SAFETY TESTS ON MICE AND GUINEA-PIGS WITH *BACILLUS SPHAERICUS* ISOLATE*

AYSE BOSGELMEZ
LÜTFÜ CAKMAKCI
SELMA YÖRÜKAN
NACI M. BOR
NURDAN ÖZER
BULENT ALTEN
BEHZAT GÜRKAN
ÖNER KOÇAK
OLUS KARAYALCIN
FERIHA GURKAN
ESIN ALTEN

SUMMARY: Mammalian toxicity tests were carried out on mice and guinea-pigs with Bacillus sphaericus isolate numbered 3302109 isolated from the Icel region of Turkey. Test animals were injected by various routes, including subcutaneous, intraperitoneal, intravenous, and percutaneous. Also ingestion, inhalation and eye irritation tests were carried out using stock suspension of 2X10^8 bacteria per/ml for each inoculation. None of the animals showed any evidence of illness, and their behavior, growth and average organ weights were normal.

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

INTRODUCTION

Spore forming bacteria of the genus *Bacillus* have become popular for biological control of insect pests because of their economic production and specifiy for the target organism. *Bacillus sphaericus* is ubiquitous and cosmopolitan in soil and soil-aquatic systems and is a promising agent for the control of mosquitoes. Many advences have been made in isolating and producing several strains of *B. sphaericus* with a wide spectum of activity against larvae of several mosquito species as tested by various researchers (4,5,8,9,12,13). In this study *B. sphaericus* isolate coded as 3302109 which, was shown to have 100% efficiency against *Aedes* aegypti. In our previous studies we investigated their mammalian pathogenicity in mice and guinea pigs.

MATERIALS AND METHODS

Sources of culture

The organism used in these experiments was isolated from soil samples of mosquito breeding sites from the Icel region of Turkey and identified as *B. sphaericus* isolate 3302109 according to its biochemical characteristics. It was cultured in a 50 ml Nutrient Broth Medium (Difco) at 32 $\pm$1°C for 72 hours. The culture was then centrifuged at 5000 rpm for 10 min. Pellts 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 general 2x10^8 per/ml suspensions were used for each inoculation.

Animals

The animals used in these studies were male and female Swiss strain mice and female guinea-pigs which were supplied by the Medical and Surgical Research Center of Hacettepe University. All animals were maintained on free-choice water and food and housed in laboratory conditions. The temperature and relative humidity were monitored during the experiments.

The mice and guinea-pigs were injected via various routes including subcutaneous, intraperitoneal, percutaneous, intravenous, oral (gavage), inhalation and eye irritation, in order to maximize the opportunity for the bacteria to behave as mammalian pathogens. A total of 270 mice (female + 30 controls, 105 male + 30 controls) were used throughout the experimental series.

* This study has been supported by Scientific and Technical Research Council of Turkey (TOAG-TARMIK) and was conducted at the Surgical Research Center, Hacettepe University Medical School, Ankara, Turkiye.

Furthermore 186 of 270 mice, of which 75 were female + 18 controls and 75 male+18 controls, were sacrificed and dissected for necropsy, organ sampling and reisolation tests after a month. The remainder were kept for a period of 3 months for subacute observations. All guinea-pigs were necropsied after 14 days.

In brief, observations included behaviour, feeding, body and organ weight during the experiments, organ and tissue sampling (liver, spleen, heart, brain, kidneys and adrenals, stomach for oral toxicity), necropsy and collection of heart blood for reisolation tests.

The experimental series are as follows:

1.1. SUBCUTANEOUS TOXICTY IN MICE

Twenty female mice and 20 male mice were used, in groups of 5 animals per cage, with 5 as controls for each sex. Injections of 0.20 ml of total culture per mouse or 0.20 ml of distilled water for the controls were made. Observations included body weight of the groups during the ensuing moth, followed by necropsy and organ sampling and reisolation tests.

1.2. SUBCUTANEOUS TOXICITY IN GUINEA-PIGS

Five female guinea-pigs of which one was left as a control were used. The same protocol as for the mice was followed (1.1) but with injections of 0.5 ml total culture per guinea-pig and 0.5 ml distilled water for the control. Necropsy was performed after 14 days.

2.1. INTRAPERITONEAL TOXICITY IN MICE

Forty (20 female, 20 male) mice, of which 5 were controls in each sex, were divided into groups of 5. Injections of 0.20 ml of total culture per mouse and 0.20 ml of distilled water for the controls were performed.

2.2. INTRAPERITONEAL TOXICITY IN GUINEA-PIGS

Five female guinea-pigs were used, of which one was the control. The same protocol as for the mice was followed (2.1) but with injections of 0.5 ml total culture per guinea-pig and 0.5 ml distilled water for the control.

3.1. ORAL TOXICITY IN MICE (GAVAGE)

Twenty female and 20 male mice, with 5 controls for each sex were used in groups of 5 animals per cage. 0.5 ml total culture per mouse was directly administered into the oesophagus, whereas controls received 0.5 ml distilled water.

3.2. ORAL TOXICITY IN GUINEA-PIGS (GAVAGE)

Five female guinea-pigs were used, of which one of them was left as a control. The same protocol as for the mice was followed (3.1) but 1 ml total culture per guinea-pig or 1 ml of distilled water to the control animal were administered.

4.1. PERCUTANEOUS APPLICATION IN MICE

Twenty female and 20 male mice, of which 5 were lift as a control for each sex were used. After shaving the abdominal skin (1 cm^2), 0.15 ml of total culture was applied to the skin. Each control received 0.15 ml distilled water. The animals were caged in groups of 5.

4.2. PERCUTANEOUS APPLICATION IN GUINEA-PIGS

Five female guinea-pigs were used, of which one was the control. The same protocol as for the mice (4.1) was followed but with injection of 1 ml of total culture per guinea-pig and 1 ml distilled water for the control.

5.1. INTROVENOUS INJECTION IN MICE

Forty mice (20 female, 20 male) were used with controls for each sex in groups of 5 animals per cage. An intravenous booster injection with 0.20 ml total culture was performed in the tail vein or 0.20 ml of distilled water for controls.

6.1 INHALATION IN MICE

Twenty female and male mice, with 5 for each sex as controls were used. An aerosol of 0.6 ml of total culture diluted with 0.6 ml of water was sprayed for five minutes into an airtight chamber containing the 20 mice. The control inhaled the same volume distilled water.

6.2. INHALATION IN GUINEA-PIGS

Four female guinea-pigs+one control were used. The same protocol as for the mice (6.1) was followed except that an aerosol of 1 ml of total culture diluted with 1 ml of water was sprayed with 1 ml distilled water for the control.

7.1. PRIMARY EYE IRRITATION TEST ON MICE

Twenty male and 20 female mice were used. The animals were then caged in groups of 5.0.1 ml = 2 droplets of total culture per mouse was inoculated into the left eye of each mouse, the other eye being used as control with 0.1 ml of distilled water for the right eye.

7.2. PRIMARY EYE IRRITATION TEST ON GUINEA-PIGS

Four female guinea-pigs were used. The same protocol as for the mice was followed (7.1)

8. SUBACUTE TOXICITY IN MICE

Of the total 270 mice, 186 (75 female + 18 controls and 75 male + 18 controls) were dissected for necropsy, organ sampling and reisolation tests one month after treatment. The remaining animals (30 female + 12 controls, 30 male mice +12 controls) were kept under observation in groups of 5 experimental animals per cage, for subacute toxicity for a period of three months. The same protocol was followed as for the mice of the experimental series. The observations included body weight of the groups during the three months, then necropsy and sampling of organs, organ weight and reisolation tests, three months after the beginning of the study.

RESULTS

The results of body weights and organ weights of various application routes tested are presented in Table 1 and 2 after one month, in Table 3 and 4 after three months for mice and in Table 5 for guinea-pigs. Cystic formation was noticed in the liver of the male mice with percutaneous application as seen in Table 2.

Table 1: Mean body and organ weights of *B. sphaericus* 3302109 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	20.5	25	29.5	28	30.5	1.52	0.14	0.13	0.42	0.19	
	σn	5.5	6.0	6.5	5.0	1.5	0.1	0.05	0.03	0.02	0.01	
	σn-1	7.7	8.48	9.19	7.07	2.12	0.14	0.07	0.05	0.03	0.015	
	EXP.MICE	27	26.3	29.4	27.3	28.2	2.04	0.13	0.21	0.38	0.19	
	σn	2.19	1.73	1.9	2.45	2.19	0.9	0.02	0.11	0.02	0.02	
	σn-1	2.3	1.82	2.01	2.58	2.32	1	0.021	0.12	0.02	0.023	
INTRAPERITONEAL	CONTROLS	20	21	26	25	28	1.20	0.11	0.09	0.38	0.15	
	σn	0	2.4	2.1	0	2	0.08	0.005	0.005	0.02	0.01	
	σn-1	0	3	2.6	0	2.8	0.1	0.007	0.007	0.03	0.012	
	EXP.MICE	20.8	22.4	26.2	24.7	26.9	1.62	0.13	0.18	0.39	0.18	
	σn	3.1	3.8	3.9	4.8	4.3	0.24	0.01	0.03	0.02	0.03	
	σn-1	3.2	4	4.1	5.1	4.5	0.25	0.018	0.04	0.028	0.031	
PRECUTANEOUS	CONTROLS	20.8	25.5	24.7	28	25.5	1.96	0.17	0.24	0.44	0.20	
	σn	3.9	3.9	3.5	6.2	4.7	0.2	0	0.04	0.005	0.02	
	σn-1	4.3	4.5	4.1	7.2	5.4	0.3	0	0.05	0.007	0.028	
	EXP.MICE	23.5	25.2	26.4	28.1	26.8	1.92	0.13	0.23	0.38	0.18	
	σn	4.2	4.4	3.8	3.4	4.2	0.3	0.019	0.05	0.05	0.02	
	σn-1	4.4	4.7	4.0	3.6	4.5	0.32	0.02	0.055	0.055	0.026	
INTRAVENOUS	CONTROLS	28	30	32.5	33.5	30.5	1.64	0.13	0.19	0.40	0.19	
	σn	3	1	2.5	3.5	0.5	0.59	0.02	0.07	0.03	0.03	
	σn-1	4.24	1.41	3.53	4.94	0.70	0.83	0.03	0.1	0.05	0.04	
	EXP.MICE	24.5	26.8	30.3	27.5	28.4	1.70	0.13	0.19	0.38	0.19	
	σn	3.6	3.6	3.4	3.2	5.08	0.54	0.01	0.09	0.03	0.03	
	σn-1	3.8	3.82	3.59	3.43	5.35	0.57	0.02	0.095	0.04	0.034	
EYE IRRITATION	EXP.MICE	23.3	27.4	28	29.1	27.5	1.99	0.16	0.22	0.40	0.19	
	σn	2.6	3.7	3.2	4.1	3.8	0.5	0.03	0.08	0.02	0.03	
	σn-1	2.7	3.9	3.4	4.3	4.06	0.54	0.04	0.084	0.028	0.033	
IINHALATION	CONTROLS	23.6	26.2	27.4	29.6	28.6	1.85	0.14	0.16	0.40	0.18	
	σn	1.2	2.13	1.74	2.41	1.49	0.32	0.02	0.04	0.01	0.008	
	σn-1	1.34	2.38	1.94	2.7	1.67	0.4	0.026	0.05	0.015	0.009	
	EXP.MICE	19.4	25.7	26.4	28.5	26.2	1.87	0.14	0.17	0.38	0.18	
	σn	2.28	3.63	2.9	3.17	4.4	0.4	0.02	0.04	0.04	0.02	
	σn-1	2.41	3.83	3.06	3.34	4.6	0.42	0.022	0.043	0.046	0.024	
ORAL ADMINISTRATION	CONTROLS	23.6	24.2	24.2	26	24.2	1.64	0.11	0.12	0.34	0.15	0.34
	σn	2.4	2.7	3.76	3.8	4.1	0.23	0.009	0.004	0.03	0.02	0.05
	σn-1	2.7	3.1	4.2	4.3	4.6	0.28	0.01	0.005	0.03	0.03	0.07
	EXP.MICE	19.3	17.5	22.7	22	22.4	1.44	0.11	0.12	0.37	0.15	0.25
	σn	3.1	4.0	3.6	4.0	4.7	0.44	0.02	0.05	0.02	0.03	0.04
	σn-1	3.2	4.3	3.8	4.2	4.9	3.46	0.03	0.054	0.02	0.036	0.046

Table 2: Mean body and organ weights of *B. sphaericus* 3302109 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	26.5	28	32	33.5	37.5	2.80	0.19	0.32	0.34	0.32	
	σn	3.5	3.0	3.0	3.5	2.5	0.01	0.02	0.005	0.015	0.03	
	σn-1	4.9	4.2	4.2	4.9	3.5	0.02	0.028	0.007	0.018	0.035	
	EXP.MICE	34.3	35.6	39.4	38.4	37.8	2.59	0.20	0.28	0.41	0.36	
	σn	6.3	6.3	6.4	5.6	4.2	0.58	0.03	0.07	0.01	0.09	
	σn-1	6.7	6.7	6.8	6.0	4.5	0.63	0.034	0.08	0.02	0.098	
IINTRAPERITONEAL	CONTROLS	27.6	36	35	37	31	2.00	0.18	0.19	0.38	0.26	
	σn	3.2	4.2	5.0	4.0	6.0	0.005	0.05	0.03	0.03	0.03	
	σn-1	4.04	5.2	7.07	5.6	8.4	0.007	0.007	0.04	0.04	0.04	
	EXP.MICE	34.3	35.5	41.2	37.2	37.4	2.20	0.18	0.18	0.41	0.28	
	σn	5.8	5.8	5.7	4.8	5.6	0.47	0.03	0.04	0.03	0.05	
	σn-1	6.18	6.15	6.01	5.15	5.98	0.5	0.034	0.05	0.04	0.054	
PRECUTANEOUS	CONTROLS	27	28	29	33	32	1.95	0.17	0.19	0.40	0.30	
	σn	3.0	3.0	3.0	0	0	0	0	0	0	0.01	
	σn-1	4.2	4.2	4.2	0	0	0	0	0	0	0.014	
	EXP.MICE	31.6	32.8	37.2	36	36.2	3.59	0.19	0.32	0.39	0.28	
	σn	4.2	4.6	3.5	5.5	4.9	1.82	0.02	0.09	0.02	0.03	
	σn-1	4.4	4.8	3.7	5.9	5.2	1.93	0.03	0.097	0.03	0.037	
INTRAVENOUS	CONTROLS	31	34.5	37.5	33.5	34	2.07	0.19	0.18	0.39	0.27	
	σn	2.0	4.5	3.5	5.5	4.0	0.64	0.05	0.05	0	0.08	
	σn-1	2.82	6.36	4.94	7.7	5.65	0.91	0.07	0.07	0	0.09	
	EXP.MICE	30.4	30.9	36.1	35.2	34.7	1.93	0.16	0.17	0.38	0.24	
	σn	6.43	4.9	5.26	4.06	4.69	0.47	0.02	0.05	0.03	0.05	
	σn-1	6.78	5.17	5.54	4.28	4.94	0.5	0.026	0.06	0.032	0.052	
EYE IRRITATION	EXP.MICE	31.6	31.9	35.5	33.2	33.1	2.09	0.15	0.28	0.38	0.23	
	σn	4.5	5.2	4.1	5.6	5.8	0.48	0.02	0.06	0.02	0.03	
	σn-1	4.7	5.4	4.4	6.03	6.1	0.51	0.028	0.07	0.03	0.034	
INHALATION	CONTROLS	28	31.5	36.5	34.5	37.5	2.71	0.16	0.23	0.39	0.25	
	σn	2.0	0.5	2.5	0.5	0.5	0.09	0	0.02	0	0.01	
	σn-1	2.8	0.7	3.5	0.7	0.7	0.12	0	0.028	0	0.014	
	EXP.MICE	33.7	35.4	39.6	36.2	36.9	2.26	0.18	0.23	0.41	0.28	
	σn	4.7	4.2	4.8	4.7	6.0	0.46	0.03	0.09	0.02	0.04	
	σn-1	5.0	4.5	5.1	5.04	6.3	0.49	0.038	0.10	0.028	0.048	
ORA ADMINISTRATION	CONTROLS	26.5	28	34	37.5	39	1.41	0.15	0.19	0.42	0.21	0.32
	σn	3.5	3.0	4.0	3.5	1.0	0.26	0.03	0.03	0.01	0.03	0.03
	σn-1	4.9	4.2	5.65	4.9	1.41	0.37	0.04	0.05	0.02	0.035	0.04
	EXP.MICE	31.9	33.4	36.7	35.7	35.8	2.22	0.17	0.24	0.41	0.26	0.37
	σn	5.2	4.4	3.7	4.1	4.9	0.42	0.02	0.04	0.02	0.03	0.06
	σn-1	5.5	4.7	3.9	4.4	5.2	0.45	0.026	0.05	0.03	0.039	0.067

Table 3: Mean body and organ weights of *B. sphaericus* 3302109 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	20.5	25	29.5	28	27.3	29	27.6	29.6	28	32	31	31	1.67	0.15	0.13	0.37	0.46	
	σn	5.5	6.0	6.5	5	1.7	1.41	2.0	0.47	0.8	0.8	0	0	0.11	0.02	0.01	0.04	0.03	
	σn-1	7.7	8.48	9.19	7.07	2.08	1.73	2.5	0.57	1.0	1.0	0	0	0.14	0.025	0.015	0.05	0.04	
	Exp. Mice	28.6	28.6	31.8	28.6	29	29.6	31.6	30.4	29.6	32.2	33.6	33.2	1.67	0.14	0.15	0.38	0.41	
	σn	1.9	1.5	2.03	2.33	2.09	1.95	1.6	1.5	1.85	1.32	3.4	2.6	0.03	0.02	0.02	0.04	0.05	
	σn-1	2.1	1.67	2.28	2.6	2.3	2.19	1.8	1.67	2.07	1.48	3.8	3	0.038	0.23	0.03	0.045	0.056	
INTRAPERITONEAL	Controls	20	21	26	25	29	27.5	30	30	32.5	31.5	32	32	1.65	0.19	0.16	0.42	0.50	
	σn	0	2.4	2.1	0	1.0	0.5	0	0	0.5	0.5	0.8	2	0.12	0.01	0.01	0	0.02	
	σn-1	0	3.0	2.6	0	1.41	0.7	0	0	0.7	0.7	1	28	0.17	0.014	0.014	0	0.03	
	Exp. Mice	24.6	25.6	29.8	27.4	29	29.6	30.4	29.7	31.2	29.2	30.8	30.6	1.49	0.15	0.14	0.39	0.42	
	σn	2.5	2.0	1.4	2.2	1.41	1.3	1.3	1.6	1.4	2.5	3.1	3.5	0.16	0.008	0.04	0.01	0.03	
	σn-1	2.8	2.2	1.6	2.5	1.58	1.5	1.5	1.9	1.6	2.7	3.5	3.9	0.17	0.01	0.05	0.02	0.04	
PRECUTANEOUS	Controls	20.8	25.5	24.7	28	25.5	24.5	24.5	25.5	23.5	25	26.5	30	1.75	0.15	0.18	0.32	0.35	
	σn	39	39	3.5	6.2	4.7	4.5	2.5	2.5	3.5	3.0	3.5	0	0	0	0	0	0	
	σn-1	4.3	4.5	4.1	7.2	5.4	6.3	3.5	3.5	4.9	4.2	5	0	0	0	0	0	0	
	Exp. Mice	24.2	23.2	25.8	30	29.6	32	32.2	32.2	31.2	32	32.6	34	2.25	0.18	0.24	0.43	0.45	
	σn	2.2	1.9	2.8	1.41	2.8	1.9	1.4	2.6	2.03	2.1	3.2	1.7	0.29	0.03	0.06	0.04	0.05	
	σn-1	2.4	2.1	3.2	1.58	3.2	2.1	1.6	2.9	2.28	3.4	4.8	2.01	0.32	0.034	0.07	0.05	0.06	
INTRAVENOUS	Controls	28	30	32.5	33.5	34.3	34.6	36.3	36.6	35.3	40	38.6	37.6	2.20	0.19	0.24	0.32	0.59	
	σn	3.1	1.0	2.5	3.5	4.7	4.49	4.9	2.86	4.9	5.7	4.9	4.49	0.39	0.035	0.01	0.12	0.08	
	σn-1	4.24	1.41	3.5	4.9	5.8	5.5	6.02	3.5	6.1	7.0	6	5.5	0.48	0.04	0.015	0.15	0.1	
	Exp. Mice	28.2	28	32.8	30.8	30	31.6	33.4	33.3	30.8	33.8	35.8	33.8	1.72	0.17	0.40	0.41	0.46	
	σn	3.4	5.4	4.3	4.4	4.19	4.4	4.45	5.3	4.5	4.9	4.79	4.5	0.22	0.03	0.04	0.05	0.06	
	σn-1	3.8	6.1	4.8	4.9	4.69	4.9	4.9	6.5	5.1	5.5	5.35	5.06	0.25	0.04	0.05	0.097	0.07	
Eye Irritation	Exp. Mice	25.8	25.6	25	26.8	26.4	28.2	30	30	29	29.7	32.7	31.2	1.59	0.13	0.16	0.36	0.44	
	σn	4.16	2.2	1.78	2.4	2.8	3.0	2.1	2.1	2.3	2.16	2.5	3.2	0.3	0.02	0.04	0.03	0.05	
	σn-1	4.6	2.5	2.0	2.6	3.2	3.5	2.4	2.5	2.7	2.31	29	3.7	0.4	0.028	0.05	0.04	0.06	
INHALATION	Controls	23.6	26.2	27.4	29.6	28.8	30	31.5	31.5	30.5	31.5	33	32.5	1.94	0.16	0.16	0.39	0.41	
	σn	1.2	2.13	1.74	2.4	1.46	0	1.5	0.5	0.5	0.5	0	0.5	0.14	0.01	0	0.04	0.02	
	σn-1	1.34	2.38	1.94	2.7	1.6	0	2.12	0.7	0.7	0.7	0	0.7	0.2	0.014	0	0.05	0.029	
	Exp. Mice	20	25	24.6	27.2	26	29.6	30.2	28.4	28.4	29.8	30.4	30	1.55	0.13	0.10	0.36	0.36	
	σn	2.09	3.0	3.0	3.3	3.03	2.5	1.93	3.4	3.6	3.6	4.9	3.4	0.26	0.02	0.02	0.03	0.05	
	σn-1	2.3	3.4	3.3	3.7	3.4	2.8	2.16	3.8	4.03	4.08	5.5	3.8	0.3	0.03	0.03	0.04	0.06	
Oral Administration	Control	23.6	24.2	24.2	26	24.2	29.5	31.5	30.5	31	34	32	32.5	2.00	0.15	0.21	0.39	0.44	0.33
	σn	2.4	2.7	3.7	3.8	4.1	3.5	2.5	2.5	3.0	4.0	4.0	2.5	0.22	0.005	0.06	0.05	0.03	0.02
	σn-1	2.7	3.1	4.2	4.3	4.6	4.9	3.5	3.5	4.2	5.6	5.6	3.5	0.31	0.007	0.09	0.07	0.04	0.03
	Exp. Mice	20.2	22.2	25.8	24.2	26.6	27.4	25.2	26.6	28.6	31	30.4	31.2	1.55	0.15	0.14	0.36	0.38	0.34
	σn	5.2	5.4	5.6	6.5	5.3	4.0	5.0	4.4	4.3	2.5	2.5	1.08	0.2	0.01	0.04	0.04	0.03	0.03
	σn-1	5.8	6.0	6.3	7.2	5.9	4.5	5.6	4.9	4.8	2.8	2.8	1.1	0.22	0.017	0.05	0.05	0.036	0.04

Table 4: Mean body and organ weights of *B. sphaericus* 3302109 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	26.3	28	32	33.5	37	36.6	38	37	37	39.6	42.6	40	2.50	0.20	0.17	0.37	0.69	
	σn	3.5	3.0	3.0	3.5	4.3	3.29	4.08	3.5	3.7	2.05	4.1	2.3	0.43	0.04	0.05	0.009	0.14	
	σn-1	4.9	4.2	4.2	4.9	5.2	4.04	5.0	4.3	4.5	2.5	5	2.8	0.53	0.05	0.06	0.01	0.17	
	Exp. Mice	40.4	38.2	43.4	41.2	39.4	38.7	42.2	37.4	39	37.5	39.7	41	2.43	0.21	0.23	0.40	0.66	
	σn	5.7	3.4	7.05	5.6	5.6	5.4	5.6	6.4	6.3	5.2	3.9	4.48	0.38	0.04	0.05	0.03	0.07	
	σn-1	6.3	3.8	7.89	6.2	6.3	6.2	6.3	7.1	7.3	5.9	4.5	5.01	0.44	0.05	0.06	0.036	0.08	
INTRAPERITONEAL	Controls	27.6	36	35	37	38	39.5	37	39	39	39	39	42	2.36	0.21	0.20	0.39	0.65	
	σn	3.2	4.2	5.0	4.0	3.0	0.5	0	0.5	0	0.5	0.5	0	0.17	0.01	0.02	0.01	0.03	
	σn-1	4.04	5.2	7.07	5.6	4.2	0.7	0	0.7	0	0.7	0.7	0	0.24	0.014	0.03	0.02	0.05	
	Exp. Mice	31.2	32	36.6	34.4	34.4	36	37	37	38	39	39.6	40.4	2.10	0.18	0.19	0.35	0.61	
	σn	7.38	4.7	4.9	4.6	3.8	2.6	3.6	1.26	0.6	0.8	1.5	1.0	0.1	0.03	0.03	0.04	0.03	
	σn-1	8.2	5.3	5.5	5.2	4.2	3.0	4.06	1.41	0.7	1.0	1.6	1.14	0.11	0.034	0.035	0.048	0.04	
PRECUTANEOUS	Controls	27	28	29	33	28.5	29	29	29.5	28.5	28.9	31	31	1.70	0.13	0.31	0.32	0.55	
	σn	3.0	3.0	3.0	0	3.5	1.0	1.0	0.5	0.5	0.5	1.0	1.0	0.03	0.02	0.07	0.005	0.04	
	σn-1	4.2	4.2	4.2	0	4.9	1.4	1.4	0.7	0.7	0.7	1.4	1.4	0.04	0.028	0.1	0.007	0.06	
	Exp. Mice	28	31.2	35.2	35	35.2	33.4	33.8	35	36.2	35	37.2	38.6	2.19	0.17	0.17	0.42	0.66	
	σn	3.2	4.4	3.8	3.9	4.7	3.0	4.3	4.1	3.4	3.5	2.5	4.2	0.44	0.005	0.01	0.01	0.12	
	σn-1	3.6	5	4.3	4.4	5.3	3.3	4.8	4.6	3.8	4	2.8	4.7	0.5	0.007	0.02	0.02	0.14	
INTRAVENOUS	Controls	31	34.5	37.5	33.5	39.6	41	42	42	39.6	42.6	43.6	43	2.22	0.21	0.18	0.40	0.67	
	σn	2	4.5	3.5	5.5	3.2	4.8	4.0	4.8	3.2	4.5	4.1	4.1	0.4	0.02	0.02	0.04	0.07	
	σn-1	2.82	6.36	4.94	7.7	4.0	6.0	5.0	6.0	4.0	5.5	5	5	0.5	0.03	0.025	0.05	0.09	
	Exp. Mice	28.2	29.8	26	30.8	31.4	31.7	32	31.5	33.2	30	32.7	32.5	1.63	0.12	0.11	0.36	0.46	
	σn	3.2	3.8	7.3	3.5	5.4	4.6	5.0	2.9	3.5	5.3	4.3	6.1	0.32	0.02	0.03	0.03	0.08	
	σn-1	3.6	4.3	9.1	3.9	6.1	5.3	5.7	3.4	4.0	6.1	4.9	7	0.36	0.03	0.04	0.04	0.1	
Eye Irritation	Exp. Mice	26.2	27.2	31.8	28.2	30.8	30.2	32.2	34.2	32.6	36	36.8	30	2.23	0.17	0.17	0.35	0.60	
	σn	5.6	6.4	5.15	4.7	5.8	3.9	5.8	4.3	5.3	4.5	5.4	3.8	0.4	0.03	0.032	0.02	0.07	
	σn-1	6.3	7.15	5.7	5.2	6.5	4.3	6.5	4.8	5.9	5.0	6	4.3	0.5	0.037	0.035	0.027	0.08	
INHALATION	Controls	28	31.5	36.5	34.5	39	37	38	36	37	35	40	37	2.49	0.18	0.35	0.29	0.77	
	σn	2	0.5	2.5	0.5	0	0	0	0	0	0	0	0	0	0	0	0	0	
	σn-1	2.8	0.7	3.5	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0	
	Exp. Mice	36	37	42.2	40.8	41.4	41.4	40.6	40.8	40	41.2	42.8	41.4	2.40	0.24	0.20	0.43	0.72	
	σn	2.6	2.6	2.7	1.9	3.0	3.2	3.3	2.31	2.6	1.46	2.3	1.2	0.25	0.02	0.03	0.03	0.06	
	σn-1	3.0	2.9	3.1	2.1	3.36	3.5	3.7	2.58	2.9	1.64	2.58	1.3	0.28	0.023	0.04	0.04	0.07	
Oral Administration	Controls	26.5	28	34	37.5	40	40.3	40	41.3	41.3	41.6	43.6	43	1.69	0.21	0.23	0.42	0.70	0.38
	σn	3.5	3.0	4.0	3.5	2.8	0.47	2.0	1.24	0.94	3.0	3.2	3.1	0.62	0.02	0.04	0.008	0.12	0.03
	σn-1	4.9	4.2	5.6	4.9	3.4	0.57	2.8	1.5	1.15	3.78	4	3.9	0.76	0.03	0.05	0.01	0.15	0.04
	Exp. Mice	32.4	32.6	34.6	31.2	32.1	32.6	36.2	34	36	40.5	40.7	41	2.36	0.20	0.30	0.40	0.61	0.34
	σn	3.13	5.8	6.3	5.8	5.4	6.8	3.2	3.2	3.7	2.1	0.8	1.2	0.34	0.007	0.03	0.04	0.04	0.07
	σn-1	3.5	6.5	7.12	6.5	5.8	7.6	3.7	3.7	4.3	2.5	0.9	1.9	0.4	0.008	0.036	0.05	0.05	0.08

Table 5: Mean body and organ weights of *B. sphaericus* 33021109 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	332	326	350	340	350	15.80	1.24	0.59	3.37	2.97	
	σn	0	0	0	0	0	0	0	0	0	0	
	σn-1	0	0	0	0	0	0	0	0	0	0	
	Guinea-pigs	396	398	418	409	420	26.37	1.47	0.68	3.23	3.14	
	σn	27.7	23.1	19.6	24.2	24.4	2.8	0.07	0.09	0.14	0.05	
	σn-1	34	28.3	24	29.7	30	3.5	0.09	0.1	0.17	0.07	
INTRAPERITONEAL	CONTROLS	394	400	418	404	400	20.60	1.49	0.63	3.55	3.31	
	σn	0	0	0	0	0	0	0	0	0	0	
	σn-1	0	0	0	0	0	0	0	0	0	0	
	Guinea-pigs	444	436	457	443	456	27.27	1.64	0.79	3.41	3.64	
	σn	27.7	23.1	16.5	23.5	12.4	1.65	0.04	0.06	0.05	0.14	
	σn-1	34	28.3	20.2	28.8	15.2	2.02	0.06	0.08	0.06	0.17	
PRECUTANEOUS	CONTROLS	406	390	404	398	410	22.52	1.55	0.58	2.87	3.27	
	σn	0	0	0	0	0	0	0	0	0	0	
	σn-1	0	0	0	0	0	0	0	0	0	0	
	Guinea-pigs	346	337	360	356	363	20.80	1.51	0.62	3.24	3.29	
	σn	36.7	31.9	33.5	28.1	33.9	2.19	0.1	0.05	0.18	0.19	
	σn-1	45	39.1	41	34.4	41.6	2.69	0.13	0.06	0.22	0.23	
EYE IRRITATION	Guinea-pigs	386	388	409	406	421	26.5	1.46	0.66	3.13	3.23	
	σn	36.1	36.1	33.9	41.2	41.3	8.8	0.06	0.08	0.19	0.4	
	σn-1	44.2	44.2	41.5	50.4	50.6	10.8	0.08	0.1	0.23	0.5	
IINHALATION	CONTROLS	356	360	370	370	380	17.39	1.24	0.55	3.29	3.23	
	σn	0	0	0	0	0	0	0	0	0	0	
	σn-1	0	0	0	0	0	0	0	0	0	0	
	Guinea-pigs	398	386	404	388	407	25.14	1.54	1.14	3.26	3.42	
	σn	25.5	16.4	21.4	22	21.7	3.59	0.08	0.6	0.12	0.19	
	σn-1	31.2	20.1	26.2	27	26.6	4.4	0.1	0.7	0.15	0.23	
ORAL ADMINISTRATION	CONTROLS	417	408	430	414	430	23.44	1.52	0.70	3.22	3.63	3.77
	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
	Guinea-pigs	390	382	408	398	410	24.80	1.55	0.64	3.51	3.64	4.04
	σn	56.7	58.2	63.2	59.3	63.6	7.3	0.22	0.08	0.06	0.7	0.5
	σn-1	69.5	71.2	77.4	72.7	77.9	8.9	0.27	0.09	0.07	0.9	0.7

There were no pathological findings after necropsy in any of the experiments. The blood samples taken from treated mice did not show development of *B. sphaericus* by culture on appropriate media.

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

According to statistical analyses, there was no difference between the experimental and control groups in organ and body weights, except for the male mice in the 3 months percutaneous and intravenous application and the female guinea-pigs in subcutaneous applications ($P<0.05$-0.01).

DISCUSSION

Our studies performed on *B. sphaericus* Icel isolate have revealed the absence of acute and prolonged toxicity. All acute toxicity tests produced negative results in all the various routes of administration; subcutaneous,

intraperitoneal, intravenous, oral, percutaneous, inhalation and eye irritation tests, using $2x10^8$ per/ml stock suspension for each inoculation. Our results indicate that the behaviour, growth and weight gain of the experimentally infected animals were within the normal range and close to the controls.

In the study carried out by WHO, the *B. sphaericus* strain 1593, which is considered as the most promising for the control of mosquito larvae, has been shown to be safe for mammals and is already used experimentally for mosquito control operations in California and Florida (1).

B. sphaericus strains SSII-1, 1404-9 and 1593-4 were tested for mammalian pathogenity and infectivity in the study of Shadduck and his co-workers. The results demonstrate that *B. sphaericus* is capable of surviving in mammalian tissue and is associated with the production of lesions under various conditions of experimental injection of high doses of the organisms into vulnerable tissues. No deaths or clinical illnesses resulted from any of the injections. They concluded that the *B. sphaericus* isolates were a virulent for mammals and considered it highly unlikely that the pose any hazard to man (7).

According to Singer, to date there appears to be no untoward effects in terms of mammalian toxicity. Dosages of *B. sphaericus* lethal to mosquitoes were relatively safe for both invertebrates and vertebrates, which are normally found in mosquito habitats. In addition, lack of any adverse effect in honey bees has been confirmed by Davidson *et al.*, and Cantwell and Lehnart which were cited in Singer's study (10,11).

Mammalian safety tests have shown no hazard to man in another study by WHO (2).

According to Mulla *et al.*, both strains of *B. sphaericus* (1593 and 2362) could be effectively utilized for the control of Culicine mosquito larvae with no adverse impact on associated aquatic biota (6).

In the study performed by the Barjac, *et al.* mammalian toxicity tests were carried out on mice with *B. sphaericus* serotype H-5a, 5b, strain 2362, isolated in Nigeria in 1984. Safety tests with whole cultures of *B. sphaericus* serotypes did not show any pathological symptoms in female mice. Behaviour, growth curves, average organ weights and necropsy findings were normal (3).

According to our results, *B. sphaericus* Icel isolate appears to be well tolerated by each of the animals used in these tests.

REFERENCES

1. Anonymous: Scientific activities, biological control of disease vectors. Bull World Hlth Org 57(6):911-914, 1979.

2. Anonymous: Data sheet on the biological control agent, Bacillus sphaericus, strain 1593. WHO/VBC/80. 777, (VBC/BCDS/80. 10, 1-16). Geneva, 1980.

3. De Barjac H, Dumanoir VC, Harmon S, Thiery I: Safety tests on mice with Bacillus sphaericus serotype H-5a, 5b, strain 2362. WHO/VBC/87. 948, Geneva, 1987.

4. Davidson EW, Singer S, Briggs JD : Pathogenesis of Bacillus sphaericus strain SSII-1 infection in Culex pipiens quinquefasciatus (=C. pipiens fatigans) larvae. J Invertabrate Pathol 25:179-184, 1975.

5. Hornby JA, Hertlein BC, Levy R, Miller Jr TW : Persistent activity of mosquito larvicidal Bacillus sphaericus 1593 in fresh water and sewage. WHO/VBC/81. 830, Geneva 1981.

6. Mulla MSK, Darwazeh HA, Davidson EW, Dulmage HT, Singer S: Larvicidal activity and field efficacy of Bacillus sphaericus strains against mosquito larvae and their safety to nontarget organisms. Mosq. News 44(3):336-342, 1984.

7. Shadduck JA, Singer S, Lause S: Lack of mammalian pathogenicity of entomocidal isolates of Bacillus sphaericus. Environ Entomal 9:403-407, 1980.

8. Sinégre G, Gaven B, Vigo G : Evaluation preliminairé de l'activite larvicide de la sauche 1593 de Bacillus sphaericus vis-a-vis de quatre especes de moustiques du littoral mediterranéen Français. WHO/VBC/80. 762. Geneva, 1980.

9. Singer S: Insecticidal activity of recent bacterial isolates and their toxins against mosquito larvae. Nature 244:110-111, 1973.

10. Singer S: Bacillus sphaericus for the control of mosquitoes. Biotechnology and Bioengineering XXII:1335-1355, 1980.

11. Singer S: Potential of spore-forming bacteria without crystals for pest control. Microbial Control of Insects, Mites and Plant Disease, H.D. Burges (ed.), Academic Press, London, 279-294, 1980.

12. Wickremesignhe RSB, Mendis CL: Evaluation of Bacillus thringiensis var. israelensis and B. sphaericus 1593 on Sri Lankan strains of larval Culex quinquefasciatus. Mosq. News 41(3):558-561, 1981.

13. Yousten AA: Bacteriophage typing of mosquito pathogenic strain of Bacillus sphaericus. J Invertebrate Pathol 43:124-125, 1984.

Correspondence:
Ayse Bosgelmez
Hacettepe University,
Faculty of Science,
Department of Biology,
Beytepe, Ankara, TÜRKIYE.