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EDGEWOOD ARSENAL
TECHNICAL REPORT

EATR 4301

TOXICITY OF O-CHLOROBENZYLIDENE MALONONITRILE (CS) IN TRIOCTYLPHOSPHATE (TOF) SOLUTIONS

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April 1969

DEPARTMENT OF THE ARMY
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Project 1B562602A079
Task 1B562602A07908

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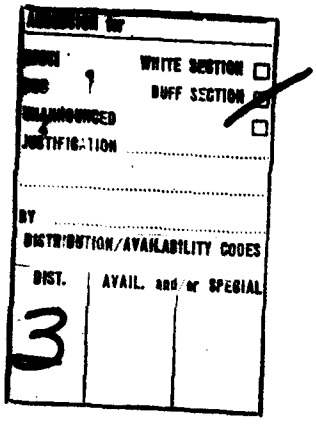

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FOREWORD

The work described in this report was authorized under Project 1B562602A079, Task 1B562602A07908, Nondefense Medical Aspects of Chemical Agents (U), Incapacitating and Riot Control Agents (U). The work was started in January 1968 and completed in December 1968.

The volunteers in these tests are enlisted US Army personnel. These tests are governed by the principles, policies, and rules for medical volunteers as established in AR 70-25.

In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal Resources, National Academy of Sciences-National Research Council.

Acknowledgments

The authors acknowledge the assistance of W. U. Thomas, R. L. Fortier, S. G. Ryan, R. P. Merkey, and J. S. Olson in conducting the investigations reported herein. Special thanks are due to Mrs. Martha A. Langan who assisted greatly in the preparation of the tables and in the arrangement of the report.

DIGEST

As solutions containing 1% o-chlorobenzylidene malononitrile (CS) in trioctylphosphate (TOF) have been proposed for use in riot control situations, a series of toxicological tests was conducted in animals. Results showed that the doses tested are not permanently injurious to the eyes. Effects on skin ranged from none to necrosis; and the affected areas usually healed within 2 weeks. When the mixtures were directly injected into the trachea, some evidence of congestion and pneumonia was seen. But the severity and duration of signs were not in a category that would warrant veterinary treatment under conditions of normal animal care.

The reported studies indicate that no irreversible damage is expected with 1% CS/TOF solutions as long as the dose ranges shown to be safe in this paper are not exceeded.

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TOXICITY OF O-CHLOROBENZYLIDENE MALONONITRILE (CS) IN TRIOCTYLPHOSPHATE (TOF) SOLUTIONS

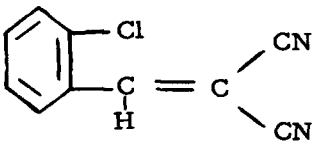
I. INTRODUCTION.

Solutions of o-chlorobenzylidene malononitrile (CS) and trioctylphosphate (TOF) have been proposed for use in riot control situations. This report brings together previously reported and new information on the toxicological properties of CS, TOF, and CS/TOF solutions in animals and man.

II. TOXICOLOGY OF CS.

A. Physical Properties.

The structural formula of CS is as follows:



CS is a white, crystalline solid that melts at 94°C and boils at 310° to 315°C (760 mm Hg). The solubility of CS in water is not listed because the compound hydrolyzes in that medium. Approximate solubilities of CS in organic solvents at room temperature are as follows:

Solvent	% Dissolved by weight
Acetone	42
Dioxane	33
Ethyl acetate	25
Methylene chloride	39
Pyridine	46
Chloroform	32
Methyl isopropyl ketone	21

B. Airborne CS.

Previous studies have shown that CS is highly potent as an irritant and as a riot control compound and has a very low toxicity. [1-3]

The effective dose for riot control for 50% of a population (ED50) is a Ct (concentration x time) of 0.1 to 10 mg min/cu m. The LD50 is estimated to be 61,000 mg min/cu m.

CS as an aerosol or vapor acts directly on mucous membranes to produce irritation, burning, and pain in the eyes and respiratory tract. The action on the eyes also causes lacrimation, blepharospasm, and conjunctivitis. The compound affects the upper and lower respiratory tracts, causing sneezing, coughing, salivation, congestion of the nose and wall of the pharynx, and a feeling of suffocation. The effects are immediate and persist for 5 to 20 minutes after one leaves the contaminated atmosphere.

On the skin, these aerosols produce a burning sensation which is greatly accentuated by moisture such as perspiration, lacrimation, rhinorrhea, and salivation. The burning sensation may persist for several hours and recurs upon washing of the exposed area.[4] Exposure of the skin to CS aerosols in high concentrations of 300 mg/cu m at a wind velocity of 5 mph, a temperature of 95°F, and a relative humidity of 95% for 30 minutes produced irritation and burning, but no blisters. Continuation of these severe exposures for 45 minutes did produce vesication.[5]

First and second degree burns were produced accidentally in 12 officers participating in field exercises. The officers were thoroughly soaked from a heavy continuous rain. They were subjected to micropulverized CS1 dispersed from a modified flamethrower. The CS seemed to soak through the wet fatigues and burn the skin. From 7 to 10 hours after exposure, erythema appeared on the V-area of the neck, the forearms, the wrists, and the calves. In 14 to 16 hours after exposure, blistering had begun in the most severe cases. All vesicles healed in about 1 week, and no sequelae were reported.[5]

Daily exposure of people to heavy concentrations of CS in manufacturing plants resulted in erythema and vesiculation.[6]

C. Patch Tests.

In 24-hour patch tests on men, dry CS on the forearm produced varying skin reactions in both Negroes and Caucasians. The effects varied from no reaction to erythema, vesication, and sloughing.[4]

D. Solutions of CS on the Skin of Animals.

The minimal dose of CS in corn oil, applied to the freshly clipped backs of rabbits, that produced any sign of irritation was

20 mg/animal. Doses of 40 to 60 mg/animal caused mild to moderate erythema or necrosis. The results of these tests are shown in table A-I, appendix. (Tables A-1 through A-XXII are in the appendix.)

E. Comparison of Skin and Eye Effects of CS With Those Caused by Other Riot Control Compounds and Mixtures.

The minimal dose of CS in corn oil, applied to the eyes of rabbits, that produced any sign of irritation was 1.0 mg/eye. Doses of up to 10 mg in the eye caused moderate to severe conjunctivitis and blepharitis. No corneal damage, ulceration, or other permanent damage was produced, and recovery from the minor signs was completed 9 days after application. The results of these tests are shown in table A-II.

A comparison of CS with other irritants in corn oil showed that CS was less damaging to the eyes and skin than are CN (chloroacetophenone), DM (diphenylaminochloroarsine), or CN-DM mixtures. These data are shown in table A-III.

F. CS in Methylene Dichloride.

Doses of 0.05 and 0.1 ml of 10% CS in methylene dichloride placed in rabbits' eyes caused immediate hyperemia, which disappeared in a few hours. There was no noticeable corneal damage. A dose of 0.1 ml of a 50% mixture of CS in methylene dichloride was placed in the eyes of 10 rabbits, but no eye damage was noted during a 14-day observation period. The eyes were treated daily with sodium sulfamide to prevent secondary infection. Methylene dichloride alone produced no persistent effects on eyes or eyelids.[2]

III. TOXICOLOGY OF TOF.

A. Physical Properties.

Trioctylphosphate, tri-(2-ethylhexyl)-phosphate, is a water-clear liquid with a specific gravity of 0.9225 at 20°C; a boiling point of 220°C at 5 mm Hg; a vapor pressure of 1.9 mm Hg at 200°C; and a pour point of -90°C.

B. Summary of LD50's by Single Exposure.

Approximate LD50 doses of TOF for a single exposure are shown in table I.

Table I. Approximate LD50 Doses for Single Exposure of TOF in Animals

Species	Route	Approximate LD50	Reference
Rabbits	Intravenous	>358 mg/kg	7
Rabbits	Intratracheal	>1,811 mg/kg	7
Rabbits	Intragastric	46 gm/kg	7
Rabbits	Cutaneous	<20 ml/kg (19 gm/kg)	9
Rats	Intragastric	>36.8 gm/kg	7
Rats	Oral	37.1 gm/kg	8
Rats	Oral	39.8 gm/kg	9
Rats	Inhalation	>93,000 mg min/cu m	7
Chickens	Intracrop	>2,500 mg/kg	7
Guinea pigs	Inhalation	ca. 30,000 mg min/cu m	7
Monkeys	Cutaneous	>75 ml/kg (70 gm/kg)	This rpt

C. Single Inhalation Exposures.

One of two dogs died after a 4-minute exposure to a concentration of 250 mg/cu m of TOF (Ct = 1,000 mg min/cu m). However, the two dogs exposed to a Ct of 500 and the two exposed to a Ct of 5,000 survived.

No deaths occurred when six rats were exposed for 8 hours to nearly saturated vapor concentrations of TOF. A mist of TOF generated at 170°C killed no rats in 30 minutes, but killed two of six rats after 1 hour.[9]

Necropsy of rats that had inhaled TOF labeled with P^{32} showed a high percentage of the compound in their stomachs with lesser amounts in their lungs, livers, brains, spleens, kidneys, bones, muscles, and fat. The compound was excreted in both urine and feces.[7]

D. Single Application to the Eyes of Rabbits.

A letter from Union Carbide Corporation[9] states: "Eye injury to rabbits: Definite Hazard." This caution has not been supported by further study.[7]

E. Single Doses in the Crops of Chickens.

One of eight chickens died after 2,500 mg/kg of TOF was introduced into their crops. All of eight chickens given a dose of 500 mg/kg survived. Although demyelinization is produced by certain phosphates, there was no gross or histological evidence of neurological damage.[7]

F. Repeated Inhalation in Guinea Pigs.

Three groups of 20 guinea pigs each were exposed to TOF in concentrations of 10.8, 26.4, and 85.0 mg/cu m, 6 hours/day, 5 days/week for 12 weeks. A control group of 20 unexposed animals was included. Death rates were 30%, 46%, 25%, and 59%, respectively, for the controls, the low, intermediate, and high doses.[7] The value of this experiment is doubtful because of the presence of a respiratory infection. In the second experiment, the death rates were 0/20, 1/20, and 1/20 for control animals and animals exposed to concentrations of 1.6 and 9.6 mg/cu m, respectively.[7] Both deaths occurred in the ninth week of exposure.

Two dogs and two monkeys at each of three concentrations, 10.8, 26.4, and 85.0 mg/cu m, survived the 12 weeks of exposure.[7]

G. Repeated Application to the Skin of Rabbits.

Repeated doses of 0.1 ml of TOF were applied to the clipped backs of rabbits 5 days/week for 2 to 4 weeks. Moderate erythema was seen after the first application. Following the fourth and fifth applications, desquamation, cracking, and bleeding were noted in the skin. Microscopic lesions in the skin included hyperkeratosis, parakeratosis, epidermal thickening, dermatitis, focal atrophy of the hair follicles, some destruction of the hair shafts, and slight degeneration of the sebaceous glands.

H. Percutaneous Effects in Man.

Union Carbide Corporation[9] reported that TOF applied to cloth patches and worn on the skin for 5 days caused erythema in 1.5% of 200 volunteers. Three weeks later the material was retested on these 200 men and removed after 48 hours. Five percent of the volunteers developed erythema.

IV. TOXICOLOGY OF CS/TOF SOLUTIONS.

A. Materials.

The CS used in these studies was obtained from Edgewood Arsenal stock, designated Lot No. 2011-73-1003. The purity was 95.4% as determined by chemical analysis performed by the Chemical Research Laboratory. The TOF used in this work was manufactured by Union Carbide Corporation. All solutions were prepared fresh each day.

B. Eye Effects.

Prior to dosing, both eyes of each animal (New Zealand white rabbits and rhesus monkeys) were examined, and any animal with eye defects or irritation was eliminated. Before and throughout the observation period, the animals were caged individually in raised pens, away from bedding and animal droppings. One day after exposure, the eyes were flushed with isotonic saline. Clinical observation of the exposed and unexposed eyes were then recorded. Following this, one drop of fluorescein sodium ophthalmic solution was instilled in each eye; then the eyes were flushed with isotonic saline and treated with one drop of 15% sodium sulfacetamide ophthalmic solution. On subsequent observations days, eyes that needed treatment were flushed and treated with ophthalmic solution.

Irritation of the eye was evaluated according to the modified Draize technique as described in the "Illustrated Guide for Grading Eye Irritation by Hazardous Substances," published by the Food and Drug Administration. The grades of ocular lesions are shown in table A-IV.

In most studies, animals were sacrificed and necropsied 3 and 30 days after exposure. To date, only the gross observations of the application sites on the 3-day animals have been completed by the pathologists, and these are reported here. (This is also true of the skin studies, which follow.)

1. Instillation in Rabbits' Eyes.

In pilot studies, 0.2 ml of 0.05% and 0.1% (w/v) solutions of CS/TOF produced no more than mild chemosis and hyperemia when instilled in the eyes of rabbits (table A-V).

A 1% CS/TOF solution* and TOF alone were tested as shown in table II. Some animals were killed and necropsied 3 days after dosing. The rest were observed for 14 days.

Table II. Experimental Design for 1% CS/TOF and TOF Alone Applied to Rabbits' Eyes

No. of rabbits exposed	Dose			Necropsied 3 days after dosing
	CS/TOF	TOF	Saline	
	ml			
10	0.2			4
8		0.2		2
2			0.2	2
6	0.1			
2		0.1		
6	0.05			
2		0.1		
10	0.025			4
4		0.025		2
2			0.025	2
6	0.01			4
2		0.01		
10	0.005			4
4		0.005		2
2			0.005	2

None of the CS/TOF mixtures produced more than mild chemosis and hyperemia. At all but the highest dose, these mild signs of irritation were gone within a week. At the highest dose, mild chemosis persisted in one rabbit for 14 days. Individual gross observations during the 14-day period are presented in table A-VI.

The highest dose of TOF alone, 0.2 ml, caused mild to moderate chemosis and hyperemia which disappeared in 13 days. The two

* The volume of each dose here and elsewhere may be converted to milligrams as follows: specific gravity of CS = ca. 1, of TOF = 0.9225; a 1% solution of CS/TOF contains 10 mg of CS per milliliter. Therefore, 0.2 ml of 1% CS/TOF contains 2 mg of CS and 186 mg of TOF.

rabbits that received 0.1 ml displayed mild chemosis for 1 week. No irritation was seen in rabbits that received lower doses. Individual observations are presented in table A-VII.

Gross pathological examination of the eyes of the rabbits that were killed 3 days after dosing showed mild hyperemia of the conjunctivae in two of the four animals that received the lowest dose of 1% CS/TOF. Conjunctivitis, usually mild, was seen in all four rabbits that received the intermediate dose of CS/TOF and in both TOF controls. At the high dose of the mixture, all four test animals had moderate to severe conjunctivitis and chemosis; the TOF controls had moderate conjunctivitis. With the exception of very mild conjunctivitis in one rabbit, no significant changes were seen grossly in the eyes of the saline control animals. The findings are summarized in table A-VIII.

2. Spray in Rabbits' Eyes.

To simulate an operational spray device, a hypodermic syringe with a 21-gauge needle was used to spray 1 or 10 ml of CS/TOF and TOF alone into one eye of restrained rabbits. (Accuracy at 6 feet was limited.) The experimental design is shown in table III.

Table III. Experimental Design for Spraying 1% CS/TOF and TOF Alone Into Rabbits' Eyes

No. of rabbits	Distance from which sprayed	Dose		Necropsied 3 days after dosing
		CS/TOF	TOF	
	ft	ml		
4	1	10		
8	3	10		4
4	3	1		4
4	6	10		
2	1		10	
4	3		10	2
2	3		1	2
2	6		10	

The mixture of CS/TOF and TOF alone (1 and 10 ml) produced moderate to severe chemosis and hyperemia, which persisted for 7 days in

the animals that were not sacrificed (tables A-IX and A-X). There were erythematous reactions on the head and ears of all rabbits.

Necropsy showed that three of the four rabbits sprayed with 1 ml of the mixture from a distance of 3 feet had mild conjunctival hyperemia, as did one of the two TOF controls. Three of the four sprayed with 10 ml of the mixture from the same distance had mild conjunctivitis, and the fourth had only mild hyperemia of the conjunctivae. The eyes of both TOF controls sprayed with 10 ml were normal (table A-X).

3. Instillation in Monkeys' Eyes.

The ocular effects of a 1% CS/TOF solution were studied in rhesus monkeys according to the design shown in table IV.

Table IV. Experimental Design for 1% CS/TOF and TOF Alone Instilled in Monkeys' Eyes

No. of monkeys exposed	Dose		Necropsied 3 days after dosing
	CS/TOF	TOF	
	ml		
8	0.1		4
8	0.025		4
8	0.005		4
4		0.1	2
4		0.025	2
4		0.005	2

Lacrimation and conjunctival hyperemia on the day of exposure were the only signs of irritation shown during the 14-day observation period, and all monkeys were affected. Necropsy of animals killed 3 days after dosing showed all eyes to be normal.

C. Cutaneous Effects.

New Zealand white rabbits and rhesus monkeys were prepared for exposure by clipping the hair from their backs. Any animal with skin abnormalities (abrasions, discoloration, etc.) was excluded from the study.

The grading system used to assess skin irritation is given in table A-XI.

1. Drops.

Discrete drops of 1% CS/TOF or TOF alone were placed on the backs of the animals with a syringe.

a. Rabbits.

In preliminary studies, the skin of groups of six rabbits each was exposed to drops (1.0 ml) of 0.05% and 0.1% CS/TOF solution. Both doses produced severe erythema and edema, followed by dehydration and necrosis. These signs disappeared in the low-dose group within 12 days. Erythema in two of the high-dose groups was still evident at the end of the 14-day observation period (table A-XII).

Table V gives the design of the experiment with 1% CS/TOF solutions.

Table V. Experimental Design for 1% CS/TOF and TOF Alone Applied in Drops to the Clipped Backs of Rabbits

No. of rabbits exposed	Dose		Necropsied 3 days after dosing
	CS/TOF	TOF	
	ml		
10	1.0		4
4		1.0	2
6	0.5		
6	0.25		
10	0.10		4
4		0.10	2
6	0.05		
10	0.025		4
4		0.025	2

All doses of CS/TOF and TOF alone produced severe erythema and edema, followed by dehydration and necrosis. At the end of the 14-day observation period, a few rabbits still had areas of cutaneous necrosis and erythema (tables A-XIII and A-XIV). The rabbits necropsied 3 days after exposure had only mild to severe erythema (table A-XV).

b. Monkeys.

The local and systemic effects of cutaneously applied 1% CS/TOF solutions were studied in monkeys according to the design shown in table VI.

Table VI. Experimental Design for 1% CS/TOF and TOF Alone Applied to the Clipped Backs of Monkeys

No. of monkeys exposed	Dose		Necropsied 3 days after dosing
	CS/TOF	TOF	
	Local effects		
8	1.0 ml		4
4		1.0 ml	2
8	0.1 ml		4
4		0.1 ml	2
8	0.025 ml		4
4		0.025 ml	2
	Systemic effects		
2		12.5 ml/kg*	
2		25.0 ml/kg*	
2		50.0 ml/kg*	
2		75.0 ml/kg*	

* The monkeys weighed about 2 kg.

The monkeys exposed to 1.0, 0.1, and 0.025 ml of CS/TOF and TOF alone showed no signs of skin irritation during the 14-day observation period. The skin of those monkeys necropsied 3 days after dosing was normal.

All the monkeys that received the large cutaneous doses of TOF developed mild erythema within 24 hours. In those that received doses of 12.5, 25, or 50 ml/kg, the erythema progressed to drying and wrinkling of the skin by the end of the first week, and the skin was normal by 3 weeks. The monkeys that received the highest dose, 75 ml/kg, developed scattered areas of necrosis, but these had healed by the end of 3 weeks. No physical or behavioral signs of neurologic damage or systemic signs were noted. The cutaneous signs are listed in table A-XVI.

2. Patch Tests.

a. Rabbits.

Patches of sateen cloth were contaminated with 0.10 and 1.0 ml of 1% CS/TOF and taped to the backs of eight rabbits per dose for 24 hours. The same procedure was followed for TOF alone except that four rabbits per dose were used. Half of the rabbits at each dose were killed and necropsied 3 days after dosing.

The CS/TOF mixture produced mild to severe erythema in all rabbits within 48 hours. Necrosis appeared as early as the third day, but had healed by the ninth day. Mild erythema was still present 10 days after dosing (table A-XVII).

Results with the patches contaminated with TOF alone were the same as those with the mixture except that necrosis appeared a day later.

Rabbits dosed with 0.1 ml of CS/TOF had mild to moderately severe erythema; the skin of one was slightly thickened. The TOF controls dosed with 0.1 ml also had mild erythema. Those rabbits that received 1.0 ml of the mixture had mild to moderate erythema, and one had a slight thickening of the skin. Both of the TOF controls at the 1.0-ml dose had moderate erythema, and the skin of one was slightly thickened.

b. Men.

Table VII shows the design and the results of a study in which 1% CS/TOF was placed on the volar surface of the forearm of human subjects. The environmental temperature during these exposures was 59°F (56° to 61°) and the relative humidity was 95% (83% to 99%).

Table VII. Effects of 1% CS/TOF Solution on Human Skin

No. of subjects	Dose	Material applied on	Results
	ml		
2	0.1	Bare skin	No effect
2	0.025	Bare skin	No effect
2	0.1	Sateen patch*	Mild stinging for 8 hr (one subject)
2	0.025	Sateen patch*	No effect

* Patch taped to skin and left in place for 24 hours.

D. Effects on Upper and Lower Respiratory Tract.

Beagle hounds that were less than 2 years old were used in these studies. Preexposure physical examinations included counts of red and white blood cells and measurement of packed cell volume, blood urea nitrogen, and rectal temperature. These measurements were repeated 72 or 96 hours after exposure. Each animal was found to be in good health before it was used.

The protocol for this series of tests is shown in table VIII.

Table VIII. Experimental Design for Determining Effects of 1% CS/TOF and TOF Administered to Dogs by Tracheal Intubation or Intratracheal Injection

No. of dogs	Dose			Necropsied 3 days after dosing	Necropsied 30 days after dosing
	CS/TOF	Saline	TOF		
		ml			
			Tracheal intubation		
8	0.50			4	4
8	0.25			4	4
8	0.10			4	4
4		0.50		2	
4		0.25		2	
4		0.10		2	
8			0.50	4	4
			Intratracheal injection		
8	0.50			4	4
4		0.50		2	

1. Tracheal Intubation.

After the dogs had been given a short-acting barbiturate, a laryngoscope was inserted and a polyethylene tube fitted to a 21-gauge needle was passed 2 inches posterior to the larynx, where the dose was deposited. The only sign was coughing when the trachea was pressed and congestion of the lungs, revealed by auscultation (table A-XVIII). Red and white blood cell count, packed cell volume, blood urea nitrogen, and rectal temperature were not affected (tables A-XIX and XX).

Mild tracheal hyperemia and minimal pulmonary congestion were seen in two of the four dogs necropsied 3 days after receiving 0.1 ml of the CS/TOF mixture (table A-XXI). A small pneumonic area was present in the lung of one dog that received 0.25 ml, and discolored areas, probably representing congestion, were seen in two other dogs. At the highest dose, three of four dogs had some hyperemia of the tracheal mucosa and different degrees of pneumonia. One of these dogs had a single consolidated nodule 2 cm in diameter while the other two had larger areas of consolidation scattered throughout the lungs. The fourth dog had patchy discoloration that was probably due to congestion but could represent early pneumonia. Two of the six saline control animals had mild hyperemia in the tracheal mucosa, while two others had only pulmonary congestion. There was no sign of tracheal damage in the TOF controls. The lungs of all four, however, showed varying degrees of congestion and pneumonia. A small pneumonic area was detected grossly in only one of the 12 dogs necropsied 30 days after receiving CS/TOF. Some evidence of pneumonia was present in those that had received TOF alone. All of these dogs were clinically normal.

2. Intratracheal Injection.

The investigators prepared this group of dogs by clipping the hair on the ventral neck and scrubbing the skin with surgical soap. The animals were tranquilized with chlorpromazine hydrochloride. Dosing was accomplished by inserting a 21-gauge needle into the trachea 1 to 2 inches caudal to the larynx and injecting the test solution.

Lesions in this group were the same as those in the group that was given the materials by intubation (table A-XXI). Blood values were not affected (table A-XIX). Moderate tracheitis was seen in one dog killed 3 days after dosing, but mild, focal pneumonia was seen in all four. Ecchymotic hemorrhages were present in all lobes of the lungs of one saline control animal. Three of the four dogs necropsied 30 days after receiving CS/TOF had at least microscopic evidence of pneumonitis, as did both saline controls.

V. CHEMICAL STABILITY OF VARIOUS SOLUTIONS OF CS IN TOF.

The stability of CS/TOF solutions stored under various conditions for 15 days was determined by ultraviolet analysis. The solvent systems were checked daily, and the changes in ultraviolet absorbance peaks at 300 mμ (peak for CS) and 228 mμ (secondary activity) were recorded. The concentrations of unreacted CS found in the solutions were compared with the control peak and the percentage of this value recorded.

The details of this experiment and the results are shown in table A-XXII. The CS/TOF solutions containing 1% CS were relatively stable when stored for various time periods at either 25° or 12°C with fluorescent light, but were degraded when the temperature was raised to 90°C. Concentrations of 0.1% or 0.06% were degraded at 25° and 12°C in room light in direct proportion to solution strength. Samples containing 1.0% CS stored in darkness were less stable than those stored at the same temperature (25°C) under fluorescent light.

VI. SUMMARY AND CONCLUSIONS.

A. Oscular Effects of CS/TOF and TOF Alone.

The highest dose (0.1 ml) of 1% CS/TOF and TOF alone instilled in the eyes of monkeys had no effect. The highest dose (0.2 ml) put in the eyes of rabbits produced very mild effects, which regressed within 2 weeks.

Severe chemosis and redness, which persisted for 1 week, was produced when 10 ml of a 1% CS/TOF solution or TOF alone was sprayed into the eyes of rabbits from a distance of 1, 3, and 6 feet. No corneal lesions were seen.

B. Cutaneous Effects of CS/TOF and TOF Alone.

The lowest dose (0.025 ml) of 1% CS/TOF and TOF alone applied in drops to the skin of rabbits caused immediate erythema that progressed to necrosis in 8 to 10 days. Higher doses caused the same reaction with the area affected being more extensive due to the greater spread of the liquid over the skin. In most instances the skin had healed by the end of 2 weeks. The highest dose (1 ml) of the CS/TOF mixture or TOF alone had no effect on the skin of monkeys.

Patch tests with 0.1 and 1.0 ml of 1% CS/TOF caused necrosis of rabbit skin that healed in 9 days. The same doses of TOF produced no reaction.

When 0.025 ml of 1% CS/TOF was applied to the bare skin of the volar forearm of men or was placed on a sateen patch that was taped to the skin of the forearm, no reactions were seen. When the dose was increased to 0.1 ml, one of two subjects experienced a mild stinging for about 8 hours.

C. Effects of CS/TOF and TOF Alone on the Upper and Lower Respiratory Tract.

When 1% CS/TOF was introduced into the trachea of dogs by either intubation or injection, only mild clinical signs were noted. These signs were not dose-related; in fact, they were most frequent at the lowest dose.

The results indicate that injection of 0.5 ml of CS/TOF or TOF alone into the trachea of the dog does not cause enough damage to be discernible by clinical observation. This dose did not result in secondary infection severe enough to cause fever, pronounced dehydration, spontaneous coughing, generalized auscultatory sounds, or elevation in white blood cell count. In general, the severity and duration of signs were not in a category that would warrant veterinary treatment under the conditions of normal animal care. This is especially significant in view of the method of exposure, i.e., direct injection into the trachea.

D. Systemic Effects of CS/TOF and TOF Alone.

The only effects seen in the dogs, rabbits, and monkeys used in the eye, skin, and lung studies were mild respiratory signs in the dogs exposed by instilling the materials directly into the trachea.

The reported studies indicate that no irreversible damage is expected with 1% CS/TOF solutions as long as the dose ranges shown to be safe in this paper are not exceeded.

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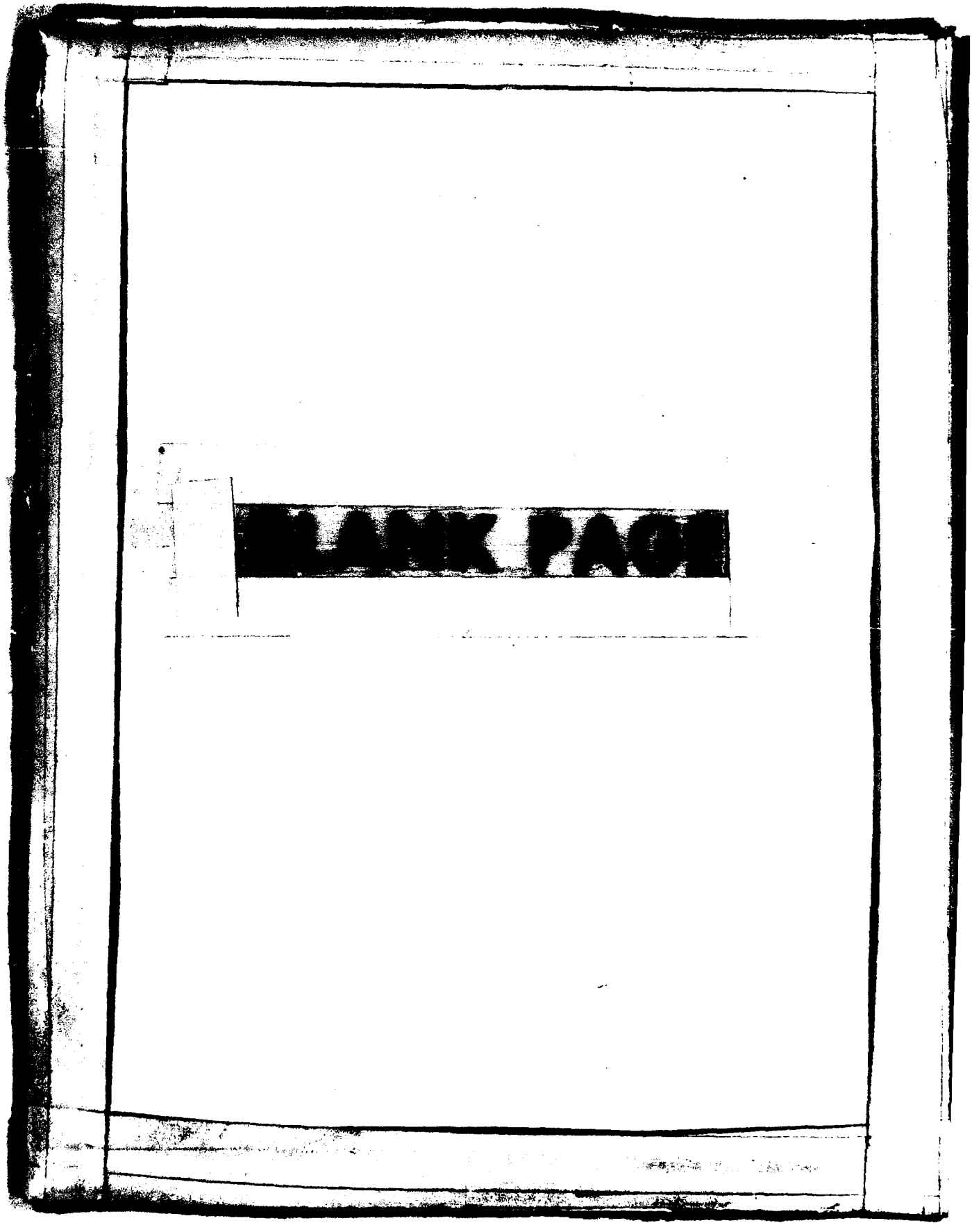
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Table A-1. The Cutaneous Effects of CS in Corn Oil on Rabbits

Rabbit no.	Dose	Suspension	Signs*				
			1**	2**	4**	7**	10**
	mg	mg/ml					
1-6	5	10	No noticeable effects	No noticeable effects	No noticeable effects	No noticeable effects	No noticeable effects
7-12	10	20	No noticeable effects	No noticeable effects	No noticeable effects	No noticeable effects	No noticeable effects
13	20	40	E-	E-	E-	Recovered	
14			E-	E-	E-	Recovered	
15			No noticeable effects	E-	E-	Recovered	
16			E-	E-	E-	Recovered	
17			E-	E	E	Recovered	
18			E-	E-	Recovered		
19-24	40	80	E	E	E	N-	Recovered
25	60	120	E	E	E	N-	N-
26			E	E	E	E, N-	N-
27			E	E	E	N-	N-
28			E	E	E	N-	N-
29			E	E	E	E, N-	N-
30			E	E	E	N-	N-

* Signs: E = Moderate erythema; E- = Mild erythema
N = Moderate necrosis; N- = Mild necrosis

** Number of days after dosing

Table A-II. Effects of CS in Corn Oil Instilled in the Eyes of Rabbits

(Volume instilled, 0.05 ml; one eye per rabbit)

Rabbit no.	Dose	Suspension	Signs*						
			1**	2**	4**	6**	7**	9**	10**
	mg	mg/ml							
1-6	0.5	10	No noticeable effects	No noticeable effects	No noticeable effects	No noticeable effects	No noticeable effects	No noticeable effects	No noticeable effects
7	1.0	20	C-	Recovered					
8			C-	Recovered					
9			C	C-	Recovered				
10			C-	Recovered					
11			C	C-	Recovered				
12			No noticeable effects	No noticeable effects	No noticeable effects	No noticeable effects	No noticeable effects	No noticeable effects	No noticeable effects
13	2.5	50	C	C-	Recovered				
14			C	C-	Recovered				
15			C-	Recovered	Recovered				
16			C	C-	Recovered				
17			C	C-	Recovered				
18			C	C	Recovered				
19	5.0	100	C+	C-	Recovered				
20			C	Recovered					
21			C+, B-	C-	Recovered				
22			C+, B-	C-	Recovered				
23			C+, B-	C-	Recovered				
24			C+, B-	C-	Recovered				
25	10.0	200	C+, B	C+, B-	C	C	C-	Recovered	
26			C+, B	C+, B-	C	C	C-	Recovered	
27			C+, B	C+, B-	C	C	C-	Recovered	
28			C+, B	C+, B-	C	C	C-	Recovered	
29			C+, B	C+, B-	C	C	C-	Recovered	
30			C+, B	C+, B-	C	C	C-	Recovered	

* Signs: C = Moderate conjunctivitis; C- = Mild conjunctivitis; C+ = Severe conjunctivitis
B = Moderate blepharitis; B- = Mild blepharitis

** Number of days after dosing

Table A-III. Doses of DM, CN, CN-DM Combinations, and CS, all Suspended in Corn Oil, Causing Cutaneous and Ocular Effects in Rabbits

Agents	Cutaneous effects			Ocular effects		
	No effect dose	Minimal effective dose	Dose causing pronounced effects	No effect dose	Minimal effective dose	Dose causing pronounced effects
	mg/animal					
DM	1.0	10.0	10.0	0.1	0.2	1.0
CN	1.0	10.0	20.0	0.5	1.0	2.5
CN-DM 1:1	1.0	5.0	20.0	0.1	0.5	1.0
CN-DM 1:5	1.0	5.0	20.0	0.05	0.1	0.5
CN-DM 1:10	1.0	5.0	20.0	0.05	0.1	0.5
CN-DM 5:1	5.0	10.0	25.0	0.05	0.1	1.0
CN-DM 10:1	5.0	10.0	25.0	0.05	0.1	1.0
CS	10.0	20.0	40.0	0.5	1.0	5.0

Table A-IV. Guide for Grading Eye Irritation

Parts of eye	Condition found	Rating scale
Cornea	No ulceration or opacity	0
	Scattered or diffuse areas of opacity (other than slight dulling of normal luster), details of iris clearly visible	[1]*
	Easily discernible translucent areas, details of iris slightly obscured	2
	Nacreous areas, no details of iris visible, size of pupil barely discernible	3
	Complete corneal opacity, iris not discernible	4
Iris	Normal	0
	Markedly deepened folds, congestion, swelling, moderate circumcorneal injection (any of these or combination of any thereof), iris still reacting to light (sluggish reaction is positive)	[1]*
	No reaction to light, hemorrhage, gross destruction (any or all of these)	2
Conjunctivae	Redness (refers to palpebral and bulbar conjunctivae excluding cornea and iris)	
	Vessels normal	0
	Some vessels definitely injected	1
	Diffuse, crimson red, individual vessels not easily discernible	[2]*
	Diffuse beefy red	3
	Chemosis	
	No swelling	0
	Any swelling above normal (includes nictitating membrane)	1
	Obvious swelling with partial eversion of lids	[2]*
	Swelling with lids about half-closed	3
	Swelling with lids more than half-closed	4

* Bracketed figures indicate lowest grade considered positive under Section 191.12 of the Federal Hazardous Substances Labeling Act Regulations.

Table A-V. The Effects of CS (0.05% or 0.1% in TOF) Instilled in Eyes of Rabbits (Pilot Studies)

(One eye per rabbit)

Rabbit no.	Ocular effects																											
	1*			2*			3*			4*			5*			6*			7*			8*			9*			10*			11*			12*			13*			14*		
	CH	R	C	CH	R	C	CH	R	C	CH	R	C	CH	R	C	CH	R	C	CH	R	C	CH	R	C	CH	R	C	CH	R	C	CH	R	C	CH	R	C	CH	R	C
0.05% CS, 0.2 ml																																								
37	0	0	0	0	0	0	0	0	0	0	0	0	<1	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
38	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	<1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
39	0	0	0	<1	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	<1	0	0	<1	0	0	<1	0	0	0	<1	0	0	<1	0	0	0	0	0
40	0	1	0	<1	0	0	0	1	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	<1	0	0	<1	0	0	0	0	0	0	0	0	0
41	0	1	0	<1	0	0	<1	1	0	0	0	0	1	0	0	1	0	0	1	0	0	0	0	0	0	0	0	1	0	0	<1	0	0	0	0	0	0	0	0	0
42	0	1	0	<1	0	0	0	1	0	0	0	0	1	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.10% CS, 0.2 ml																																								
43	0	1	0	1	1	0	1	1	0	<1	0	0	1	0	0	1	0	0	1	0	0	0	0	0	<1	0	0	<1	0	0	<1	0	0	0	0	0	0	0	0	0
44	0	1	0	<1	0	0	1	0	0	0	1	0	2	0	0	1	0	0	1	1	0	0	1	0	2	1	0	1	0	0	<1	0	0	<1	0	0	<1	0	0	<1	0	0
45	1	1	0	1	1	0	1	1	0	0	0	0	<2	0	0	1	0	0	1	1	0	1	0	0	<1	0	0	0	1	0	0	<1	0	0	0	0	0	0	0	0	0	0
46	0	0	0	<1	0	0	1	0	0	0	0	0	1	0	0	2	0	0	1	0	0	<1	0	0	0	0	0	0	0	0	0	<1	0	0	0	0	0	0	0	0	0	0
47	0	0	0	<1	0	0	0	0	0	0	0	0	1	0	0	1	0	0	1	0	0	1	0	0	<1	0	0	<1	0	0	<1	0	0	0	0	0	0	0	0	0	0	0
48	0	0	0	<1	0	0	0	0	0	0	0	0	1	0	0	<1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

NOTES: The grading system used in this table is shown in table A-IV.

CH = Chemosis
R = Redness
C = Cornea
< = Questionable

* Number of days after dosing

Table A-VI. Effects of CS (1% in TOF) Instilled in Eyes of Rabbits

(One eye per rabbit)

Dose	Rabbit no.	1*			2*			3*			4*			5*			6*			7*			8*			9*			10*			11*			12*			13*			14*		
		CH	R	C	CH	R	C	CH	R	C	CH	R	C	CH	R	C	CH	R	C	CH	R	C	CH	R	C	CH	R	C	CH	R	C	CH	R	C	CH	R	C	CH	R	C	CH	R	C
ml																																											
0.2	49	0	0	0	0	0	0	0	0	0	0	1	0	<1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.2	50	0	1	0	<1	0	0	<1	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.2	51	0	1	0	0	0	0	0	0	0	0	1	0	1	0	0	1	0	0	1	0	0	<1	0	0	<1	0	0	<1	0	0	<1	0	0	<1	0	0	<1	0	0	<1	0	0
0.2	52	0	1	0	<1	0	0	<1	0	0	0	0	0	<1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.2	53	0	1	0	<1	0	0	<1	0	0	<1	0	0	1	0	0	1	0	0	1	1	0	<1	<1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.2	54	0	1	0	<1	0	0	<1	0	0	0	1	0	1	0	0	1	0	0	1	1	0	<1	<1	0	<1	0	0	<1	0	0	<1	0	0	0	0	0	0	0	0	0	0	0
0.1	61	<1	0	0	0	0	0	0	0	0	1	0	0	1	0	0	<1	<1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.1	62	0	0	0	0	0	0	0	0	0	<1	0	0	<1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.1	63	<1	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.1	64	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	<1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.1	65	<1	0	0	0	0	0	0	0	0	1	0	0	1	0	0	1	0	0	<1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.1	66	<1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	Dead**											
0.05	69	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.05	70	<1	0	0	0	0	0	0	0	0	<1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.05	71	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.05	72	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	<1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.05	73	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.05	74	<1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.025	77	0	0	0	0	<1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.025	78	<1	0	0	0	0	0	0	0	0	<1	0	0	<1	0	0	<1	0	0	<1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.025	79	<1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.025	80	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.025	81	<1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.025	82	<1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.010	85	<1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	<1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.010	86	<1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	Dead**																							
0.010	87	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.010	88	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.010	89	<1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.010	90	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.005	93	0	0	0	0	<1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.005	94	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.005	95	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.005	96	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.005	97	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.005	98	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

NOTES: The grading system used in this table is shown in table A-IV.

CH = Chemosis
R = Redness
C = Cornea
< = Questionable

* Number of days after dosing

** Death not agent induced

Table A-VII. The Effects of TOF Instilled in Eyes of Rabbits

(One eye per rabbit)

Dose	Rabbit no.	1*			2*			3*			4*			5*			6*			7*			8*			9*			10*			11*			12*			13*			14*		
		CH	R	C	CH	R	C	CH	R	C	CH	R	C	CH	R	C	CH	R	C	CH	R	C	CH	R	C	CH	R	C	CH	R	C	CH	R	C	CH	R	C	CH	R	C	CH	R	C
ml																																											
0.2	55	0	1	0	<1	0	0	<1	0	0	<1	0	0	1	0	0	1	0	0	1	0	0	1	0	0	<1	0	0	<1	0	0	<1	0	0	<1	0	0	0	0	0	0	0	0
0.2	56	0	0	0	<1	0	0	1	0	0	1	0	0	<2	0	0	1	0	0	1	1	0	1	0	0	<1	0	0	<1	0	0	<1	0	0	0	0	0	0	0	0	0	0	0
0.2	57	0	1	0	1	0	0	1	0	0	1	0	0	2	0	0	1	0	0	1	1	0	<1	0	0	<1	0	0	<1	0	0	<1	0	0	0	0	0	0	0	0	0	0	0
0.2	58	0	0	0	<1	0	0	0	1	0	<1	0	0	1	0	0	1	0	0	1	0	0	<1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.2	59	0	1	0	<1	0	0	1	0	0	1	0	0	2	0	0	1	0	0	<1	1	0	<1	0	0	<1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.2	60	0	1	0	<1	0	0	1	0	0	1	0	0	2	0	0	1	0	0	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.1	67	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.1	68	<1	0	0	0	0	0	0	0	0	<1	0	0	<1	0	0	<1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.05	75	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.05	76	0	0	0	0	0	0	0	0	0	<1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.025	83	<1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.025	84	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.010	91	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.010	92	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.005	99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.005	100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

NOTES: The grading system used in this table is shown in table A-IV.

CH = Chemosis
R = Redness
C = Cornea
< = Questionable

* Number of days after dosing

Table A-VIII. Effects of 1% CS/TOF and TOF Alone Instilled in Eyes of Rabbits

(These rabbits were sacrificed and necropsied 3 days after dosing)

Dose	Rabbit no.	Ocular effects									Necropsy findings
		1*			2*			3*			
		CH	R	C	CH	R	C	CH	R	C	
ml											
						CS/TOF					
0.2	119	2	0	0	0	0	0	0	<1	0	4/4 Moderate to severe conjunctivitis and chemosis
0.2	120	1	0	0	0	0	0	<1	0	0	
0.2	121	1	0	0	0	0	0	<1	0	0	
0.2	122	1	0	0	<1	0	0	1	0	0	
0.025	123	0	1	0	0	0	0	0	<1	0	4/4 Mild conjunctivitis
0.025	124	<1	0	0	0	0	0	1	<1	0	
0.025	125	1	0	0	0	1	0	<1	0	0	
0.025	126	1	0	0	0	1	0	0	1	0	
0.005	127	0	1	0	0	0	0	0	1	0	2/4 Conjunctival hyperemia
0.005	128	0	0	0	0	0	0	0	0	0	
0.005	129	0	0	0	0	0	0	0	0	0	
0.005	130	<1	0	0	0	0	0	0	<1	0	
						TOF					
0.2	137	1	0	0	0	0	0	0	<1	0	2/2 Moderate conjunctivitis
0.2	138	1	0	0	0	0	0	<1	0	0	
0.025	139	0	1	0	0	<1	0	0	<1	0	2/2 Mild conjunctivitis
0.025	140	0	<1	0	0	0	0	0	<1	0	
0.005	141	0	0	0	0	0	0	0	0	0	1/2 Mild conjunctivitis
0.005	142	0	0	0	0	0	0	0	<1	0	
						Saline					
0.2	131	0	0	0	0	0	0	0	0	0	None
0.2	132	0	0	0	0	0	0	0	0	0	
0.025	133	0	0	0	0	0		0	0	0	None
0.025	134	0	0	0	0	0		0	0	0	
0.005	135	0	0	0	0	0		0	0	0	None
0.005	136	0	0	0	0	0		0	0	0	

NOTES: CH = Chemosis
R = Redness
C = Cornea

The grading system used in this table is shown in table A-IV.

* Number of days after dosing

Table A-IX. The Ocular Effects of 1% CS in TOF and TOF Alone in Rabbits

(Ten mililiters sprayed at one eye from varying distances)

Distance	Rabbit no.	1*			2*			3*			4*			5*			6*			7*		
		CH	R	C	CH	R	C	CH	R	C	CH	R	C	CH	R	C	CH	R	C	CH	R	C
ft																						
										CS/TOF												
1	209	2	2	0	3	2	0	2	2	0	3	2	0	2	2	0	3	2	0	2	2	0
1	210	2	2	0	3	2	0	2	2	0	3	2	0	3	2	0	2	2	0	2	1	0
1	211	2	2	0	3	2	0	2	2	0	3	2	0	4	2	0	1	1	0	1	1	0
1	212	1	2	0	2	2	0	2	2	0	2	2	0	1	1	0	1	1	0	1	<1	0
3	213	2	2	0	4	2	0	3	2	0	3	2	0	2	1	0	2	1	0	2	1	0
3	214	1	2	0	3	2	0	3	2	0	3	2	0	3	2	0	3	1	0	3	1	0
3	215	1	2	0	3	2	0	3	2	0	3	2	0	3	2	0	3	1	0	2	1	0
3	216	1	2	0	2	2	0	2	2	0	2	2	0	2	1	0	2	1	0	1	<1	0
6	217	1	2	0	2	2	0	2	2	0	3	2	0	2	2	0	2	1	0	2	1	0
6	218	1	2	0	2	2	0	3	2	0	3	2	0	3	1	0	1	1	0	1	1	0
6	219	1	2	0	2	2	0	3	2	0	2	2	0	2	2	0	2	<1	0	1	<1	0
6	220	1	2	0	2	2	0	3	2	0	2	1	0	1	1	0	1	<1	0	1	<1	0
										TOF												
1	221	1	2	0	3	2	0	3	2	0	3	2	0	2	2	0	1	1	0	1	1	0
1	222	1	2	0	2	2	0	3	2	0	2	2	0	2	2	0	1	1	0	1	<1	0
3	223	1	2	0	3	2	0	3	2	0	2	2	0	3	2	0	2	1	0	1	<1	0
3	224	1	2	0	2	2	0	3	2	0	3	2	0	3	2	0	2	1	0	2	1	0
6	225	1	2	0	1	1	0	1	2	0	1	1	0	1	1	0	<1	<1	0	0	0	0
6	226	1	2	0	3	2	0	2	2	0	3	2	0	3	1	0	3	1	0	3	<1	0

NOTES: The grading system used in this table is shown in table A-IV.

CH = Chemosis
R = Redness
C = Cornea

* Number of days after dosing

Table A-X. The Ocular Effects of 1% CS in TOF and TOF Alone in Rabbits Necropsied 3 Days After Dosing

(Sprayed at one eye from a distance of 3 feet)

Dose	Rabbit no.	1*			2*			3*			Necropsy findings
		CH	R	C	CH	R	C	CH	R	C	
ml											
1	265	2	2	0	2	2	0	2	2	0	3/4 Mild conjunctival
1	266	2	2	0	2	2	0	2	2	0	hyperemia
1	267	2	2	0	3	2	0	3	2	0	
1	268	2	2	0	2	2	0	2	2	0	
1	273**	3	2	0	4	2	0	4	2	0	1/2 Mild conjunctival
1	274**	2	2	0	2	2	0	3	2	0	hyperemia
10	269	3	2	0	3	2	0	3	2	0	3/4 Mild conjunctivitis
10	270	3	2	0	3	2	0	3	2	0	1/4 Mild conjunctival
10	271	3	2	0	3	2	0	3	2	0	hyperemia
10	272	3	2	0	4	2	0	4	2	0	
10	275**	3	2	0	2	2	0	3	2	0	None
10	276**	2	2	0	2	2	0	3	2	0	

NOTES: The grading system used in this table is shown in table A-IV.

CH = Chemosis
R = Redness
C = Cornea

* Number of days after dosing

** TOF controls

Table A-XI. Grading System for Assessing Skin Irritation

Effect	Degree of effect	Rating scale
Erythema	No erythema	0
	Mild erythema	1
	Moderate erythema	2
	Severe erythema	3
	Erythema with edema	4
Necrosis	No necrotic tissue	0
	Less than 50% necrotic tissue	1
	50%-100% necrotic tissue	2
	100% necrotic tissue with well defined eschar formation	3
Dehydration and/or desquamation	No dehydration or desquamation	0
	Mild dehydration or desquamation	1
	Moderate dehydration or desquamation	2
	Severe dehydration or desquamation	3

Table A-XII. Cutaneous Effects of CS (0.5% or 0.1%) in TOW on Rabbits

Solution strength (% of CS)	Dose	Rabbit no.	Cutaneous effects																																			
			1*			2*			3*			4*			5*			6*			7*			8*			9*			10*			11*			12*			13*			14*		
			E	N	D	E	N	D	E	N	D	E	N	D	E	N	D	E	N	D	E	N	D	E	N	D	E	N	D	E	N	D	E	N	D	E	N	D	E	N	D
	ml																																							
0.05	1.0	37	3	0	0	2	0	0	3	0	0	4	0	0	2	0	2	2	0	2	2	0	2	1	0	2	1	0	2	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0
0.05	1.0	38	3	0	0	2	0	0	3	0	0	4	2	0	2	2	1	2	2	2	3	2	2	2	0	2	3	0	2	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.05	1.0	39	2	0	0	3	0	0	3	0	0	4	2	0	2	2	1	3	0	2	3	0	2	3	0	2	2	2	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.05	1.0	40	3	0	0	3	0	0	3	0	0	3	0	0	1	0	0	0	0	2	2	0	2	2	0	2	2	0	2	2	0	2	2	0	2	0	0	0	0	0	0	0	0	0
0.05	1.0	41	3	0	0	2	0	0	3	0	0	2	0	0	1	0	1	2	0	0	2	0	0	2	0	2	1	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0
0.05	1.0	42	2	0	0	2	0	0	3	0	0	4	0	0	1	0	2	1	0	2	1	0	2	1	0	2	1	0	2	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0
0.10	1.0	43	3	0	0	3	0	0	3	0	0	4	0	0	2	0	2	2	2	2	2	0	2	2	0	2	2	1	2	2	0	0	2	0	0	0	0	0	0	0	0	0	0	0
0.10	1.0	44	3	0	0	2	0	0	3	0	0	4	2	0	2	0	3	2	2	2	2	2	2	3	0	2	2	0	0	2	0	0	2	0	0	2	0	0	2	0	0	2	0	0
0.10	1.0	45	3	0	0	3	0	0	3	0	0	4	2	0	3	2	3	2	2	2	2	2	2	3	0	2	2	0	2	2	0	0	2	0	0	1	0	0	0	1	0	0	1	0
0.10	1.0	46	3	0	0	3	0	0	3	0	0	4	2	0	2	1	1	2	2	2	2	0	2	2	0	2	1	0	1	2	0	0	1	0	0	0	0	0	0	0	0	0	0	0
0.10	1.0	47	2	0	0	2	0	0	0	0	0	0	0	0	1	0	3	2	0	2	2	0	1	1	0	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.10	1.0	48	3	0	0	2	0	0	1	0	0	4	0	0	1	0	0	1	0	1	1	0	1	1	0	1	1	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

NOTES: The grading system used in this table is shown in table A-XI.

E = Erythema

N = Necrosis

D = Dehydration and/or desquamation

* Number of days after dosing

Table A-XIII. The Cutaneous Effects of CS (1% in TOF) in Rabbits
(Solution applied to clipped skin of each rabbit)

Dose	Rabbit no.	Cutaneous effects 1* E	1* N	1* D	2* E	2* N	2* D	3* E	3* N	3* D	4* E	4* N	4* D	5* E	5* N	5* D	6* E	6* N	6* D	7* E	7* N	7* D
ml																						
1.0	49	2	0	0	2	0	0	2	0	0	2	0	0	2	0	0	2	0	2	2	0	2
1.0	50	2	0	0	3	0	0	3	0	0	4	0	0	3	0	0	2	0	2	2	0	2
1.0	51	3	0	0	2	0	0	3	0	0	4	0	0	3	0	0	2	0	2	2	0	2
1.0	52	3	0	0	3	0	0	2	0	0	4	1	0	3	0	1	2	0	0	2	0	2
1.0	53	3	0	0	3	0	0	3	0	0	4	1	0	3	0	3	2	0	2	3	0	2
1.0	54	1	0	0	1	0	0	2	0	0	4	0	0	2	0	2	2	0	2	3	0	2
0.5	61	1	0	0	2	0	0	4	0	0	2	0	2	2	0	2	2	0	1	1	0	2
0.5	62	1	0	0	2	0	0	4	0	0	3	0	2	2	0	2	2	0	1	1	0	2
0.5	63	2	0	0	2	0	0	4	0	0	2	0	3	2	0	2	2	0	2	2	0	2
0.5	64	0	0	0	3	0	0	4	0	0	2	0	0	1	0	0	2	0	2	2	0	2
0.5	65	1	0	0	1	0	0	2	0	0	2	0	3	1	0	2	2	0	2	2	0	2
0.5	66	1	0	0	1	0	0	4	0	0	2	0	0	1	0	0	2	0	0	2	0	1
0.25	69	2	0	0	3	0	0	3	0	0	3	0	0	3	0	0	2	0	0	2	0	1
0.25	70	1	0	0	1	0	0	2	0	0	2	0	0	2	0	0	2	0	1	1	0	1
0.25	71	2	0	0	1	0	0	2	0	0	2	0	0	2	0	0	2	0	2	2	0	2
0.25	72	1	0	0	1	0	0	2	0	0	3	0	0	2	0	0	2	0	2	2	0	1
0.25	73	2	0	0	2	0	0	4	0	0	4	0	1	4	0	0	4	0	0	2	0	1
0.25	74	2	0	0	1	0	0	4	0	0	4	0	0	4	0	0	4	0	0	2	0	1
0.10	77	1	0	0	2	0	0	2	0	0	3	0	0	2	0	0	2	0	0	1	0	2
0.10	78	1	0	0	2	0	0	3	0	0	3	0	0	2	0	0	2	0	0	1	0	2
0.10	79	1	0	0	2	0	0	1	0	0	2	0	0	2	0	0	1	0	0	1	0	1
0.10	80	2	0	0	2	0	0	3	0	0	3	0	0	2	0	0	4	0	1	2	0	0
0.10	81	2	0	0	2	0	0	4	0	0	2	0	0	2	0	0	2	0	2	2	0	1
0.10	82	1	0	0	3	0	0	2	0	0	2	0	0	2	0	0	2	0	2	2	0	2
0.05	85	2	0	0	2	0	0	3	0	0	3	0	0	2	0	0	4	0	0	2	0	0
0.05	86	1	0	0	1	0	0	1	0	0	2	0	0	1	0	0	1	0	0	Dead**		
0.05	87	1	0	0	1	0	0	1	0	0	3	0	0	2	0	0	1	0	0	2	0	0
0.05	88	1	0	0	3	0	0	4	0	0	4	0	0	4	0	0	2	0	0	1	0	2
0.05	89	1	0	0	3	0	0	4	0	0	3	0	0	2	0	0	2	0	0	1	0	1
0.05	90	2	0	0	3	0	0	4	0	0	3	0	2	3	0	2	1	0	1	1	0	1
0.025	93	1	0	0	2	0	0	2	0	0	2	0	0	1	0	0	2	0	2	1	0	1
0.025	94	1	0	0	1	0	0	4	0	0	2	0	0	2	0	0	2	0	0	2	0	2
0.025	95	1	0	0	1	0	0	2	0	0	2	0	0	2	0	0	2	0	0	1	0	0
0.025	96	1	0	0	1	0	0	4	0	0	2	0	0	2	0	0	2	0	0	1	0	0
0.025	97	1	0	0	1	0	0	1	0	0	1	0	0	2	0	0	1	0	0	1	0	1
0.025	98	1	0	0	2	0	0	4	0	0	4	0	0	4	0	0	4	0	2	2	0	1

Dose	Rabbit no.	Cutaneous effects 8* E	8* N	8* D	9* E	9* N	9* D	10* E	10* N	10* D	11* E	11* N	11* D	12* E	12* N	12* D	13* E	13* N	13* D	14* E	14* N	14* D
ml																						
1.0	49	0	0	1	2	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0
1.0	50	2	0	2	2	0	1	2	0	0	1	0	0	0	0	0	0	0	0	0	0	0
1.0	51	2	0	2	2	0	1	2	0	0	1	0	0	0	0	0	0	0	0	0	0	0
1.0	52	2	0	2	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1.0	53	2	0	0	2	0	0	2	0	0	2	0	0	0	0	0	0	0	0	0	0	0
1.0	54	1	0	2	2	0	1	2	0	0	2	0	0	0	0	0	0	0	0	0	0	0
0.5	61	2	0	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.5	62	2	0	1	2	0	0	2	0	0	0	1	0	0	1	0	0	0	0	0	0	0
0.5	63	2	0	1	2	1	0	2	0	0	0	1	0	0	1	0	0	0	0	0	0	0
0.5	64	2	0	1	2	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.5	65	1	0	1	1	0	0	[illegible]	0	0	0	1	0	0	1	0	0	0	0	0	0	0
0.5	66	1	0	1	1	0	0	1	0	0	Dead**											
0.25	69	2	0	0	2	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0
0.25	70	1	0	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.25	71	2	0	1	2	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.25	72	1	0	0	1	0	0	1	0	0	1	0	0	0	0	0	0	1	0	0	1	0
0.25	73	1	0	1	2	0	1	2	0	0	1	1	0	1	0	0	1	0	0	1	0	0
0.25	74	1	0	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.10	77	1	0	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.10	78	1	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.10	79	1	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	1	0
0.10	80	1	0	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.10	81	2	0	2	2	0	0	1	0	0	0	1	0	0	1	0	0	0	0	0	0	0
0.10	82	2	0	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.05	85	2	0	0	2	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.05	86																					
0.05	87	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.05	88	1	0	1	1	0	0	1	0	0	0	1	0	0	1	0	0	1	0	1	0	0
0.05	89	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.05	90	1	0	1	1	0	0	1	0	0	1	0	0	1	0	0	1	0	0	1	0	0
0.025	93	1	0	1	1	0	0	1	0	0	1	0	0	1	0	0	1	0	0	1	0	0
0.025	94	2	1	0	1	0	0	1	0	0	0	0	0	0	0	0	1	0	0	1	0	0
0.025	95	1	1	0	1	1	0	1	0	0	0	1	0	0	1	0	0	1	0	1	0	0
0.025	96	1	0	0	1	0	0	1	0	0	0	1	0	0	1	0	Dead**					
0.025	97	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	2	0	0
0.025	98	2	0	1	2	0	0	1	0	0	0	1	0	0	1	0	0	1	0	1	0	0

NOTES: The grading system used in this table is shown in table A-XI.

E = Erythema
N = Necrosis
D = Dehydration and/or desquamation

* Number of days after dosing

** Death not agent induced

Table A-XIV. The Cutaneous Effects of TOF in Rabbits

(Solution applied to clipped skin of each rabbit)

Dose	Rabbit no.	1*			2*			3*			4*			5*			6*			7*			8*			9*			10*			11*			12*			13*			14*		
		E	N	D	E	N	D	E	N	D	E	N	D	E	N	D	E	N	D	E	N	D	E	N	D	E	N	D	E	N	D	E	N	D	E	N	D	E	N	D	E	N	D
ml																																											
1.0	55	2	0	0	2	0	0	3	0	0	4	0	0	2	0	2	2	0	2	2	0	1	2	0	1	3	0	0	2	0	0	2	0	0	0	0	0	0	0	0	0	0	0
1.0	56	3	0	0	2	0	0	3	0	0	3	0	0	2	0	0	2	0	2	2	0	2	2	0	2	2	0	2	2	0	0	2	0	0	0	0	0	0	0	0	0	1	0
1.0	57	2	0	0	1	0	0	1	0	0	4	0	0	0	0	1	2	0	2	1	0	2	1	0	2	2	0	2	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1.0	58	1	0	0	1	0	0	1	0	0	4	0	0	2	0	2	2	0	2	2	0	1	1	0	1	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1.0	59	2	0	0	2	0	0	2	0	0	4	0	0	2	0	1	1	0	2	2	0	2	1	0	1	2	0	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1.0	60	2	0	0	2	0	0	1	0	0	4	0	0	2	0	2	2	0	2	2	0	1	1	0	1	2	0	2	2	0	0	1	0	0	0	0	0	0	0	0	0	0	0
0.5	67	2	0	0	1	0	0	4	0	0	2	0	1	2	0	1	2	0	2	2	0	1	1	0	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.5	68	1	0	0	1	0	0	4	0	0	2	0	0	2	0	2	2	0	2	1	0	1	1	0	1	1	0	0	0	1	0	0	1	0	0	1	0	0	0	0	0	0	0
0.25	75	2	0	0	2	0	0	4	0	0	4	0	0	4	0	0	4	0	0	1	0	2	1	0	1	1	0	0	1	0	0	0	0	0	0	0	0	0	1	0	1	0	0
0.25	76	1	0	0	2	0	0	4	0	0	4	0	0	4	0	0	4	0	0	2	0	2	2	0	2	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.10	83	2	0	0	3	0	0	2	0	0	2	0	0	1	0	0	2	0	2	2	0	1	1	0	2	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.10	84	1	0	0	2	0	0	4	0	0	4	0	0	4	0	0	4	0	1	1	0	1	1	0	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.05	91	2	0	0	2	0	0	2	0	0	3	0	2	2	0	2	1	0	2	1	0	1	1	0	1	1	0	0	1	0	0	1	0	0	1	0	0	0	0	0	0	0	0
0.05	92	1	0	0	2	0	0	2	0	0	2	0	0	2	0	0	1	0	1	1	0	1	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0
0.025	99	1	0	0	2	0	0	2	0	0	2	0	0	3	0	0	2	0	2	2	0	2	2	0	1	1	0	0	0	1	0	0	1	0	0	1	0	0	1	0	1	0	0
0.025	100	1	0	0	1	0	0	4	0	0	2	0	0	1	0	0	1	0	1	1	0	1	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

NOTES: The grading system used in this table is shown in table A-XI.

E = Erythema
N = Necrosis
D = Dehydration and/or desquamation

* Number of days after dosing

Table A-XV. Cutaneous Effects of 1% CS in TOF and TOF Alone in Rabbits

(These rabbits were sacrificed and necropsied 3 days after dosing)

Dose	Cutaneous effects									Necropsy findings
	1*			2*			3*			
	E	N	D	E	N	D	E	N	D	
ml										
						CS/TOF				
1.0	3	0	0	2	0	0	2	0	0	Moderate to severe erythema
	3	0	0	3	0	0	3	0	0	
	3	0	0	4	0	0	3	0	0	
	2	0	0	2	0	0	3	0	0	
1.0	1	0	0	2	0	0	3	0	0	Moderate erythema
	1	0	0	2	0	0	3	0	0	
	2	0	0	2	0	0	2	0	0	
	3	0	0	3	0	0	3	0	0	
0.025	1	0	0	1	0	0	3	0	0	Moderate erythema
	1	0	0	1	0	0	3	0	0	
	1	0	0	3	0	0	3	0	0	
	1	0	0	2	0	0	3	0	0	
						TOF				
1.0	1	0	0	1	0	0	2	0	0	Moderate erythema
	1	0	0	3	0	0	2	0	0	
0.1	0	0	0	1	0	0	3	2	0	Moderate erythema
	1	0	0	3	0	0	3	2	0	
0.025	0	0	0	1	0	0	3	0	0	Mild to moderate erythema
	0	0	0	3	0	0	3	0	0	
						Saline				
1.0	0	0	0	0	0	0	0	0	0	None
	0	0	0	0	0	0	0	0	0	
0.1	0	0	0	0	0	0	0	0	0	None
	0	0	0	0	0	0	0	0	0	
0.025	0	0	0	0	0	0	0	0	0	None
	0	0	0	0	0	0	0	0	0	

NOTES: E = Erythema
N = Necrosis
D = Dehydration and/or desquamation

The grading system used in this table is shown in table A-XI.

* Number of days after dosing

Table A-XVI. The Cutaneous Effects of TOF in Monkeys

(Average weight - 2.0 kg)

Dose	Monkey no.	1*			2*			3*			4*			5*			8*			9*			10*			11*			12*		
		E	N	D	E	N	D	E	N	D	E	N	D	E	N	D	E	N	D	E	N	D	E	N	D	E	N	D	E	N	D
ml/kg																															
12.5	C8-75	1	0	0	1	0	0	1	0	0	1	0	0	1	0	0	0	0	2	0	0	2	0	0	2	0	0	2	0	0	1
12.5	C8-76	1	0	0	1	0	0	1	0	0	1	0	0	1	0	0	0	0	2	0	0	2	0	0	2	0	0	2	0	0	1
25	C8-83	1	0	0	1	0	0	1	0	0	1	0	0	1	0	0	0	0	2	0	0	2	0	0	2	0	0	2	0	0	1
25	C8-82	1	0	0	1	0	0	1	0	0	1	0	0	1	0	0	0	0	2	0	0	2	0	0	2	0	0	2	0	0	1
50	C8-74	1	0	0	1	0	0	1	0	0	1	0	0	1	0	0	0	0	2	0	0	2	0	0	2	0	0	2	0	0	1
50	C8-77	1	0	0	1	0	0	1	0	0	1	0	0	1	0	0	0	0	2	0	0	2	0	0	2	0	0	2	0	0	1
75	C8-65	1	0	0	1	0	0	1	0	0	1	0	0	1	0	0	0	1	0	0	1	0	0	1	0	0	1	0	0	1	0
75	C8-63	1	0	0	1	0	0	1	0	0	1	0	0	1	0	0	0	1	0	0	1	0	0	1	0	0	1	0	0	1	0

NOTES: The grading system used in this table is shown in table A-XI.

E = Erythema
N = Necrosis
D = Dehydration and/or desquamation

* Number of days after dosing

Table A-XVII. The Cutaneous Effects of CS (1% in TOF) in Rabbits

(Formulation applied to sateen patch taped to skin of rabbits for 24 hr)

Dose	Rabbit no.	1a/			2a/			3a/			4a/			5a/			6a/			7a/			8a/			9a/			10a/		
		E	N	D	E	N	D	E	N	D	E	N	D	E	N	D	E	N	D	E	N	D	E	N	D	E	N	D	E	N	D
ml																															
1.0	193	2	0	0	2	0	0	2	0	0	2	0	0	2	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0
1.0	194	2	0	0	1	0	0	1	0	0	1	0	0	1	0	0	1	1	0	1	1	0	1	0	0	1	0	0	1	0	0
1.0	195	1	0	0	1	0	0	1	0	0	3	0	0	2	2	0	1	1	0	1	1	0	1	0	0	1	0	0	1	0	0
1.0	196	2	0	0	2	0	0	2	0	0	2	0	0	2	0	0	1	1	0	1	1	0	1	0	0	1	0	0	1	0	0
1.0	201 b/	2	0	0	2	0	0	2	0	0	2	0	0	2	1	0	1	2	0	1	1	0	1	0	0	1	0	0	1	0	0
1.0	202 b/	2	0	0	2	0	0	2	0	0	1	3	0	1	2	0	1	2	0	1	2	0	1	0	0	1	0	0	1	0	0
1.0	239	1	0	0	3	0	0	3	0	0 c/																					
1.0	240	0	0	0	2	0	0	2	0	0 c/																					
1.0	241	1	0	0	3	0	0	2	0	0 c/																					
1.0	242	2	0	0	2	0	0	3	0	0 c/																					
1.0	243 b/	1	0	0	1	0	0	1	0	0 c/																					
1.0	244 b/	1	0	0	3	0	0	3	0	0 c/																					
0.1	197	1	0	0	1	0	0	1	0	0	2	0	0	2	0	0	2	1	0	2	0	0	1	0	0	1	0	0	1	0	0
0.1	198	1	0	0	1	0	0	1	0	0	1	2	0	1	2	0	1	1	0	1	0	0	1	0	0	1	0	0	1	0	0
0.1	199	1	0	0	2	0	0	2	0	0	2	0	0	2	0	0	1	1	0	1	0	0	1	0	0	1	0	0	1	0	0
0.1	200	1	0	0	1	0	0	3	0	0	2	2	0	1	1	0	1	1	0	1	1	0	1	1	0	1	0	0	1	0	0
0.1	203 b/	2	0	0	1	0	0	2	0	0	3	0	0	1	0	0	1	1	0	1	0	0	1	0	0	1	0	0	1	0	0
0.1	204 b/	2	0	0	1	0	0	1	0	0	2	0	0	1	0	0	1	1	0	1	1	0	1	0	0	1	0	0	1	0	0
0.1	245	1	0	0	2	0	0	2	0	0 c/																					
0.1	246	1	0	0	3	0	0	3	1	0 c/																					
0.1	247	1	0	0	2	0	0	3	2	0 c/																					
0.1	248	1	0	0	1	0	0	2	0	0 c/																					
0.1	249 b/	0	0	0	0	0	0	2	0	0 c/																					
0.1	250 b/	1	0	0	1	0	0	1	0	0 c/																					

NOTES: The grading system used in this table are shown in table A-XI.

E = Erythema
N = Necrosis
D = Dehydration and/or desquamation

a/ = Number of days after dosing

b/ = TOF control

c/ = Sacrificed and necropsied 3 days after exposure

Table A-XVIII. Summary of Signs Seen in Dogs Following Administration of 1% CS/TOF or TOF Alone by Tracheal Intubation or Intratracheal Injection

Dose	Test solution	Route of administration	Time of examination	Signs		
				Dehydrated	Cough on tracheal pressure	Ausculatory sounds
ml						
0.50	CS/TOF	Tracheal intubation	72 Hours after exposure	0/8	1/8	1/8
0.25				0/8	4/8	4/8
0.10				0/8	3/8	1/8
0.50	Saline	Tracheal intubation	72 Hours after exposure	0/4	1/4	0/4
0.25				0/4	0/4	1/4
0.10				0/4	0/4	0/4
0.5	TOF alone	Tracheal intubation	72 Hours after exposure	0/8	0/8	0/8
0.5	CS/TOF	Intratracheal injection	96 Hours after exposure	0/8	1/8	1/8
0.5	Saline	Intratracheal injection	96 Hours after exposure	1/4	0/4	0/4

Table A-XIX. Average Blood Chemistry Values in Dogs Following Administration of 1% CS/TOF by Tracheal Intubation or Intratracheal Injection

Dose	Route of administration	No. tested	Sample time	Blood values			
				RBC ($\times 10^6$)	WBC - ($\times 10^3$)	PCV (%)*	BUN-SF units**
ml							
0.50	Tracheal intubation	8	Preexposure	7.1	13.5	50.3	16.4
			72-hours after exposure	8.0	16.5	51.3	16.8
0.25	Tracheal intubation	8	Preexposure	6.7	9.8	47.4	17.4
			72 hours after exposure	7.5	14.0	48.0	15.5
0.10	Tracheal intubation	8	Preexposure	7.6	15.9	51.8	16.3
			72 hours after exposure	8.0	14.6	51.2	16.0
Grouped saline controls		12	Preexposure	7.4	13.9	48.8	16.9
			72 hours after exposure	7.4	12.4	47.5	17.1
0.50	Intratracheal injection	8	Preexposure	6.8	16.4	46.0	18.0
			96 hours after exposure	7.3	15.5	48.0	15.0

* PCV = Packed cell volume

** BUN = Blood urea nitrogen

Table A-XX. Rectal Temperatures of Dogs After Administration of 1% CS/TOF and TOF Alone by Tracheal Intubation

Dose	Test solution	No. of animals	Rectal temperature (average)	
			Preexposure	72 Hours after exposure
ml			°F	
0.5	CS/TOF	8	102.3	102.5
0.25	CS/TOF	8	102.0	102.6
0.10	CS/TOF	8	102.2	102.7
0.5	TOF	8	Not taken	101.6

Table A-XXI. Gross Pathological Observations in Dogs Following Administration of 1% CS/TOF and TOF Alone by Tracheal Intubation and Intratracheal Injection

(3 Days after dosing)

Solution	Dose	Gross pathological observations			
		Trachea	No. responding	Lungs	No. responding
	ml				
		Tracheal Intubation			
CS/TOF	0.1	Mild hyperemia	2/4	Slight congestion	2/4
	0.25	No significant lesions		Focal pneumonia	1/4
				Congestion	2/4
	0.5	Mild to moderate hyperemia	3/4	Pneumonia	3/4
				Congestion	1/4
TOF	0.5	No significant lesions		Pneumonia	4/4
Saline	All doses	Mild hyperemia	2/6	Congestion	2/6
		Intratracheal Injection			
CS/TOF	0.5	Moderate tracheitis	1/4	Mild, focal pneumonia	4/4
Saline	0.5	None		Ecchymotic hemorrhage	1/2

Table A-XXII. Stability of CS in TOF Under Various Storage Conditions as Determined by Ultraviolet Analysis

Sample no.	1	2	3	4	5	6	7	8	9
Storage condition (°C)	25*	25*	25*	12*	12*	12*	25**	90*	25*
Solution concn (%) (w/v)	1	0.1	0.06	1	0.1	0.06	1	1	1
Day no.	% Control peak								
0	100	100	100	100	100	100	100	100	100
1	-	-	-	-	-	-	94	82	90
2	-	-	-	-	-	-	97	88	100
3	103	94	80	100	97	87	88	78	90
4	100	91	80	100	94	83	94	73	90
5	100	88	83	100	91	83	91	69	88
6	97	88	77	100	88	80	91	71	90
7	100	88	77	100	94	87	88	67	88
8	100	88	77	103	88	83	85	65	85
9	97	97	80	103	94	87	85	64	82
10	97	91	77	107	94	87	91	64	88
11	100	91	80	103	94	87	-	-	-
12	97	88	77	107	97	87	-	-	-
13	107	83	-	118	91	83	-	-	-
14	100	88	-	100	94	-	-	-	-
15	91	88	-	100	94	-	-	-	-
16-17	94	88	-	97	91	-	91	50	85
24	91	83	-	97	91	-	85	41	85
31	91	81	-	100	91	-	82	34	82
38	91	81	-	93	91	-	88	26	85
45	91	78	-	100	91	-	-	-	-
47	-	-	-	-	-	-	-	21	-
52	-	-	-	-	-	-	82	Hydrolyzed absorption peak shifted to 270 mμ	78
54	91	-	-	-					
59	86	-	-	93	-	-	85		80
66	84	-	-	93	-	-	-		-

* Fluorescent light

** No light

UNCLASSIFIED

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11. SUPPLEMENTARY NOTES	12. SPONSORING MILITARY ACTIVITY
Nondefense medical aspects of chemical agents Incapacitating and riot control agents	

13. ABSTRACT

(U) As solutions containing 1% o-chlorobenzylidene malononitrile (CS) in trioctylphosphate (TOF) have been proposed for use in riot control situations, a series of toxicological tests was conducted in animals. Results showed that the doses tested are not permanently injurious to the eyes. Effects on skin ranged from none to necrosis, and the affected areas usually healed within 2 weeks. When the mixtures were directly injected into the trachea, some evidence of congestion and pneumonia was seen. But the severity and duration of signs were not in a category that would warrant veterinary treatment under conditions of normal animal care. The reported studies indicate that no irreversible damage is expected with 1% CS/TOF solutions as long as the dose ranges shown to be safe in this paper are not exceeded.

DD FORM 1 NOV 68 1473 REPLACES DD FORM 1473, 1 JAN 64, WHICH IS OBSOLETE FOR ARMY USE.

UNCLASSIFIED

Security Classification

14. KEY WORDS	LINK A		LINK B		LINK C	
	ROLE	WT	ROLE	WT	ROLE	WT
CS						
TOF						
o-chlorobenzalmalononitrile						
o-chlorobenzylidene malononitrile						
Trioctylphosphate						
Riot control						
Cutaneous effects						
Ocular effects						
Upper and lower respiratory tract effects						
Tracheal intubation						
Tracheal injection						
Toxicity						
Dogs						
Rabbits						
Monkeys						
Man						