SAFETY TEST ON MICE WITH *BACILLUS THURINGIENSIS* ISOLATE*

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SUMMARY: The toxicological studies performed on Bacillus isolate 310109 isolated from the Iskenderun region of Turkey, by experimental infection of mice have revealed the absence of acute and prolonged toxicity. All acute and prolonged toxicity tests produced negative results with all the various routes, subcutaneous, intraperitoneal, intravenous injection oral (gavage), inhalation and percutaneous application and eye irritation test, using $2x10^8$ bacteria per/ml suspension for each inoculation. Neither pathological symptoms nor mortality were observed. The behaviour and weight gain of the experimentally infected animals were within the normal range and close to those of the control animals.

Key Words: Safety tests, biological control, Bacillus thuringiensis.

INTRODUCTION

The most promising alternatives to chemical insecticides against mosquito vectors of diseases are *Bacillus* isolates as biocontrol agents. Various isolates and formulations of *Bacillus thuringiensis* were reported to possess high levels of activity against larvae of several mosquito species in the laboratory and under natural conditions (5,6,7,9). We have tested 510 *Bacillus* preparations isolated from the water and soil samples of different regions in Turkey. Among them in this study *B. thuringiensis* isolate 310109, which was shown to have 96.8% efficiency against *Culex tritaeniorchynchus*, was tested in mice to examine the mammalian pathogenicity.

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MATERIALS AND METHODS

All innocuity test were carried out on the *Bacillus* isolate number 310109, which were isolated from the soil and water samples of mosquito breeding sites, and identified according to their biochemical characteristics in Microbiology Laboratories of the Agricultural Faculty of Ankara University. The bacteria was grown in 0.50 ml. Nutrient Broth Medium (Difco) at 32 ±1°C for 72 hours. It was then centrifugated at 5000 rpm for 10 min. Pellets were washed three times with double distilled sterile water, and the optical density was measured. At the some time the number of spore and crystal proteins were determined by direct microscopic count. In general $2X10^8$ per/ml suspension were used for each inoculation.

Twenty female mice and 20 male mice (5 controls for each sex) 5 per cage were used for each inoculation route. The mice were supplied by the Surgical Research Center of Hacettepe University. All animals were maintained on free choice water and food and kept in a conventional laboratory environment.

The mice were injected by various routes including subcuta-

neous, intraperitoneal, percutaneous, intravenous, oral (gavage), inhalation and eye irritation in order to maximize the oppourtunity for the bacteria to behave as mammalian pathogens. Volumes injected were 0.20 ml of total culture subcutaneously, 0.20 ml intraperitoneally, 0.5 ml orally 0.15 ml percutaneously, 0.20 ml intravenously, 0.6 ml with inhalation and 0.1

Table 1: Mean body and organ weights of *B. thuringiensis* israelensis 310109 treated female mice one month after treatment.

	Week After Injection	Mean Body Weights in g.					Mean Organ weights in g.					
		0	1	2	3	4	Liver	Heart	Spleen	Brain	Kidneys+Adrenals	Stomach
SUBCUTANEOUS	CONTROLS	14.2	20.6	21	24	26.2	1.48	0.14	0.16	0.37	0.33	
	σn	3.8	2.3	1.2	2.4	2.2	0.1	0.01	0	0.005	0.03	
	σn-1	4.3	2.6	1.4	2.8	2.6	0.17	0.014	0	0.007	0.04	
	EXP.MICE	17.2	20.2	20.6	22.7	24.5	1.73	0.12	0.23	0.41	0.35	
	σn	2.9	0.9	1.9	3.1	2.6	0.3	0.02	0.09	0.01	0.05	
	σn-1	3.1	1.1	2.2	3.6	3	0.4	0.025	0.1	0.02	0.06	
INTRAPERITONEAL	CONTROLS	18.6	21	20.2	22.8	23	2.06	0.12	0.17	0.36	0.36	
	σn	0.8	1.09	0.7	1.4	0.9	0.4	0.02	0.05	0.01	0.02	
	σn-1	0.9	1.22	0.8	1.6	1.0	0.6	0.028	0.07	0.014	0.03	
	EXP.MICE	15.8	22.4	22.1	25.2	26.9	1.84	0.13	0.18	0.37	0.40	
	σn	7.0	4.7	4.4	4.2	4.2	0.2	0.02	0.04	0.02	0.03	
	σn-1	7.5	5	4.6	4.4	4.4	0.26	0.024	0.043	0.03	0.035	
PRECUTANEOUS	CONTROLS	21.2	25	22.4	24.6	25.4	1.31	0.11	0.12	0.37	0.33	
	σn	1.9	2.9	1.3	3.5	3.2	0.04	0.01	0.02	0.03	0.01	
	σn-1	2.1	3.3	1.5	3.9	3.5	0.05	0.017	0.03	0.04	0.02	
	EXP.MICE	16.6	22.5	21.7	26.1	26.1	2.01	0.13	0.17	0.35	0.35	
	σn	4.8	5.0	4.6	4.5	4.4	0.46	0.02	0.03	0.02	0.04	
	σn-1	5.0	5.4	5.0	4.8	4.8	0.5	0.025	0.035	0.023	0.05	
INTRAPVENOUS	CONTROLS	21.8	28	27.8	30.6	31.2	2.18	0.16	0.18	0.39	0.42	
	σn	1.9	3.0	3.1	2.3	2.3	0.3	0.01	0.03	0.02	0.05	
	σn-1	2.1	3.4	33.5	2.6	2.5	0.4	0.017	0.04	0.03	0.06	
	EXP.MICE	25	28.8	28.8	31	30.6	2.46	0.17	0.29	0.38	0.41	
	σn	2.7	2.07	2.4	4.03	3.01	0.6	0.02	0.14	0.02	0.09	
	σn-1	2.9	2.2	2.6	4.3	3.2	0.65	0.03	0.15	0.021	0.1	
EYE IRRITATION	EXP.MICE	22.4	25.3	25.3	26.5	26.7	1.76	0.15	0.18	0.38	0.17	
	σn	2.28	3.6	4.19	4.19	3.6	0.26	0.02	0.05	0.03	0.02	
	σn-1	2.41	3.8	4.42	4.44	3.8	0.27	0.02	0.06	0.04	0.02	
INHALATION	CONTROLS	24	27.6	28.4	29.2	29.4	1.74	0.22	0.16	0.42	0.39	
	σn	2.9	1.3	2.6	1.1	1.7	0.18	0.16	0.02	0.02	0.03	
	σn-1	3.3	1.5	2.9	1.3	1.9	0.22	0.18	0.03	0.025	0.04	
	EXP.MICE	22.4	28.3	27.2	27	30	1.88	0.16	0.17	0.40	0.39	
	σn	3.4	1.2	2.9	2.9	4.3	0.2	0.02	0.04	0.04	0.05	
	σn-1	3.9	1.5	3.3	3.3	4.8	0.24	0.027	0.047	0.045	0.06	
ORAL ADMINISTRATION	CONTROLS	22	22.6	25.2	28	26.4	1.66	0.14	0.16	0.37	0.18	0.32
	σn	2.9	3.5	3.5	3.09	2.4	0.13	0.004	0.02	0.02	0.01	0.05
	σn-1	3.3	3.9	3.9	3.4	2.7	0.16	0.005	0.025	0.03	0.013	0.06
	EXP.MICE	20.8	24.2	24	26.4	27.1	1.90	0.13	0.16	0.38	0.18	0.30
	σn	2.07	3.9	5.2	4.8	3.6	0.2	0.01	0.03	0.3	0.03	0.03
	σn-1	2.2	4.1	5.5	5.1	3.8	0.3	0.017	0.04	0.04	0.032	0.035

ml=2 droplets with eye irritation tests per experimental mouse. On the other hand the controls were injected with the same volume of distilled water for each route.

Body weight, feeding and behaviour were observed during the following month for acute toxicity, then necropsy was performed on representative animals and tissues from each major organ

Table 2: Mean body and organ weights of *B. thuringiensis* israelensis 310109 treated male mice one month after treatment.

	Week After Injection	Mean Body Weights in g.					Mean Organ weights in g.					
		0	1	2	3	4	Liver	Heart	Spleen	Brain	Kidneys+Adrenals	Stomach
SUBCUTANEOUS	CONTROLS	22.6	27.6	27.2	30.8	29	2.18	0.16	0.31	0.36	0.24	
	σn	4.3	3.4	3.4	2.13	3.5	0.45	0.004	0.13	0.03	0.03	
	σn-1	4.8	3.9	3.8	2.4	3.9	0.55	0.005	0.16	0.037	0.033	
	EXP.MICE	21.1	27.7	27.2	31.3	29.7	1.83	0.15	0.23	0.39	0.26	
	σn	1.51	2.7	3.9	4.3	3.32	0.32	0.03	0.1	0.03	0.09	
	σn-1	1.59	2.9	4.14	4.58	3.52	0.34	0.036	0.1	0.038	0.095	
INTRAPERITONEAL	CONTROLS	24	29.8	29.8	32.2	30	2.00	0.18	0.23	0.41	0.30	
	σn	3.03	3.7	4.9	6.4	4	0.35	0.01	0.08	0.03	0.05	
	σn-1	3.4	4.14	5.5	7.2	4.5	0.45	0.015	0.1	0.037	0.058	
	EXP.MICE	24	28.9	30.1	33.4	31.4	2.41	0.17	0.27	0.41	0.26	
	σn	3.8	3.9	3.9	3.8	3.3	0.64	0.04	0.11	0.02	0.04	
	σn-1	4.08	4.1	4.16	4.06	3.5	0.67	0.046	0.12	0.024	0.048	
PRECUTANEOUS	CONTROLS	20.2	23.6	22.6	22	27	1.79	0.13	0.15	0.36	0.23	
	σn	3.4	2.07	6.0	4.3	3.5	0	0	0	0	0	
	σn-1	3.8	2.1	7.3	5.2	4.3	0	0	0	0	0	
	EXP.MICE	24.9	28	27.5	29.6	28.7	1.92	0.14	0.25	0.37	0.23	
	σn	4.2	4.5	3.5	3.4	4.3	0.3	0.02	0.18	0.01	0.04	
	σn-1	4.4	4.8	3.7	3.6	4.6	0.4	0.025	0.19	0.017	0.04	
INTRAPVENOUS	CONTROLS	24.6	30	31.4	31.4	31.8	4.09	0.19	0.22	0.37	0.26	
	σn	2.8	3.16	3.4	1.01	3.24	0.86	0.02	0.03	0.03	0.01	
	σn-1	2.9	3.53	3.8	1.14	3.6	1.06	0.025	0.04	0.037	0.01	
	EXP.MICE	26.2	28.1	27.3	31.8	34.2	2.29	0.16	0.24	0.38	0.27	
	σn	4.9	6.3	7.0	5.2	5.0	0.45	0.02	0.04	0.02	0.04	
	σn-1	5.2	6.6	7.4	5.6	5.4	0.48	0.027	0.044	0.03	0.047	
EYE IRRITATION	EXP.MICE	21.2	26.1	27.4	30.7	30.1	1.81	0.15	0.18	0.36	0.24	
	σn	4.6	4.3	4.7	4.2	5.7	0.4	0.01	0.05	0.04	0.04	
	σn-1	4.9	4.6	5.2	4.4	6.0	0.46	0.01	0.057	0.05	0.043	
INHALATION	CONTROLS	16.6	22	23.3	27.3	32	2.21	0.19	0.23	0.36	0.25	
	σn	3.3	0.8	1.2	5.2	0	0	0	0	0	0	
	σn-1	3.7	1.0	1.5	6.4	0	0	0	0	0	0	
	EXP.MICE	26.7	31.8	31.2	34.2	32.5	2.13	0.19	0.23	0.39	0.26	
	σn	4.6	5.0	5.2	4.7	5.0	0.3	0.03	0.06	0.04	0.05	
	σn-1	4.8	5.3	5.5	4.9	5.3	0.35	0.032	0.067	0.046	0.055	
ORAL ADMINISTRATION	CONTROLS	3.2	33.8	33.6	33.2	36.6	2.35	0.19	0.28	0.38	0.54	0.32
	σn	1.4	1.4	1.4	3.5	1.1	0.24	0.02	0.1	0.03	0.1	0.06
	σn-1	1.5	1.6	1.6	3.5	1.2	0.29	0.03	0.11	0.04	0.14	0.08
	EXP.MICE	24.8	27	28.4	29.6	30.6	1.93	0.16	0.15	0.39	0.45	0.27
	σn	4.7	4.6	6.7	7.4	8.3	0.4	0.04	0.03	0.02	0.09	0.06
	σn-1	5.2	5.0	7.5	8.3	9.3	0.5	0.048	0.034	0.03	0.1	0.07

system were examined histologically and the main organs (liver, spleen, heart, brain, kidneys + adrenals, stomach for oral toxicity) were sampled and weighed. For reisolation tests, 1 ml heart blood of two selected mice for each route were collected in test tubes.

Table 3: Mean body and organ weights of B. thuringiensis 310109 treated female mice three months after treatment.

	Weeks After Injection	Mean Body Weights in g.												Mean Organ weights in g.					
		0	1	2	3	4	5	6	7	8	9	10	11	Liver	Heart	Spleen	Brain	Kid.+Ad.	Stomach
SUBCUTANEOUS	Controls	14.2	20.6	21	24	26.2	27	28.5	25.5	31	30	31.5	32.5	1.84	0.16	0.12	0.35	0.43	
	σn	3.8	2.3	1.2	2.4	2.2	3.0	1.5	2.5	3.0	2.0	2.5	2.5	0.3	0.02	0.02	0.005	0.09	
	σn-1	4.3	2.6	1.4	2.8	2.6	4.2	2.1	3.5	4.2	2.8	3.5	3.9	0.5	0.03	0.03	0.007	0.13	
	Exp. Mice	17.4	20.4	21	22.8	24.8	25.6	27.4	24.2	29.6	29.1	29.6	30.4	1.77	0.13	0.23	0.41	0.52	
	σn	1.14	0.8	1.1	1.9	2.9	3	2.5	3.3	2	1.6	1.6	2.4	0.06	0.01	0.01	0.02	0.2	
	σn-1	1.8	0.9	1.2	2.1	3.2	3.3	2.8	3.7	2.3	1.8	1.8	2.7	0.07	0.015	0.02	0.03	0.3	
INTRAPERITONEAL	Controls	18.6	21	20.2	22.8	23	23.5	25	26.5	26.5	26.5	26.5	28	2.06	0.12	0.17	0.36	0.36	
	σn	0.8	1.09	0.7	1.4	0.9	0.005	1.0	6.5	1.5	1.5	1.5	2	0.4	0.02	0.05	0.01	0.02	
	σn-1	0.9	1.22	0.8	1.6	1.0	0.007	1.4	9.2	2.1	2.1	2.1	2.8	0.6	0.03	0.07	0.014	0.03	
	Exp. Mice	19.8	24.2	22.4	23.4	25.2	24.8	25	24.4	26.2	26.2	26	26.4	1.55	0.12	0.15	0.35	0.36	
	σn	2.3	3.0	1.3	1.9	2.9	3.8	3.6	2.4	4.0	4.0	4.4	4.8	0.3	0.01	0.03	0.03	0.03	
	σ-1	2.5	3.4	1.5	2.1	3.2	4.3	4.1	2.8	4.5	4.5	4.9	5.3	0.4	0.015	0.04	0.036	0.035	
PRECUTANEOUS	Controls	21.2	25	22.4	24.6	25.4	21	25.5	27.5	27.5	28.5	29	30	1.88	0.15	0.21	0.40	0.43	
	σn	1.9	2.9	1.3	3.5	3.2	1.0	2.5	1.5	1.5	0.005	1.0	0	0.03	0	0.03	0.01	0.1	
	σn-1	2.1	3.3	1.5	3.9	3.5	1.4	3.5	2.1	2.1	0.007	1.2	0	0.04	0	0.05	0.014	0.14	
	Exp. Mice	17.8	21	21	23.6	25.8	26.8	27.8	26	26	28.4	28.4	30	1.96	0.14	0.27	0.38	0.37	
	σn	1.4	1.4	1.2	2.2	3	2.9	3	3.2	2.3	3.8	3.8	3.8	0.5	0.02	0.15	0.01	0.07	
	σ-1	1.5	1.5	1.4	2.5	3.5	3.3	3.4	3.7	2.6	4.3	4.3	4.3	0.54	0.022	0.16	0.02	0.08	
INTRAVENOUS	Controls	21.8	28	27.8	30.6	30.5	32.5	29.5	34.5	34.5	34.5	34.5	34.5	2.04	0.15	0.16	0.38	0.43	
	σn	1.9	3	3.1	2.3	2.3	0.5	1.5	0.5	1.5	0.5	0.5	0.5	0.01	0.01	0.005	0.01	0.01	
	σn-1	2.1	3.4	3.5	2.6	2.5	0.7	2.1	0.7	2.1	0.7	0.7	0.7	0.05	0.014	0.007	0.02	0.02	
	Exp. Mice	26	29.2	28.4	31.6	32.2	32.4	32.2	29.4	33.2	33.6	33.2	33.8	2.74	0.19	0.37	0.37	0.52	
	σn	2.5	2.9	2.0	2.3	3.3	2.2	1.0	0.8	1.16	1.0	2.9	4.1	0.5	0.04	0.08	0.06	0.06	
	σ-1	2.8	3.2	2.3	2.6	3.8	2.5	1.2	0.9	1.3	1.14	3	4.6	0.8	0.05	0.09	0.07	0.07	
Eye Irri tation	Exp. Mice	25.2	26.2	27.4	30.2	28.8	31.6	33	32.2	31	34.4	33.8	35	1.79	0.16	0.14	0.39	0.41	
	σn	1.3	1.9	1.2	0.4	2.6	1.3	0.9	0.7	2.0	2.5	3.9	2.4	0.18	0.02	0.02	0.04	0.05	
	σ-1	1.4	2.1	3	0.44	2.9	1.5	1.0	0.8	2.3	3.2	4.3	2.7	0.2	0.03	0.03	0.046	0.06	
INHALATION	Controls	24	27.6	28.4	29.2	29.4	29	29	30.5	30	32	32	32	2.09	0.16	0.21	0.45	0.47	
	σn	2.9	1.3	2.6	1.1	1.7	1.0	1.0	0.5	0	0	0	0	0.1	0.005	0.02	0.05	0.03	
	σn-1	3.3	1.5	2.9	1.3	1.9	1.4	1.4	0.7	0	0	0	0	0.15	0.007	0.03	0.07	0.04	
	Exp. Mice	22.4	28.3	27.2	27	30	30.6	32.5	32	31	31.6	32.4	33	1.81	0.16	0.15	0.42	0.45	
	σn	3.4	1.2	2.9	2.9	4.3	3.4	2.9	3.1	3.0	3.2	3.2	1.0	0.3	0.04	0.08	0.02	0.03	
	σ-1	3.9	1.5	3.3	3.3	4.8	3.8	3.4	3.4	3.4	3.5	3.9	1.4	0.45	0.05	0.1	0.03	0.04	
Oral Administration	Controls	22	22.6	25.2	28	26.4	27	27	28.5	25	25.5	33	29.5	1.72	0.14	0.19	0.39	0.37	0.28
	σn	2.9	3.5	2.0	3.0	2.4	3.0	3.0	3.5	3.0	3.5	1.0	4.5	0.07	0.03	0.005	0.04	0.04	0.04
	σn-1	3.3	3.9	3.9	3.4	2.7	4.2	4.2	4.9	4.2	4.9	1.4	6.3	0.1	0.04	0.007	0.06	0.06	0.05
	Exp. Mice	20.6	21.4	21.6	22.6	24.4	27.8	29	28.5	25.6	25	28.6	26.6	1.41	0.12	0.14	0.36	0.35	0.31
	σn	1.6	1.49	1.49	1.8	2.4	2.9	2.0	2.9	2.1	2.7	3.2	3.2	0.2	0.01	0.03	0.01	0.04	0.04
	σ-1	1.8	1.67	1.67	2.07	2.7	3.2	2.2	3.4	2.4	3	3.5	3.6	0.23	0.013	0.03	0.02	0.05	0.05

A total of 270 mice (105 female+30 controls, 105 male+30 controls) were used throughout the experimental series. 186 of 270 mice, of which 75 were female +18 controls and 75 male + 18 controls, were necropsied for acute toxicity after one month.

Table 4: Mean body and organ weights of *B. thuringiensis* 310109 treated male mice three months after treatment.

	Weeks After Injection	Mean Body Weights in g.												Mean Organ weights in g.					
		0	1	2	3	4	5	6	7	8	9	10	11	Liver	Heart	Spleen	Brain	Kid.+Ad.	Stomach
SUBCUTANEOUS	Controls	22.6	27.6	27.2	30.8	29	29.5	31.5	34.5	33.5	34.5	39	36.5	2.07	0.21	0.19	0.29	0.54	
	σn	4.3	3.5	3.5	2.13	3.5	2.5	1.5	2.5	3.5	2.5	3	3.5	0.05	0.005	0.02	0.08	0.02	
	σn-1	4.8	3.9	3.9	2.4	3.9	3.5	2.1	3.5	4.9	3.5	4.2	4.9	0.07	0.007	0.03	0.09	0.03	
	Exp. Mice	23	27.8	27.2	30.8	29.8	33.4	35.8	35.6	34.6	35.8	39.2	37.2	2.08	0.18	0.16	0.40	0.56	
	σn	2.0	2.4	2.7	2.0	2.0	3.2	3.1	3.0	2.8	1.6	2.4	3.2	0.2	0.03	0.03	0.02	0.05	
	σn-1	2.3	2.8	3.0	2.4	2.4	3.6	3.5	3.6	3.2	1.7	2.7	3.8	0.26	0.034	0.037	0.03	0.06	
INTRAPERITONEAL	Controls	24	29.8	29.8	32.2	30	27.5	33	32	31	32	32	32	2.02	0.20	0.14	0.36	0.58	
	σn	3.0	3.7	4.9	6.5	4.0	3.5	0	0	0	0	0	0	0	0	0	0	0	
	σn-1	3.4	4.1	5.5	7.2	4.6	4.9	0	0	0	0	0	0	0	0	0	0	0	
	Exp. Mice	25	31	33.2	36	34.8	37.4	39.2	39	38.4	39	40.8	38.2	2.51	0.20	0.18	0.40	0.63	
	σn	3.5	3.7	2.4	2.2	2.4	1.7	0.9	1.09	1.8	2	0.9	3.5	0.54	0.03	0.02	0.02	0.04	
	σ-1	3.9	4.1	2.6	2.4	2.6	1.9	1.09	1.22	2	2.3	1.09	3.9	0.63	0.032	0.024	0.03	0.045	
PRECUTANEOUS	Controls	20.2	23.6	22.6	22	27	31	29	31	30.5	33	35	35	2.15	0.21	0.17	0.39	0.54	
	σn	3.4	4.9	6.0	4.3	3.5	7.0	5.0	6.0	3.5	4.7	1.0	3.0	0.1	0.01	0.03	0.01	0.04	
	σn-1	3.8	5.5	7.3	5.3	4.3	9.8	7	8.4	4.9	5.4	1.4	4.2	0.14	0.01	0.05	0.02	0.05	
	Exp. Mice	25	29.8	29.8	32	30.6	34.3	36	37.7	32	38.3	40	40	2.25	0.20	0.18	0.39	0.55	
	σn	2.9	3.8	5.0	3.3	1.2	1.7	2.5	2.4	5.7	1.2	1.6	1.8	0.09	0.02	0.03	0.02	0.09	
	σ-1	3.3	4.3	5.6	3.7	1.3	2	2.9	2.8	6.6	1.5	2	2.3	0.12	0.03	0.04	0.026	0.11	
INTRAVENOUS	Controls	24.6	30	31.4	31.4	31.7	39	41.5	39	39.5	37.5	35	46	7.64	0.28	0.23	0.42	0.68	
	σn	3.1	3.16	3.4	1.0	3.3	3	1.5	1.0	0.5	3.5	4.0	0	0	0	0	0	0	
	σn-1	3.5	3.5	3.8	1.14	3.8	4.2	2.1	1.4	0.7	4.9	4.7	0	0	0	0	0	0	
	Exp. Mice	30.6	33.8	33.6	34.2	36.2	38.6	40.4	40.4	39.4	41	43.6	44.5	2.71	0.22	0.40	0.39	0.73	
	σn	5.8	5.8	6.9	5.4	4.3	5.5	4.8	5.8	5.6	4.6	4.4	4.7	0.5	0.01	0.07	0.04	0.07	
	σ-1	6.5	6.5	7.8	6	4.8	6.1	5.4	6.5	6.2	5.3	4.7	5.4	0.6	0.02	0.08	0.05	0.09	
Eye Irri tation	Exp. Mice	23.4	27.8	28	31.8	33.2	33.2	35.6	37.4	33.4	36.5	39.2	37.2	2.42	0.17	0.19	0.41	0.55	
	σn	3.6	5.6	5.3	3.6	3.6	3.7	3.8	1.8	2	1.9	1	1.2	0.07	0.01	0.01	0.008	0.01	
	σ-1	4	6.2	6	4	4	4.1	4.2	2.07	2.4	2.2	1.2	1.4	0.08	0.012	0.012	0.01	0.02	
INHALATION	Controls	16.6	22	23.3	27.3	32	35	38	40	35	40	43	42	2.83	0.24	0.13	0.42	0.77	
	σn	3.3	0.8	1.2	5.2	0	0	0	0	0	0	0	0	0	0	0	0	0	
	σn-1	3.7	1	1.5	6.4	0	0	0	0	0	0	0	0	0	0	0	0	0	
	Exp. Mice	24.6	28.4	30.4	34.2	33	36.2	39	38.6	36.8	38	39.2	37.2	2.08	0.18	0.16	0.38	0.55	
	σn	4.0	4.1	3.3	3.6	3.7	2.4	1.5	4.2	2.1	3.1	3.4	3.4	0.35	0.01	0.03	0.006	0.11	
	σ-1	4.5	5.2	3.7	4.0	4.1	2.7	1.7	4.7	2.3	3.3	3.9	3.8	0.4	0.017	0.04	0.007	0.13	
Oral Administration	Controls	32	33.8	33.6	32.2	36.6	39	39	39.5	40	40	41	40	2.76	0.22	0.22	0.43	0.73	0.38
	σn	1.4	1.4	1.4	3.1	1.1	1.0	1.0	0.5	1.5	1.5	1.7	1.5	0.05	0.02	0.02	0.005	0.01	0.03
	σn-1	1.5	1.6	1.6	3.5	1.2	1.4	1.4	0.7	2.1	2.1	2.4	2.1	0.07	0.03	0.03	0.007	0.02	0.04
	Exp. Mice	24.8	27	28.4	29.6	30.6	35.5	37.2	34.4	37.8	40	41	40	2.29	0.21	0.27	0.43	0.66	0.28
	σn	4.7	4.6	6.7	7.4	8.3	1.8	2.0	2.3	0.7	1.2	0.8	1.2	0.15	0.04	0.07	0.02	0.02	0.06
	σ-1	5.2	5.0	7.5	8.3	9.3	2.0	2.2	2.6	0.8	1.4	1.0	1.4	0.18	0.047	0.08	0.023	0.03	0.07

The remaining mice were kept for a period of 3 months for prolonged toxicity, then the same procedures were carried out.

RESULTS

The results of body weight and organ weight examinations for each injection after one month are presented in Tables 1 and 2, and after three months in Tables 3 and 4. Cystic formation was noticed in the liver of the control group of intravenous applica tion as seen in Tables 2 and 4.

The pathological findings after necropsy were normal in all the experiments.

The reisolation tests of *B. thuringiensis* in blood samples from treated mice were all negative.

The results of the male one month oral and three month percutaneous groups showed a statistically significant difference compared to the respective control groups ($P<0.05$). No statistically significant difference in the other groups and the female groups were determined.

DISCUSSION

Larvicide formulations based on the endotoxin of *B. thuringiensis* serotype H-14 were shown to have a very large safety margin for aquatic non-target organisms except for chironomids, in the study of Sinegre, *et al.* (8).

According to the WHO data sheet on the biological control agent, no harmful effects have been recorded in safety tests with bees, vertebrates, mammals and man (1).

In other papers by WHO, it was demonstrated that injection of *B. thuringiensis* var. *israelensis* was not harmful unless more than 10^6 viable organisms were introduced directly into the brain (3,10).

In 1980, the Informal Consultation Group on mammalian safety of microbial control agents for vector control concluded that the organism had passed the necessary tests to warrant its application in large-scale field trials after reviewing the status of safety testing of *B. thuringiensis* var. *israelensis* (2).

According to Margalet and Dean, over 240 tons of *B. thuringiensis* var. *israelensis* were used operationally in West Africa in 1982 without causing any adverse effect (4).

In our studies, the behaviour, feeding and weight gain of the experimentally infected animals with *B. thuringiensis* isolate number 310109 and the weight or organs after necropsy, were within the normal range and close to the controls. As a result, *B. thuringiensis* 310109 appears to be perfectly well tolerated by each of the animals used in these experiments.

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