SAFETY TESTS ON MICE AND GUINEA-PIGS WITH *BACILLUS THURINGIENSIS* ISRAELENSIS*

NURDAN OZER
SELMA YORUKAN
LUTFU CAKMAKCI
AYSE BOSGELMEZ
BULENT ALTEN
BEHZAT GURKAN
ONER KOCAK
FERIHA GURKAN
OLUS KARAYALCIN
ESIN ALTEN
GÖNÜL ÇETINKAYA
NACI M. BOR

SUMMARY: Mammalian toxicity tests were carried out on mice and guinea-pigs with Bacillus thuringiensis israelensis number 330211, isolated from the Icel region of Turkey. Test animals were injected by various routes including subcutaneous intraperitoneal, intravenous, oral, percutaneous, inhalation and eye irritation test, using stock suspension of $2X10^8$ per/ml bacteria for each inoculation. None of the animals showed any evidence of illness and behaviour, growth, average organ and body weights were normal.

Key Words: Safety tests, mice, guinea-pig, biological control, Bacillus thuringiensis israelensis.

INTRODUCTION

Because of the present concern about the effects of insecticides on the environment, vector resistance to the traditional insecticides and their high costs, the use of biological control agents is becoming more popular. Among the most promising alternatives to chemical insecticides against mosquito vectors of diseases are *Bacillus* products. To date an estimate of over 5,000 tons of products containing *B. thuringiensis israelensis* have been used without harmful effects (1,5-8,10-13). In this study, *B. thuringiensis israelensis* 330211 which was shown to have 94.4% efficiency against *Anopheles sacharovi larvae* in our previous studies, were tested against mice and guinea-pigs in order to examine the potential mammalian pathogenecity.

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MATERIALS AND METHODS

The organism used in these experiments was isolated from soil samples of mosquito breeding sites from the Içel region of Turkey and identified as *B. thuringiensis israelensis* (isolate number 330211) according to its biochemical characteristics. It was grown in a 50 ml. Nutrient Broth Medium (Difco) at $32 \pm 1°C$ for 72 hours, then centrifuged at 5000 rpm for 10 min. Pellets were washed three times with double distilled sterile water and the optical density was then measured. At the same time, the number of spore and crystal proteins were determined by direct microscopic count. In genaral $2X10^8$ per/ml suspension were used for each inoculation.

The animals used in studies were male and female Swiss strain mice and female guinea-pigs which were obtained from the Surgical Research Center of Hacettepe University. All animals were maintained on free choice water and food and housed in conventional laboratory environments. The temperature and relative humidity during tests were recorded daily.

Twenty female mice and 20 male mice (with 5 as controls for each sex) 5 per cage were used for each route. In addition the same protocol was followed for the mice and the guinea-pigs except that five female guinea-pigs (one of them as control) were used.

The mice and guinea-pigs were injected by various routes in order to maximize the opportunity for the *B. thuringiensis* 330211 to behave as mammalian pathogens. Volumes injected were 0.20 ml total culture subcutaneously, 0.20 ml intraperitoneally, 0.5 ml orally, 0.15 ml percutaneously, 0.20 ml intravenously, 0.6 ml with 0.6 ml of water inhalation and 0.1 ml=2 droplets with eye irritation tests per mice. All controls were injected with the same amounts of distilled water, volumes injected were 0.5 ml total culture subcutaneously, 0.5 ml intraperitoneally, 1ml orally, 1ml percutaneously, 1ml + 1ml water with inhalation and 0.1 ml with eye irritation tests respectively in the guineapigs.

Body weight and feeding were observed for a period of one month for acute toxicity in mice, three months for prolonged toxicity in mice and fourteen days for acute toxicity in female guinea-pigs. Necropsy was then performed on representative animals and tissues from each major organ system were examined histologically, and the main organs (liver, spleen, heart, brain, kidneys + adrenals, stomach for oral toxicity) were sampled and weighed. Finally, 1 ml of heart blood of selected animals were collected for reisolation tests.

A total of 270 mice (105 female + 30 controls, 105 male + 30 controls) were used throughout the experimental series. 186 of 270 mice (75 female + 18 controls and 75 male + 18 controls) were dissected for general observations one month later. The remainder of the mice were kept for a period of 3 months for prolonged toxicity then the same procedure was carried out. All guinea-pigs (4+1 control for each application) were necropsied after 14 days.

RESULTS

The results of body and organ weights of various application routes tested are presented in Table 1 and 2 for the one month period, in Tables 3 and 4 for the three months period for mice, and in Table 5 for guinea-pigs.

No pathological findings were seen after necropsy in any of the experiments.

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

A statistically significant difference ($P<0.05$) was found between the one-month female inhalation group, male subcutaneous and intraperitoneal groups and three-month male subcutaneous group and their respective control groups. No significant difference was noted in the other groups.

DISCUSSION

According to the WHO Data Sheet on the Biological Control agents, no harmful effects have been recorded in safety tests with bees, vertebrates, mammals and man (1).

Investigations carried out with Coleoptera larvae and adults (Berosus sp.), Copepods (Cylops sp.), Cladocera (*Daphnia magna*), dragonfly larvae (Cordulla sp.), shrimp (*Artemia salina*), Chrinomid larvae (Chironomus sp.), Chaoborid larvae, fish (*Gambusia affinis*) and oysters (*Ostirea edulis*) which live in mosquito larvae breeding sites, indicated the total innocuousness of the *B. thuringiensis* endotoxin. Larvicide formulations based on the endotoxin of this agent seem therefore to have a very large safety margin for aquatic non-target organisms except for the chironomids (15).

According to maximum challenge or maximum hazard experiments which were carried out in order to determine extremes of high doses and vulnerable tisues, injection of *B. thuringiensis* H-14 was not harmful unless more than 10^6 viable organisms were introduced directly into the brain (14).

Both unformulated and formulated products, containing either *B. thuringiensis* H-14 or the agricultural strains, have proved to be remarkably safe for mammals and man, and non-target flora and fauna. This has been demonstrated in repeated conventional safety tests, many environmental studies and the commercial use of use of at least 7000 tons up to 1979, much of it on food crops (3).

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

In Dr. Margalit and Dean's records, *B. thuringiensis* did not show a single case of human toxicity after over 23 years of operational use. Maximum dosages were applied by the conventional oral, parenteral, respiratory and dermal routes, together with allergenity tests and mutagenity screening, all of which confirmed that use of *B.*

thuringiensis israelensis was not hazardous. Over 240 tons of *B. thuringiensis israelensis* were used operationally in West Africa in 1982 without causing any adverse effects (9).

The results of Benz and Altwegg indicate that none of the *B. thuringiensis* treatments had an adverse effect on

Table 1: Mean body and organ weights of *B. thuringiensis israelensis* 310211 treated female mice one month after treatment.

	Weeks 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	22.2	23	24	27.4	28.6	1.60	0.15	0.14	0.45	0.34	
	σn	1.3	1.9	1.6	1.9	1.7	0.2	0.009	0.01	0.02	0.04	
	σn-1	1.4	2.01	1.8	2.1	1.9	0.24	0.01	0.02	0.03	0.05	
	EXP. MICE	26.3	27	27.1	29	30.8	1.93	0.14	0.18	0.37	0.37	
	σn	2.7	2.9	2.2	1.2	2.08	0.35	0.01	0.04	0.04	0.04	
	σn-1	2.8	3.1	2.3	1.3	2.2	0.37	0.015	0.05	0.045	0.047	
INTRAPERITONEAL	CONTROLS	18.4	20	19.6	23.6	25.6	1.77	0.13	0.19	0.38	0.37	
	σn	1.6	0.9	3	3.9	3.5	0.23	0.02	0.06	0.02	0.02	
	σn-1	1.8	1.2	3.4	4.3	5.1	0.29	0.026	0.07	0.03	0.028	
	EXP. MICE	21	21.5	22.2	24.7	25.8	1.62	0.13	0.15	0.38	0.32	
	σn	3.2	3.3	3.4	2.8	3.2	0.31	0.02	0.030	0.02	0.04	
	σn-1	3.4	3.6	3.6	3.0	3.4	0.33	0.03	0.036	0.023	0.05	
PRECUTANEOUS	CONTROLS	19.8	20	22	27	29.6	1.81	0.18	0.15	0.43	0.38	
	σn	4.4	4.6	4.1	0.8	0.4	0	0	0	0	0	
	σn-1	4.9	5.0	4.8	1.0	0.5	0	0	0	0	0	
	EXP.MICE	21.6	22	22.4	24.1	27.1	1.71	0.12	0.16	0.35	0.33	
	σn	1.6	2.0	3.7	4.8	6.1	0.39	0.04	0.06	0.05	0.05	
	σn-1	1.8	2.1	3.9	5.1	6.4	0.42	0.042	0.07	0.06	0.06	
INTRAVENOUS	CONTROLS	25.2	23.8	26.2	28.8	31	1.96	0.17	0.25	0.40	0.33	
	σn	0.4	1.3	1.3	0.9	2	0.2	0.03	0.07	0.04	0.02	
	σn-1	0.45	0.4	1.4	1.0	2.4	0.3	0.04	0.09	0.05	0.03	
	EXP. MICE	25.2	24	26.8	28.6	31.6	2.03	0.14	0.28	0.36	0.40	
	σn	1.16	1.7	1.6	2.6	2.0	0.3	0.02	0.2	0.04	0.06	
	σn-1	1.3	1.9	1.7	2.9	2.6	0.4	0.024	0.25	0.045	0.07	
EYE IRRITATION	EXP.MICE	21.4	24	24.8	25.2	28.8	1.77	0.13	0.18	0.38	0.34	
	σn	1.4	0.8	2.3	2.7	2.4	0.2	0.02	0.06	0.02	0.06	
	σn-1	1.6	0.9	2.5	3	2.6	0.3	0.026	0.07	0.027	0.07	
INHALATION	CONTROLS	22	22.5	21.4	23.6	25	1.54	0.12	0.17	0.35	0.30	
	σn	2.7	2.8	2.8	2.5	3.4	0.06	0.009	0.05	0.012	0.02	
	σn-1	3.0	3.2	3.1	2.8	3.8	0.07	0.01	0.06	0.015	0.03	
	EXP. MICE	28.4	28	28	30.1	31.1	2.09	0.14	0.20	0.39	0.38	
	σn	3.8	2.7	2.8	4.3	3.9	0.7	0.02	0.09	0.02	0.05	
	σn-1	4.0	2.9	3.0	4.5	4.2	0.8	0.022	0.1	0.023	0.057	
ORAL ADMINISTRATION	CONTROLS	27.6	26	28.2	29.4	31.2	1.85	0.13	0.25	0.38	0.38	0.36
	σn	1.1	1.9	1.9	2.3	2.03	0.1	0.02	0.01	0.02	0.05	0.01
	σn-1	1.3	2.1	2.1	2.6	2.28	0.2	0.028	0.014	0.026	0.06	0.013
	EXP. MICE	31.8	26.5	30.8	30.6	32.4	1.84	0.13	0.17	0.37	0.35	0.29
	σn	2.1	2.7	0.7	2.8	3.2	0.3	0	0.02	0.03	0.03	0.03
	σn-1	2.3	3.1	0.8	3.1	3.5	0.4	0	0.026	0.04	0.04	0.04

the eathworm population in the treated plots. Although no quantitative data concerning other invertebrates have been collected, e.g. snails, forficula, myriapodes and wood-lice, no evident differences in the density of these species could be observed (4).

In our studies observations after one month of inocula-

Table 2: Mean body and organ weights of *B. thuringiensis israelensis* 310211 treated male mice one month after treatment.

	Weeks 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	16.8	24.4	25.6	21	26.5	1.90	0.16	0.16	0.34	0.44	
	σn	1.4	1.7	1.7	2.2	1.5	0.18	0.009	0.02	0.03	0.02	
	σn-1	1.6	1.9	1.9	2.5	2.1	0.23	0.01	0.03	0.04	0.026	
	EXP. MICE	24.7	25	30.9	32.2	33.8	2.09	0.18	0.18	0.38	0.51	
	σn	3.4	3.2	4.7	4.4	3.8	0.3	0.02	0.03	0.02	0.09	
	σn-1	3.6	3.4	4.9	4.6	4.1	0.32	0.023	0.04	0.021	1	
INTRAPERITONEAL	CONTROLS	26.6	31	35.2	33	35	1.69	0.14	0.10	0.36	0.43	
	σn	2.8	4.2	3.5	4.0	3.7	0.3	0.02	0.01	0.01	0.08	
	σn-1	3.2	4.7	3.9	4.4	3.9	0.4	0.03	0.02	0.02	0.12	
	EXP. MICE	15	15.5	24.3	27.4	29.8	1.90	0.14	0.17	0.38	0.47	
	σn	3	3.1	4.3	1.6	1.7	0.3	0.01	0.02	0.02	0.06	
	σn-1	3.1	3.4	4.6	1.8	1.8	0.34	0.016	0.03	0.03	0.07	
PERCUTANEOUS	CONTROLS	25	25.4	28	29.7	32	1.91	0.20	0.18	0.42	0.43	
	σn	0.8	1.4	1.4	3.2	2.1	0.2	0.03	0.02	0.04	0.05	
	σn-1	1.6	1.8	2.1	4.0	2.8	0.28	0.04	0.03	0.06	0.07	
	EXP. MICE	26	26.2	27	28.4	31	1.89	0.17	0.18	0.41	0.45	
	σn	1.4	2.1	0.8	1.0	2.4	0.2	0.05	0.02	0.04	0.05	
	σn-1	1.8	2.4	1.2	1.4	3.2	0.3	0.06	0.03	0.06	0.07	
INTRAVENOUS	CONTROLS	28.6	29	35	35.6	37.8	2.80	0.18	0.31	0.40	0.58	
	σn	2.1	2.7	1.26	1.2	1.9	0.7	0.03	0.1	0.003	0.02	
	σn-1	2.4	3.1	1.41	1.3	2.1	0.8	0.04	0.14	0.004	0.03	
	EXP. MICE	28.8	35.3	36.9	36.8	38.6	2.30	0.19	0.29	0.37	0.52	
	σn	3.8	3.8	4.0	4.1	3.6	0.28	0.04	0.12	0.03	0.12	
	σn-1	4.0	4.0	4.2	4.3	3.8	0.3	0.043	0.13	0.034	0.13	
EYE IRRITATION	EXP. MICE	22.7	23	26.7	31.5	33.6	2.06	0.16	0.17	0.40	0.47	
	σn	5.1	4.4	4.4	3.5	3.6	0.19	0.02	0.04	0.02	0.07	
	σn-1	5.4	4.8	5.1	4.1	3.9	0.21	0.027	0.05	0.027	0.08	
INHALATION	CONTROLS	23.8	24	28.2	30.6	34.8	2.17	0.15	0.15	0.40	0.46	
	σn	4.4	3.9	3.6	3.4	2.9	0.12	0.03	0.02	0.009	0.06	
	σn-1	5.0	4.6	4.08	3.8	3.2	0.14	0.04	0.03	0.01	0.07	
	EXP. MICE	24	25	28.2	30.9	34	2.04	0.16	0.16	0.40	0.49	
	σn	3.8	3.2	3.3	2.9	4.0	0.4	0.02	0.06	0.02	0.05	
	σn-1	4.0	4.0	3.5	3.1	4.2	0.5	0.03	0.65	0.03	0.056	
ORAL ADMINISTRATION	CONTROLS	24.4	25	33.7	35.5	36.2	2.29	0.20	0.29	0.38	0.54	0.36
	σn	3.6	2.8	4.6	3.8	4.1	0.06	0	0.08	0.01	0.02	0.02
	σn-1	4.03	3.2	5.3	4.4	4.7	0.08	0	0.1	0.02	0.03	0.03
	EXP. MICE	29.8	30	34.3	36.5	38	2.49	0.16	0.27	0.39	0.60	0.36
	σn	4.7	4.6	4.0	4.0	3.4	0.6	0.02	0.12	0.01	0.19	0.07
	σn-1	5.0	5.1	4.3	4.3	3.7	0.7	0.05	0.13	0.02	0.21	0.076

Table 3: Mean body and organ weights of *B. thuringiensis israelensis* 330211 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	22.2	24	26.7	28.6	31	32	35.5	35	37	31	35	35	1.96	0.17	0.17	0.45	0.39	
	σn	1.3	1.6	1.6	1.7	3.0	2.0	2.5	3.0	3.0	3.0	3.0	3.1	0.4	0.03	0.03	0.01	0.07	
	σn-1	1.4	1.8	1.9	1.9	4.2	2.8	3.5	4.2	4.2	4.2	4.2	4.3	0.5	0.04	0.04	0.02	0.08	
	Exp. Mice	23.8	25	27.2	29.4	28.8	28.4	30.2	30.6	32	29.3	31.2	32	1.78	0.15	0.15	0.37	0.38	
	σn	2.4	2.09	2.4	3.7	2.9	2.4	2.5	2.4	2.5	2.7	2.4	2.5	0.2	0.02	0.03	0.02	0.05	
	σn-1	2.7	2.3	2.6	4.1	3.3	2.7	2.8	2.7	2.8	3.0	2.6	2.8	0.25	0.03	0.04	0.03	0.06	
INTRAPERITONEAL	Controls	18.4	19.6	23.6	24.8	25	24.5	27.5	28.5	29	27	30	30	1.79	0.14	0.25	0.38	0.38	
	σn	1.6	3	3.9	4.2	1.0	2.5	1.5	0.5	1.0	1.0	2	2	0.24	0.02	0.07	0.02	0.02	
	σn-1	1.8	3.4	4.4	4.7	1.4	3.5	2.1	0.7	1.4	1.4	2.8	2.8	0.26	0.03	0.08	0.027	0.024	
	Exp. Mice	23.6	26.6	25.8	31.2	29.8	32	32.4	29.6	30	33.6	33.6	32	2.01	0.15	0.22	0.40	0.41	
	σn	2.7	3.5	2.7	2.0	2.3	2.4	2.8	1.4	2.5	2.4	2.9	2.1	0.2	0.03	0.05	0.03	0.03	
	σ-1	3.0	3.9	3.0	2.2	2.5	2.7	3.2	1.6	2.8	2.7	3.2	2.4	0.23	0.036	0.056	0.04	0.04	
PERCUTANEOUS	Controls	13.1	22	27	30	29	31	33.5	33	31	33	33	33	1.81	0.14	0.10	0.42	0.39	
	σn	4.8	4.1	0.8	0	1.0	1.0	0.5	1.0	1.0	0	1.0	0	0.06	0.02	0.03	0.008	0.02	
	σn-1	5.0	4.8	1.0	0	1.4	1.4	0.7	1.4	1.4	0	1.4	0	0.07	0.03	0.037	0.01	0.028	
	Exp. Mice	22.8	25.4	28	26.4	26.6	29	24.7	33	30.5	34.2	33.5	28.7	2.0	0.19	0.19	0.45	0.46	
	σn	3.9	5.4	5.6	5.2	6.8	6.1	2.7	3.3	2.9	4.8	2.9	1.9	0.3	0.02	0.03	0.04	0.04	
	σ-1	4.4	6.06	6.3	5.8	7.6	6.9	3.2	3.8	3.4	5.5	3.4	2.2	0.4	0.03	0.04	0.046	0.05	
INTRAVENOUS	Controls	25.2	23.8	26.2	28.8	31	27	30	31	31	31	31	31	2.08	0.15	0.25	0.36	0.46	
	σn	0.4	1.3	1.3	0.9	2.0	1.0	0	1.0	1.0	1.0	1.0	1.0	0	0	0	0	0	
	σn-1	0.45	1.4	1.4	1.0	2.4	1.4	0	1.4	1.4	1.4	1.4	1.4	0	0	0	0	0	
	Exp. Mice	25.2	24	26.8	28.6	31.6	28.8	31.4	32	32	33	34	33	2.2	0.15	0.17	0.41	0.44	
	σn	1.16	1.7	1.6	2.6	2.0	3.7	2.6	2.1	3.4	3.7	2.1	2.1	0.2	0.02	0.06	0.02	0.08	
	σ-1	1.3	1.9	1.7	2.9	2.6	4.1	2.9	2.7	4.2	4.0	2.8	2.9	0.3	0.03	0.07	0.03	0.09	
Eye Irritation	Exp. Mice	21.4	24	24.8	25.2	28.8	24.5	28	28.4	28.6	27.8	29	30.5	1.85	0.16	0.17	0.38	0.37	
	σn	1.4	0.8	2.3	2.7	2.4	2.5	2.4	2.9	2.6	2.4	6.9	3.2	0.2	0.1	0.04	0.01	0.01	
	σ-1	1.6	0.9	2.5	3.0	2.6	3.0	2.7	3.2	2.9	2.7	7	3.6	0.3	0.13	0.05	0.017	0.02	
INHALATION	Controls	22	21.8	23.6	25	25.2	25	25	26	26.5	27	28	28	1.49	0.16	0.17	0.29	0.31	
	σn	2.7	2.9	2.5	3.4	3.2	0	0	0	0	0	0	0	0	0	0	0	0	
	σn-1	3.0	3.3	2.8	3.8	3.6	0	0	0	0	0	0	0	0	0	0	0	0	
	Exp. Mice	30.8	32.4	33	34.4	33.8	34.4	33.6	35.6	32.4	33.6	34.4	34	1.99	0.17	0.16	0.39	0.45	
	σn	2.4	2.7	2.7	3.2	3	3.5	1.8	2.9	2.6	3.1	2.6	3.1	0.2	0.05	0.01	0.03	0.04	
	σ-1	2.7	3.2	3.0	3.5	3.4	3.9	2.0	3.2	2.9	3.5	2.9	3.5	0.3	0.06	0.016	0.04	0.05	
Oral Administration	Controls	27.8	26	28.2	29.4	31.2	29	32	33	36	35	33	33	2.13	0.14	0.14	0.35	0.48	0.29
	σn	1.1	1.9	1.9	2.3	2.03	1.0	1.0	1.5	2.2	1.0	1.0	1.0	0.18	0.04	0.005	0.02	0.03	0.06
	σn-1	1.3	2.1	2.1	2.6	2.28	1.4	1.4	2.1	3.1	1.4	1.4	1.4	0.25	0.05	0.07	0.03	0.04	0.08
	Exp. Mice	31.8	26.5	30.8	30.6	32.4	34.8	31.6	33	33.6	34	34	33.5	2.00	0.16	0.17	0.41	0.44	0.28
	σn	2.1	2.7	0.7	2.8	3.2	3.1	4.2	3.0	1.4	0.8	2.1	1.8	0.14	0.01	0.03	0.008	0.06	0.01
	σ-1	2.3	3.1	0.8	3.1	3.5	3.6	4.6	3.4	1.6	1.0	2.5	2	0.15	0.02	0.04	0.01	0.07	0.016

Table 4: Mean body and organ weights of *B. thuringiensis* i*sraelensis* 330211 treated male mice three months after teatment.

	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	16.8	24.4	25.8	20.8	26.5	28	28	28	28	29	29	30	1.80	0.16	0.18	0.33	0.43	
	σn	1.4	1.7	1.7	2.4	1.5	2.0	2.0	2.0	2.0	1.0	1.0	1.5	0.2	0.02	0.06	0.03	0.019	
	σn-1	1.6	1.9	1.9	2.7	2.1	2.8	2.8	2.8	2.8	1.4	1.4	2.1	0.23	0.029	0.07	0.034	0.02	
	Exp. Mice	23.6	33	34.8	36.4	38	32.4	37.2	39.2	39.4	40.4	37.6	39.8	2.83	0.20	0.26	0.35	0.64	
	σn	3.3	5.2	4.8	5.2	5.8	12.4	5.5	5.5	5.7	4.9	5.1	5.7	0.6	0.04	0.03	0.07	0.13	
	σn-1	3.7	5.8	5.4	5.8	6.5	13	6.2	6.1	6.4	5.5	5.7	6.4	0.7	0.05	0.04	0.078	0.15	
INTRAPERITONEAL	Controls	26.6	31	35.2	36	32	33	33	34	36	35	35	36	1.90	0.16	0.13	0.37	0.47	
	σn	2.8	4.2	3.5	2.0	1.0	3.0	1.0	1.0	0	1.0	1.0	1.5	0.3	0.02	0.03	0.01	0.07	
	σn-1	3.2	4.7	3.9	2.2	1.4	4.2	1.4	1.4	0	1.4	1.4	2.1	0.34	0.03	0.035	0.017	0.08	
	Exp. Mice	17.2	26.6	27.6	30.2	31.4	32.8	33	35	30.8	34.8	32	35.2	2.13	0.18	0.35	0.34	0.52	
	σn	2.9	2.4	2.2	2.4	3.2	3.2	2.6	2.6	2.0	2.7	2.1	2.3	0.2	0.04	0.02	0.03	0.01	
	σn-1	3.3	2.7	2.5	2.7	3.5	3.6	3.0	3.0	2.2	3.0	2.4	2.5	0.3	0.05	0.026	0.035	0.02	
PRECUTANEOUS	Controls	25	25.4	28	29.7	32	33	33	33	33.7	34	34	34	2.21	0.17	0.19	0.38	0.42	
	σn	0.8	1.4	1.4	3.2	2.1	1.0	1.4	1.0	1.4	0	0	0	0.8	0.02	0.03	0.05	0.05	
	σn-1	1.6	1.8	2.1	4.0	2.8	1.4	1.8	1.4	2.1	0	0	0	1.2	0.03	0.04	0.07	0.07	
	Exp. Mice	26	26.2	27	28.4	31	32	32	34	34.5	35	36	36.7	1.92	0.18	0.22	0.44	0.53	
	σn	1.4	2.1	0.8	1.0	2.4	0	1.0	3.0	3.9	1.0	1.5	1.5	0.2	0.02	0.05	0.04	0.03	
	σn-1	1.8	2.4	1.2	1.4	3.2	0	1.4	3.5	4.2	1.4	2.1	2.1	0.3	0.03	0.07	0.06	0.06	
INTRAPENOUS	Controls	28.6	35	35.6	38.6	41.5	41	39	39.5	40	42	38	41	2.71	0.19	0.27	0.40	0.60	
	σn	2.1	1.2	1.2	1.3	0.5	1.0	1.0	1.5	1.4	0	0	0	0.6	0.03	0.12	0.004	0.04	
	σn-1	2.4	1.4	1.3	1.5	0.7	1.4	1.4	2.1	2.2	0	0	0	0.7	0.037	0.14	0.005	0.047	
	Exp. Mice	26.6	33	33.8	34.8	37.4	37.6	36.2	37.8	37.6	37.8	39	39	2.17	0.21	0.21	0.36	0.58	
	σn	3.0	2.0	2.4	2.4	1.6	1.4	2.1	2.3	2.6	2.1	1.6	2	0.17	0.03	0.05	0.02	0.04	
	σ-1	3.4	2.3	2.7	2.5	1.8	1.6	2.3	2.5	2.9	2.4	1.8	2.1	0.19	0.033	0.06	0.026	0.05	
Eye Irritation	Exp. Mice	24.4	27.4	31	32.6	32	32.2	34.4	29	37.2	34.8	38.4	37.8	2.31	0.20	0.26	0.44	0.60	
	σn	2.3	1.7	1.2	2.6	4.8	3.7	3.2	3.0	2.4	2.9	1.5	1.4	0.3	0.01	0.09	0.03	0.08	
	σ-1	2.6	1.9	1.4	2.9	5.4	4.2	4.0	3.4	2.6	3.3	1.6	1.6	0.4	0.015	0.1	0.037	0.09	
INHALATION	Controls	25.2	28.2	30.6	34.8	29	33	34	33.5	35	37.5	39	38.5	2.27	0.21	0.31	0.33	0.53	
	σn	3.8	3.6	3.4	2.9	1.0	1.0	1.0	1.5	1.0	0.5	1.0	1.5	0.18	0.05	0.02	0.05	0.03	
	σn-1	4.4	4.0	3.8	3.2	1.4	1.4	1.4	2.1	1.4	0.7	1.4	2.1	0.2	0.07	0.028	0.07	0.05	
	Exp. Mice	26.6	27.4	34.2	36.8	35.6	36.6	33.2	37.2	38	39.2	40	40	2.54	0.20	0.26	0.40	0.61	
	σn	4.0	2.9	2.8	2.7	3.1	4.9	5.1	7.8	4.5	4.8	5.2	4.8	0.2	0.02	0.07	0.02	0.07	
	σ-1	4.5	3.2	3.1	3.0	3.5	5.5	5.8	8.7	5.0	5.4	5.8	5.6	0.3	0.023	0.08	0.025	0.08	
Oral Administration	Controls	24.4	33.7	35.5	36.2	35.5	39	38	38.5	39	40	37	39.5	2.29	0.18	0.21	0.38	0.56	0.33
	σn	3.6	4.6	3.8	4.1	4.5	5.0	6.0	6.5	7.0	6.0	5.0	5.5	0.19	0.04	0.02	0.01	0.06	0.03
	σn-1	4.03	5.3	4.4	4.7	6.3	7.07	8.4	9.1	9.0	8.4	7	7.7	0.22	0.047	0.028	0.014	0.07	0.035
	Exp. Mice	29	34.5	36	37.2	40.2	38.5	39.5	40	34	38	37	41	2.58	0.21	0.21	0.38	0.65	0.37
	σn	2.6	2.3	1.2	3.1	2.5	2.5	3.8	3.0	3.4	4.8	5.3	5.3	0.69	0.02	0.07	0.02	0.08	0.04
	σ-1	3.0	2.6	1.4	3.5	2.9	3.0	4.4	3.5	4.0	5.6	6.2	6.2	0.8	0.03	0.08	0.023	0.09	0.05

Table 5: Mean body and organ weights of *B. thuringiensis israelensis* 330211 treated guinea-pigs.

	Days After Injection	Mean Body Weights in g.					Mean Organ weights in g.					
		0	3	6	10	14	Liver	Heart	Spleen	Brain	Kidneys+Adrenals	Stomach
SUBCUTANEOUS	CONTROLS	450	440	415	470	475	14.24	1.56	0.74	3.23	3.93	
	σn	0	0	0	0	0	0	0	0	0	0	
	σn-1	0	0	0	0	0	0	0	0	0	0	
	Exp. Guinea-pigs	350	353	351	413	415	15.54	1.32	0.77	3.06	3.63	
	σn	8.16	9.4	6.23	12.4	13.2	2.9	0.09	0.20	0.20	0.30	
	σn-1	10	11.5	7.6	15.2	16.1	3.6	0.10	0.30	0.28	0.40	
INTRAPERITONEAL	CONTROLS	460	450	455	520	525	21.09	1.64	0.90	3.19	4.04	
	σn	0	0	0	0	0	0	0	0	0	0	
	σn-1	0	0	0	0	0	0	0	0	0	0	
	Exp. Guinea-pigs	306	370	311	361	370	13.06	1.32	0.75	3.12	3.35	
	σn	9.42	36	11.7	14.3	24	0.9	0.05	0.19	0.07	0.28	
	σn-1	11.5	44	14.4	17.5	28.8	1.14	0.07	0.23	0.08	0.34	
PERCUTANEOUS	CONTROLS	445	420	430	480	510	16.35	1.54	0.82	2.88	3.73	
	σn	0	0	0	0	0	0	0	0	0	0	
	σn-1	0	0	0	0	0	0	0	0	0	0	
	Exp. Guinea-pigs	455	440	448	508	510	19.02	1.59	0.93	3.06	3.50	
	σn	36.7	28.2	32.7	31.04	33	1.03	0.20	0.13	0.24	0.30	
	σn-1	45	34.6	40.1	38	39.6	1.26	0.25	0.16	0.29	0.40	
Eye Irritation	Exp. Guinea-pigs	393	400	393	461	460	16.2	1.60	1.23	3.29	4.06	
	σn	41.8	36.2	28.6	39.2	39	1.23	0.05	0.4	0.15	0.43	
	σn-1	51.3	38.4	35.1	48	46	1.51	0.06	0.57	0.19	0.53	
INHALATION	CONTROLS	300	300	310	350	360	11.52	1.27	0.63	3.09	3.44	
	σn	0	0	0	0	0	0	0	0	0	0	
	σn-1	0	0	0	0	0	0	0	0	0	0	
	Exp. Guinea-pigs	378	400	390	443	450	15.83	1.55	0.92	2.92	3.82	
	σn	53.9	35.5	28.2	37.2	38	2.39	0.27	0.10	0.10	0.40	
	σn-1	66	43.5	34.6	45.6	48.6	2.90	0.34	0.14	0.13	0.47	
Oral Administration	CONTROLS	420	410	410	470	490	15.56	1.45	0.72	3.07	383	4.54
	σn	0	0	0	0	0	0	0	0	0	0	0
	σn-1	0	0	0	0	0	0	0	0	0	0	0
	Exp. Guinea-pigs	378	426	431	476	480	21.12	1.56	1.15	3.27	4.09	4.71
	σn	55.7	20.5	6.23	12.4	13.6	3.80	0.08	0.36	0.32	0.30	0.21
	σn-1	68.2	25.1	7.63	15.2	17.4	4.60	0.10	0.44	0.39	0.40	0.26

tion showed that there was no incidence of either mortality or symptoms of poisoning in controls or in animals treated with *B. thuringiensis israelensis* 330211. During the experimental period the behaviour and feeding were close to the controls and the organ weights were the normal range.

REFERENCES

1. Anonymous: Data Sheet on the Biological Control Agent Bacillus thuringiensis serotype H-14 (de Barjac 1978). WHO/VBC/79. 750 Rev. 1, Geneva, 1979.

2. Anonymous: Mammalian safety or microbiol control agents for vector control. WHO/VBC/81. 820, Geneva, 1981.

3. Anonymous: Informal Consultation on Standardization of Bacillus thuringiensis H-14. WHO/VBC/81. 1-15, Geneva, 1981.

4. Benz G, Altwegg A : Safety of Bacillus thuringiensis for earthworms. J Invertebrate Pathol 26:125-126, 1975.

5. Chihg-Ning S, Georghiou GP, Weiss K : Toxicity of Bacillus thuringiensis var. israelensis to mosquito larvae variously resistant to conventional insecticides, Mosq News 40(4):614-617, 1980.

6. Erdridge BF, Callicrate J : Efficacy of Bacillus thuringiensis var. israelensis de Barjac for mosquito control in a western Oregon Log. Pond. Mosq News 42 (1):102-105, 1982.

7. Ignoffo CM, Garcia C, Kroha MJ, Fukuda T, Couch TL : Laboratory tests to evaluate the potential efficacy of Bacillus thuringiensis var. israelensis for use against mosquitoes. Mosq News 41(1):85-93, 1981.

8. Lacey LA, Lacey JM : The larvicidal activity of Bacillus thuringiensis var. israelensis (H-14) against mosquitoes of the Central Amazon Basin. Mosq News 41(2):266-270, 1981.

9. Margalit J, Dean D : The story of Bacillus thuringiensis var. israelensis. J Am Mosq Control Assoc 1(1):1-7, 1985.

10. Mc Laughlin RE, Fukuda T, Willis OR, Billodeaux J : Effectiveness of Bacillus thuringiensis serotype H-14 against Anopheles crucians. Mosq News 42(3):370-373, 1982.

11. Mulla MS, Darwazeh HA : Larvicidal efficacy or various formulation of Bacillus thuringiensis serotype H-14 against mosquitoes. Bull Soc Vector Ecol 9(1):51-58, 1984.

12. Purcell BH : Effects of Bacillus thuringiensis var. israelensis on Aedes taeniorhynchus and some non-target organisms in the salt marsh. Mosq. News 41(3):476-483, 1981.

13. Sebastian RJ, Brust RA : An evaluation of two formulations of Bacillus thuringiensis var. israelensis for larval mosquito control in sod-lined simulated pools. Mosq News 41(3):508-511, 1981.

14. Shadduck JA : Bacillus thuringiensis serotype H-14 maximum challenge and eye irritation safety tests in mammals. WHO/VBC/80. 763, Geneva, 1980.

15. Sinegre G, Gaven B, Jullien JL : Safety of Bacillus thuringiensis serotype H-14 for non-target organisms in mosguito breeding sites of the French Mediterranean Coast. WHO/VBC/79. 742, Geneva, 1979.

Correspondence:
Nurdan Ozer
Hacettepe University
Faculty of Science
Dept. of Biology
Beytepe, Ankara,
TÜRKIYE.