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Technical Information

Summary of Toxicity Studies on Bensultap

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DESCRIPTION OF THE TEST CHEMICAL

Bensultap, an insecticide with contact and stomach poison, is a derivative of nereistoxin, which was found in a marine annelid or segmented worm, *Lumbrineris brevicirra*. Its insecticidial mode of action is blocking depressively the synaptic transmission in the insect's central nervous system by occupying competitively the acetylcholine receptor in the postsynaptic membrane.

Bensultap shows excellent efficacy against Coleopterous and Lepidopterous insects.

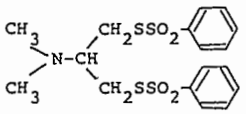
This article reviews various toxicological studies of bensultap.

The chemical structure and physicochemical properties of the insecticide are as follows:

Common name: bensultap

Chemical name: *S*,*S*′-2-dimethylaminotrimethylene di(benzenethiosulfonate)

Structural formula:



Molecular formula: $C_{17}H_{21}NO_4S_4$

Molecular weight: 431.63

Appearance: Pale yellow crystalline powder

Density: 1.33

Melting point: 83–84°C (decomposed at *ca.* 150°C)

Partition coefficient (log $P_{o/w}$): 1.92×10^2 (25°C)

Solubility (g/*l*): Water: 7×10^{-4}–8×10^{-4} (30°C)

Methanol: 25 (25°C)
Xylene: 77 (25°C)
Ethanol: 13 (25°C)
n-hexane: 0.17 (25°C)
Acetone: >1000 (25°C)
Chloroform: >1000 (25°C)
N,*N*-dimethylformamide: >1000 (25°C)
Acetonitrile: >1000 (25°C)

Stability: Stable on exposure to acids (<pH 5), unstable in alkalis (>pH 7)

ACUTE TOXICITY STUDIES

Bensultap Technical

Species	Route of administration	Sex	LD_{50} or LC_{50}[a] (mg/kg) (mg/*l*)
Rat	Oral[1]	M	1105
		F	1120
	Subcutaneous[1]	M	1180
		F	1160
	Intraperitoneal[1]	M	503
		F	438
	Inhalation[2]	M	>0 70[a]
		F	>0 70[a]
Mouse	Oral[3]	M	516
		F	484
	Subcutaneous[3]	M	1202
		F	1726
	Intraperitoneal[3]	M	442
		F	343
Rabbit	Dermal[4]	M	>2000
		F	>2000

[a] mg/*l* of air, as gravimetric concentration

[1] Nippon Experimental Medical Research Institute Co., Ltd., 1978
[2] Hazleton Laboratories America, Inc., 1984.
[3] The Institute of Environmental Toxicology, 1981
[4] Hazleton Laboratories America, Inc., 1983

Bensultap 50% Wettable Powder

Species	Route of administration	Sex	LD_{50} or LC_{50}[a] (mg/kg) (mg/l)
Rat	Oral[1]	M	2125
		F	1342
	Inhalation[2]	M	>1.16[a]
		F	>1.16[a]
Rabbit	Dermal[1]	M	>2000
		F	>2000

[a] mg/l of air, as gravimetric concentration.
[1] Hazleton Laboratories America, Inc., 1983
[2] Hazleton Laboratories America, Inc., 1984.

Bensultap 25% Emulsifiable Concentration

Species	Route of administration	Sex	LD_{50} or LC_{50}[a] (mg/kg) (mg/l)
Rat	Oral[1]	M	1658
		F	1670
	Dermal[1]	M	>2000
		F	>2000
	Inhalation[2]	M	>5.9[a]
		F	>5.9[a]
Mouse	Oral[1]	M	2083
		F	2094

[a] mg/l of air, as gravimetric concentration.
[1] Medical Scientific Research Laboratory, 1986
[2] International Research and Development Corporation, 1987.

Toxicological signs of treated rats were included ptosis, depression, hyperactivity, tremor, urine stains, soft feces, rough coat, lacrimation, salivation and convulsion.

IRRITATION STUDIES

1. Eye irritation study of bensultap technical

One group of 5 male albino rabbits for 5-minute exposure and another group of 3 male albino rabbits for 24-hour exposure were applied 50 mg of bensultap technical into the conjunctival sac of the right eye. Five minutes or 24 hours after instillation, the eye was washed for 2 minutes with 300 ml of physiological saline. The untreated left eye served as a control.

The conjunctival redness and chemosis were observed from 1 hour through 48 hours after instillation in 5-minute exposure group and from 1 hour through 72 hours in 24-hour exposure group.

Bensultap technical may be irritant but no-corrosive to the eye.

(Takeda Chemical Industries, Ltd., 1978)

2. Skin irritation study of bensultap technical

One group of 6 male albino rabbits was applied with 0.5 g of bensultap technical for 4 hours to a pair of testing area on the back, of which one site was abraded by use of a needle and another site was intact. Skin reactions were examined at 24 and 48 hours post-application.

No skin reactions were observed. The index of irritation was zero and bensultap technical caused no skin irritation.

(Takeda Chemical Industries, Ltd., 1978)

DERMAL SENSITIZATION STUDIES

1. Bensultap technical

The study was performed by the modified method of guinea pigs maximization test developed by Magnusson and Kligman (1969). One group of 20 male Hartley guinea pigs was applied with bensultap technical at a concentration of 1% for induction and challenge. The inductions were performed on day 0 and 7, and challenge was performed on day 21. Skin reaction was examined on day 23 and 24. DNCB (0.1%) was used as a positive control.

Slight erythema was observed at 24 and 48 hours after the challenge. Bensultap technical might be a mild allergen.

(Medical Scientific Research Laboratory, 1983)

2. Bensultap 50% WP

The study was performed by the method of Magnusson and Kligman. One group of 5 Hartley strain guinea pigs/sex was applied with bensultap 50%WP to the dorsal shoulder at concentrations of 50% for induction and 25% for challenge. The inductions were performed on day 0 and 7, and challenge was performed on day 21. Skin reaction was examined on day 23 and 24.

No skin reactions were observed in any test animals. Therefore, bensultap 50%WP is considered not to be a sensitizing agent in guinea pigs.

(Hazleton Laboratories America, Inc., 1984)

SUBACUTE TOXICITY STUDIES

1. Rats

Groups of 20 Sprague-Dawley (Jcl: SD) rats/sex/dose were given diets containing 0, 250, 1000 and 2000 ppm bensultap technical for 3 months.

With the exception of pale ear and rough hair observed in the animals treated with 2000 ppm, no toxic signs were observed. Dose-related suppression of body weight gain in both sexes of the 1000 and 2000 ppm groups, and the decrease in food consumption in accordance with the changes of body weight gain at 2000 ppm throughout the experimental period were noted. The low food efficiencies were observed for some period of experiment at 2000 ppm. In the hematological examination, dose-related decreases in hemoglobin and hematocrit values in the 1000 and 2000 ppm groups were observed. In blood biochemical examination, slight increases in levels of total protein, BUN, K^+, Ca^{2+} and GPT and slight decreases in levels of blood glucose, Na^+ and Cl^- were noted in the male rats of 2000 ppm group. Increases of absolute and relative weights of liver and kidney were observed in 1000 and 2000 ppm groups. Discoloration in the kidney was observed in 1000 and 2000 ppm groups. There were no treatment-related alternations in urinalysis and histopathological examinations.

Based on the above results, the no-effect level in this study was 250 ppm (equal to 17.9 mg/kg of body weight in males, 21.4 mg/kg of body weight in females).

(Medical Scientific Research Laboratory, 1983)

2. Mice

Groups of 20 ICR (Crj: CD-1) mice (5-week old)/sex/dose were given diets containing 0, 40, 100, 300, 1000 and 3000 ppm of bensultap technical for 13 weeks.

No treatment-related clinical abnormalities were observed in any animals. Only one male death occurred in 3000 ppm group. Significant depression of body weight gain with decreased food consumption was observed in 3000 ppm group. In males and females of 3000 ppm group, markedly decreased values of hematocrit, hemoglobin, erythrocyte count and mean corpuscular volume and increased values of mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration and reticulocyte count were noted. In males of 100 and 300 ppm groups, significantly but slightly increased values of hematocrit, hemoglobin, and erythrocyte count were noted. Histopathological examination revealed centrilobular cloudy swelling of hepatocytes, mucosal hyperkeratosis and epithelial hyperplasia of the forestomach, epithelial hyperplasia of urinary bladder and/or increased hematopoiesis of spleen and bone marrow in males of 300 ppm group and in males and females of 1000 and 3000 ppm groups.

Based on these results, the no-effect levels in this study were 40 ppm (4.64 mg/kg/day) in males and 300 ppm (37.0 mg/kg/day) in females. (The Institute of Environmental Toxicology, 1982)

CHRONIC TOXICITY STUDIES

1. Combined chronic toxicity and oncogenicity study in rats

Groups of 50 Charles River CD rats/sex/dose were given diets containing bensultap technical at levels of 0, 10, 30 and 90 mg/kg of body weight for 104 weeks. Additional groups of 12 rats/sex/dose were given the same diets for 52 weeks for interim examination.

Survival in males of 90 mg/kg group was significantly reduced. The growth rate and terminal body weights of both sexes at 90 mg/kg were reduced. No treatment-related clinical signs were noted. The values of food consumption were not affected by the treatment. Decreased mean hematocrit and hemoglobin values were frequently significant in males of 90 mg/kg group and were occasionally significant in males of 10 and 30 mg/kg groups. The increase of mean total cholesterol value in males of 90 mg/kg group, which was often significant, was observed throughout the study. No treatment-related abnormalities were noted in the urinalysis.

In gross pathology, an apparent dose-related trend of testes of unequal size was noted in the male groups sacrificed at termination. The mean kidney weight was significantly increased in males of 30 and 90 mg/kg groups at week 52. Significant increases of mean absolute

kidney weights and mean absolute and relative weights of heart and liver were noted in treated males sacrificed at week 105.

Compound-related increases in nonneoplastic lesions of the liver (including bile duct hyperplasia, cholangiofibrosis, spongiosis hepatis and cholangiectasis, necrosis of the individual hepatocytes and centrilobular hepatocellular swelling) and cortical cysts in the kidney were predominantly observed in 30 and 90 mg/kg groups. No other treatment-relationship was indicated by the incidence of any individual tumor type or by overall incidence of tumors.

Based on these results, the no-effect level of bensultap technical in rats was 10 mg/kg of body weight.

(Hazleton Laboratories America, Inc., 1984)

2. *Combined chronic toxicity and oncogenicity study in mice*

Groups of 70 Crj: ICR(CD-1) mice/sex/dose were given diets containing 0, 40, 200 and 1000 ppm of bensultap technical for 104 weeks. Additional groups of 10 mice/sex/dose were given the same diets for 52 weeks for interim sacrifice.

No treatment-related clinical signs were observed in any test group during the study. There were no significant changes related to the treatment in mortality, food and water consumptions, blood biochemistry, and urinalysis. A significant depression of body weight gain was observed in both sexes of 200 and 1000 ppm groups. The significant decrease in erythrocyte count and increased mean corpuscular volume were observed in male mice of the 1000 ppm group at week 52. In addition, significantly lower leukocyte count was observed in males and significantly higher values of mean corpuscular volume and mean corpuscular hemoglobin were noted in females of 1000 ppm group at week 104. The relative weight of liver was increased without histological evidence in males and females of 1000 ppm group at week 52. Marked mucosal hyperkeratosis and epithelial hyperplasia in forestomach were noted in 1000 ppm group and a significantly high incidence of mucosal epithelial hyperplasia of the urinary bladder were observed in males of 1000 ppm group at week 52. These findings appeared to be related to the treatment. All the neoplastic lesions noted in this study were considered to be spontaneous tumors occurring in this strain of mice.

In conclusion, bensultap technical had no carcinogenicity and the no-effect level to ICR mice was 40 ppm (equal to 3.64 mg/kg of body weight/day in males and 3.42 mg/kg/day in females). (The Institute of Environmental Toxicology, 1984)

3. *Chronic toxicity study in dogs*

Groups of 4 beagle dogs/sex/dose were given diets containing 0, 200, 600 and 2000 ppm bensultap technical for 52 weeks.

Ataxia, ptyalism, fasciculations, tremors, muscle weakness and poor reflexes were observed in the 2000 ppm group. At week 36, one male dog in the 2000 ppm group was sacrificed in a moribund condition. The other animals were all survived till the study termination. Mean body weight and body weight gains in males of the 2000 ppm group and food consumptions in both sexes of 2000 ppm group were significantly lower than those of the control group. The values of erythrocyte count, hematocrit and hemoglobin were significantly decreased in the both sexes of 2000 ppm. The decreases in the values of albumin, calcium, albumin/globulin ratio and cholesterol, and the increase of glucose level were observed in 2000 ppm group. Absolute liver weights were increased in both sexes of the 2000 ppm group. In histopathological examination, liver changes including the pigmentation in hepatocytes and Kupffer cells and hepatocellular hypertrophy were observed in the one moribund male in the 2000 ppm group. Liver changes in the one moribund animals and higher absolute liver weight in 2000 ppm animals may reflect enzyme induction. There was no treatment-related effect on ophthalmic findings.

The no-effect level appears to be 600 ppm (approximately 15.52 mg/kg of body weight/day for males and 15.88 mg/kg of body weight/day for females).

(Hazleton Laboratories America, Inc., 1986)

TERATOGENICITY STUDIES

1. Rats

Groups of 22 or 23 pregnant Slc: Wistar rats/dose were orally dosed bensultap technical at levels of 0, 20, 60 and 180 mg/kg of body weight from gestation day 7 to 17. On day 21 of gestation fetuses were removed by cesarean section and were examined for fetal toxicity and teratogenicity.

Mortality and general conditions of the pregnant rats were observed during the study. Body weight of the pregnant rats was recorded on gestation day 0, 4 and 7 through 21, and food and water consumptions were recorded on gestation day 1, 4 and 7 through 21.

Significant decreases in the food and water consumption and remarkable suppression of body weight gain were observed in rats of the 180 mg/kg group. Severe toxic signs and deaths from gestation day 10 to 15 were observed in 4 rats of 180 mg/kg group. No gross lesions were observed in surviving rats of 180 mg/kg group. No compound related effects on implantation and survival embryos and fetuses were recognized in all treated groups. Ossification of the cervical corpus vertebrae of fetuses was delayed significantly in the 60 and 180 mg/kg groups.

These results indicate that bensultap technical caused slight retardation in the ossification, but bensultap technical did not have any teratogenic effect on fetuses when pregnant rats were dosed via oral route.

(Nippon Institute for Biological Science, 1983)

2. Rabbits

Groups of 17 pregnant New Zealand White rabbits/dose were orally dosed bensultap technical at levels of 0, 10, 25 and 60 mg/kg of body weight from gestation day 7 to 19. On day 29 of gestation fetuses were removed by cesarean section and were examined for fetal toxicity and teratogenicity.

One female in 10 mg/kg group and two female in 60 mg/kg group were died on day 29 of gestation. A possible treatment-related increase in anorexia was noted. The mean body weight gain in 25 mg/kg group was significantly decreased during gestation. Gravid uterine weights in 60 mg/kg group were less than the control weights. No external, visceral and skeletal anomalies of fetuses were observed in the treated groups. An increase in skeletal variants, attributable primarily to an increased number of pups with thickened rib ends, was noted in the 25 and 60 mg/kg groups. The biological significance of that findings was unclear.

From the results, bensultap technical had no teratogenic potential in rabbits.

(Hazleton Laboratories America, Inc., 1984)

REPRODUCTION STUDY IN RATS

Groups of 25 Jcl; Wistar rats/sex/dose were given diets containing 0, 5, 40 and 300 ppm of bensultap technical for 2 consecutive generations and effects of this chemical on the reproductive performance were investigated.

No death attributable to the treatment was noted throughout the study. The decreases in body weight gains were consistently observed in F_1 and F_2 generations of 300 ppm group. Hematological examinations revealed that values of hematocrit, hemoglobin, mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration were significantly lower in F_1 and F_2 generations at 300 ppm than those of the control group. The increases of absolute or relative weights of the kidney and the heart were observed in males of 300 ppm group in F_1 and F_2 generations. In gross- and histopathological examinations, no abnormalities related to the test compound were observed. The reproductive performance (including the mating, fertility and gestation incidences and the length of pregnancy) in the treated groups were not influenced with the test compound.

In conclusion, reproductive performance was not influenced with bensultap technical even at the level of 300 ppm.

(Nippon Institute for Biological Science, 1984)

MUTAGENICITY STUDIES

1. Reverse mutation test

Escherichia coli WP2 *uvr*A (hcr) and 5 strains of *Salmonella typhimurium* TA1535, TA1537, TA1538, TA98 and TA100 were used to evaluate the mutagenic potential of bensultap technical at levels of 0, 1, 5, 10, 50, 100, 500, 1000 and 5000 μg/plate with or without metabolic

activation.

Bensultap technical induced no appreciable increases in the numbers of revertant colonies of any strain at any dose, compared with those of the corresponding control, either with or without metabolic activation.

Based on the results, bensultap technical had no reverse mutation inducing capability.

(The Institute of Environmental Toxicology, 1983)

2. DNA repair test (rec assay)

Using the recombination-wild (H17) and -deficient (M 45) strains of *Bacillus subtilis*, the DNA-damaging potential of bensultap technical was evaluated at levels of 0, 50, 100, 200, 500, 1000, 2000, 5000 and 10000 μg/disk.

Bensultap technical did not cause any inhibitory zone in either strain even at the highest dose of 10000 μg/disk.

It is concluded that bensultap technical had no DNA-damaging capability.

(The Institute of Environmental Toxicology, 1983)

3. CHO/HGPRT forward mutation assay

Using Chinese hamster ovary (CHO) cells, CHO/HGPRT forward mutation assay was performed at levels of 10, 20, 30, 40, 50 and 60 μg/ml with or without metabolic activation.

Bensultap did not exhibit a significant mutagenic effect or a significant dose-related mutagenic response in the presence or absence of metabolic activation. Therefore, bensultap technical is considered negative in the CHO/HGPRT forward mutation assay.

(Hazleton Laboratories America, Inc., 1984)

4. In vitro sister chromatid exchange in Chinese hamster ovary cells

Chinese hamster ovary cells (CHO) were exposed to bensultap technical at levels of 0.25, 0.85, 2.5, 8.5 and 25 μg/ml with or without metabolic activation.

No statistically significant increases in the frequency of sister chromatid exchanges (SCE) were observed either with or without metabolic activation. Therefore, bensultap technical had no potential of sister chromatid exchange.

(Hazleton Laboratories America, Inc., 1984)

5. Micronucleus test

Groups of 5 (C3H×3WV) F_1 male mice/dose were dosed bensultap technical at levels of 0, 20 and 200 mg/kg once, or 0, 10 and 100 mg/kg daily for 5 successive days. Animals that received single dose were killed 30 hours postdose, and those that received 5 consecutive doses were killed 6 hours after the final applications. Collecting bonemarrow cells from the femur, erythrocytes were examined for the presence of micronuclei on the Giemsa-stained slides and for the frequencies of reticulocytes on the new methyleneblue stained slides.

The frequency of bone-marrow micronucleated erythrocytes in each group did not differ significantly when compared with the control. These findings indicate that bensultap technical is not mutagenic in this test system.

(Takeda Chemical Industries, Ltd., 1980)

6. Dominant lethal evaluation in rats

Groups of 15 male rats/dose were orally dosed bensultap technical at levels of 0, 10, 30 and 100 mg/kg/day for 5 consecutive days before mating with untreated females. Thirteen days after the mid-week of cohabitation, the females were sacrificed and the uterine and ovarian contents (implantation and corpora lutea) were evaluated.

Mean male fertility, female pregnancy rate and the mean numbers of preimplantation losses, total implants and live implants (evaluated per male) were comparable for all groups.

Bensultap technical did not appear to produce a dominant lethal effect in this test system.

(Hazleton Laboratories America, Inc., 1986)

7. Unscheduled DNA synthesis rat hepatocyte assay

Bensultap technical was evaluated the potential of unscheduled DNA synthesis in rat hepatocyte at levels of 10, 15, 20, 40 and 60 μg/ml. The mean nuclear grain count of cells from each dose level was individually compared to the appropriate solvent control (DMSO), and the value whose mean grain count did not exceed three standard deviations beyond the mean solvent control value was considered to have no significant effect.

The results showed that bensultap technical did not produce a significant positive response

at any level tested when compared to the DMSO solvent control. Therefore, bensultap is negative in the rat hepatocyte unscheduled DNA synthesis assay under the conditions of this assay.

(Hazleton Laboratories America, Inc., 1984)

SUMMARY

A wide variety of toxicological study on bensultap have been conducted to assess its safety.

Bensultap is a relatively low toxic substance, and the toxicological signs of treated animals included ptosis, depression, hyperactivity, tremor, urine stains, soft feces, rough coat, lacrimation, salivation and convulsion in acute toxicity studies.

Bensultap technical might be a weak irritant but no-corrosive substance to the rabbit eye, and it caused no skin irritation. Bensultap technical might be a mild allergen, but its formulated product was not to be a sensitizing agent in guinea pig.

In chronic toxicity and oncogenicity studies, main toxicological findings related to the treatment of bensultap were including depression of body weight, decreased hematocrit and hemoglobin values, increased kidney weight and centrilobular hepatocellular swelling in the high dose groups. No treatment-related neoplastic lesions were observed in rats and mice.

The reproductive performance was not influenced with bensultap in rats, and no teratogenic potential was observed in rat and rabbit studies. Bensultap did not indicate a mutagenic potential in any assay.

Bensultap and its formulation were registered on April 14, 1986 in Japan.

The withhold standards for pesticide registration are listed below.

Rice	0.2 ppm
Wheat and others	0.2 ppm
Vegetables	3.0 ppm
Tea	20.0 ppm

Contact

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