing gynandromorphism and sex mosaicism by the mutagenic agent thalimide in *Culex pipiens molestus*.

The present study reports on two kinds of gynandromorphism from an inbreeding laboratory strain of *Culex pipiens fatigans*. Attempts have been made to investigate the possible genetic basis and the probable mechanism of origin of these abnormalities.

Materials and method. In a laboratory strain of *Culex pipiens fatigans* which was being searched for genetic variations, two kinds of gynandromorphs were discovered. One type was partly female and partly male, the anterior part being that of a male while the posterior was typical of a female. On the anterior part, i.e., on the head, feathery antennae, long palpi and a sucking type proboscis, all characteristics of a male, were present. The posterior part showed a broad abdomen with membranous pleurae and long wings, characteristics of female last abdominal segment or slightly further, and the last abdominal segment had female genitalia. This appearance is designated as simple gynandromorph *(sgm)* (fig. 1). The second type of gynander was similar to *sgm* in all respects except palpi, where it showed somatic mosaicism. The palp of one side was always longer, extending beyond the proboscis, whereas the palp of the other side was very short (fig. 2). This type was called gynandromosaic *(gm)*.

In spite of repeated efforts these gynanders did not take a blood meal. With mouth parts being typically those of a male (the proboscis being the sucking type) this was to be expected. Techniques and details of rearing, breeding experiments and crossings were similar to those described earlier[12]. All experi-