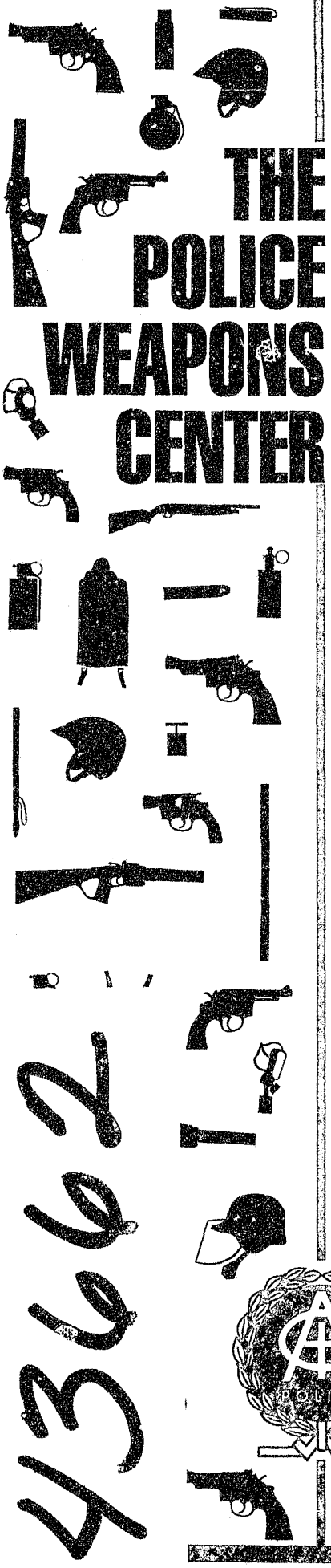
PWC EVALUATION REPORT



THE POLICE WEAPONS CENTER

EVALUATION OF SELECTED AEROSOL IRRITANT PROJECTOR FORMULATIONS

- CHEMICAL MACE (MK-IV)
- CHEMICAL WEAPON (118cc)
- FEDERAL STREAMER (No. 280)

INTERNATIONAL ASSOCIATION OF CHIEFS OF POLICE

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EVALUATION OF SELECTED AEROSAL IRRITANT PROJECTOR FORMULATIONS

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INTRODUCTION

Chloroacetophenone, a chemical which has irritant properties on exposed mucous membrane surfaces producing profuse lacrimation, uncontrollable blepherospasm and to a lesser extent dermal and respiratory irritation, has found considerable usage by numerous law enforcement agencies. The material, dispensed by a variety of methods, is used to temporarily incapacitate individuals exposed to it. Until the recent introduction of a non-explosive aerosol hand dispensing canister, "tear gas" was dispersed using some form of explosive or pyrotechnic dispersion system. The popular acceptance of this innovation has led to the marketing of aerosol irritant projectors under several brand names.

The determination of risk of using one of the many aerosol irritant projectors has relied chiefly on biological criteria designed primarily to establish the risk potential of packaged products that might be used or stored in the home environment. Such biological criteria, as set forth in the Federal Hazardous Substances Act (i. e. the absence of oral, ocular, dermal or inhalation toxicity) were not intended for the control of substances, the efficacy of which are dependent upon reversible irritating, disorienting and otherwise toxic effects imparted to an individual intentionally exposed to them. However, these tests, with minor modifications, have proven to be valuable tools in preventing the selection of products for widespread use which have the potential of inducing needless and totally undesirable prolonged or irreversible ocular, dermal, or respiratory effects.

It was the purpose of this study, to evaluate a number of test procedures in order to:

- identify and evaluate any potential risk involved in the use of the aerosol irritant projectors and attempt to

- establish test procedures which may prove useful in determining the comparative efficacy of the currently available and anticipated products.

To accomplish these goals, three currently marketed brands of chloroacetophenone aerosol irritant projectors were evaluated using four experimental systems and three laboratory animal species.

SAMPLES

The following test materials for use in this study were received April 20, 1971 from Mr. Thompson S. Crockett, Assistant Director, Management and Research Division, International Association of Chiefs of Police.

Material	Manufacturer
Chemical Mace MK-IV	General Ordinance Equipment Corporation P.O. Box 11211 Pittsburgh, Pa. 15238
Federal Streamer No. 280	Federal Laboratories, Inc. Saltsburg, Pa. 15681
Chemical Weapon (118CC)	Middle West Marketing Co. 216 South Hoyne Chicago, Illinois

In addition to the above active materials, inert training units containing the propellants used in Chemical Mace MK-IV and Federal Streamer were also received and were used as controls throughout the studies.

SUMMARY

A four-step biological screen of three formulations containing chloroacetophenone (CN) utilizing three laboratory animal species was completed. The sequence of testing and the species used were as follows:

- acute inhalation toxicity (rat)
- primary dermal irritation (rabbit)
- ocular irritation (monkey)
- multiple-exposure (monkey)

Preliminary trials conducted in rabbits and monkeys indicated that a convenient and accurately directed exposure could be obtained using one-second bursts at a distance of one-foot from the animal subject. Consequently all exposures were of one-second duration. In all but the acute inhalation toxicity study, canisters were held one-foot from the subject during exposure.

Acute Inhalation Toxicity

No mortalities among animals exposed to any of the three formulations in a closed chamber were recorded. Observed responses during the exposure period were limited chiefly to lacrimination and rhinorrhea. All symptoms disappeared upon removal of animals from the exposure chamber. Food consumption, fluid intake and growth patterns were normal during a 14-day post exposure observation period. No gross lesions, attributable to exposure to any of the materials tested, were seen in animals sacrificed at the conclusion of the

observation period.

Primary Dermal Irritation

Intact and abraded skin sites were exposed to each of the three forumlations. In one series of studies, skin sites were uncovered both during and after exposure. In a second series, skin sites were covered with a surgical gauze pad prior to exposure. Pads were left in place for one hour subsequent to exposure.

Irritation scores for animals with skin sites covered (loose clothing) were generally higher than those for rabbits without occlusive patches. However, scores for each exposure system were minimal. No retardation of healing at abraded skin sites was observed under either condition of exposure.

A procedure modeled after this study is recommended for inclusion in the Police Chemical Agents Manual.

Ocular Irritation

In monkeys exposed to a full-face burst from each of the product canisters, significant ocular irritation was seen 1-, 10- and 60-minutes after exposure. Lingering or irreversible effects, evidenced by persistent conjunctivitis, disturbance of the corneal epithelium or frank opacities were not observed. The ocular irritation noted at the 1-, 10- and 60-minute observation intervals consisted chiefly of conjunctivitis and lacrimination.

Persistent irritation of the facial skin, equivalent to a first degree sunburn, was seen in all monkeys exposed to each of the formulations. The folds of skin at the palpebral angles were particularly affected.

Based upon our experience with this study design, it appears that useful information relating efficacy and safety can be obtained from this type of study design. Consequently, the test is recommended for inclusion in the Manual. A system of scoring and interpreting the results is suggested.

Multiple Exposure Study

The effects of four exposures to each formulation studied, spaced at weekly intervals, were evaluated in groups of three monkeys. Ocular and dermal responses were similar to those seen in animal subjects of the ocular irritation study. Physiologic measurements included heart and respiratory rate measurements made during the fourth exposure. Transient increases in both parameters were considered to be the response to a non-specific stress situation. No clear-cut indication of total incapacitation of exposed monkeys was obtained. However, some evidence of pain and acute distress was visible.

No gross evidence of any local or systemic pathology was seen in monkeys sacrificed 1 hour, 4 and 8 weeks after the last exposure. The results of the microscopic review of several tissues

at autopsy are not yet available.

From the results obtained during this phase of the investigation, we have suggested a test system which may, as more experience is gained and corresponding refinements made, prove valuable in predicting the potential for temporary incapacitation and/or disorientation of the chemical weapon product.

From the results of the studies conducted on three chloroacetophenone formulations (CN), Chemical Mace MK-IV, Federal Streamer and Chemical Weapon, the following conclusions were drawn:

- the formulations appeared to be equally effective lacrimogens when tested in the monkey
- the degree and duration of dermal irritation seen following exposure to each formulation was greater in the monkey than in the rabbit
- each formulation demonstrated a relatively low order of toxicity in the rat and the monkey exposed to reasonable concentrations
- three tests, which have potential utility in evaluating the efficacy and safety of currently marketed as well as newly developed chemical weapon products, were identified

METHODS

A biological evaluation of four steps and encompassing three species of laboratory animals was completed. Each of the products submitted was studied in the following experimental systems:

- acute inhalation toxicity (rats)
- primary dermal irritation (rabbits)
- ocular irritation (monkeys)
- replicate exposure (monkeys)

Identical procedures were used in the evaluation of each of the three brands of chemical weapons evaluated. Where indicated, experiments were controlled using the appropriate and available inert training unit. Each material was dispensed from the canister as produced by the manufacturer.

Acute Inhalation Toxicity (rats)

A procedure modeled after that suggested in CFR Title 21, Section 191.1 (4) (f) (2) was followed. A clear lucite chamber having a volume of 10.8 cubic feet (306 liters) was used for each exposure. Continuous visual inspection of the animals during a one-hour exposure period was possible. For the evaluation of each product, five male and five female rats weighing approximately 200 grams were placed in the chamber and an air-flow of five liters per minute was introduced through the chamber (equivalent to one air change per hour).

Animals were individually restrained in the chamber and were arranged so that a one-second burst from the test canister could be aimed directly at each animal in the chamber. The test compound was sprayed directly at each of the animals through an inlet in the front panel of the chamber. The distance from the inlet to the rats was approximately three feet. Exhaust air was vented to the exterior of the laboratory.

Exposure concentration was determed from the ratio of weight loss of the canister to the chamber air flow.

At the conclusion of the exposure, animals were removed from the chamber, and examined for signs of toxicity to eyes, nasal passages and mouth. Animals were returned to individual cages (food and water ad libitum) and observed for mortality over a 14-day post exposure period. Body weights were recorded prior to exposure and again at the conclusion of the observation period. At sacrifice, surviving animals were autopsied for evidence of any gross lesion formation. One group of ten rats (5M:5F) were similarly exposed to the contents of a Chemical Mace training unit. An additional group of ten animals (5M:5F) was exposed to ambient air and served as controls.

Primary Dermal Irritation (rabbits)

For the evaluation of each test material, twelve adult albino

New Zealand White rabbits, weighing approximately 2.5 kilograms were used. Animals were randomly placed into two groups of six animals each.

The backs of all animals were clipped free of hair (intact skin sites). Three animals in each of the two groups were further prepared by making minor incisions through the stratum corneum (abraded skin sites).

To one group of six rabbits, three with intact and three with abraded skin sites, the assigned test material was applied directly to the prepared skin sites by spraying for one second from a distance of one foot. Treated sites were not covered subsequent to exposure. However, the eyes of the animals were protected by covering patches during the exposure.

Intact and abraded skin sites of animals of the second group were covered with a gauze pad, fixed to the skin with a non-irritating adhesive tape. Eyes of the animals were covered with protective patches. A one-second burst from the canister of test material was applied from a distance of one foot to each of the gauze pads.

All animals were restrained in individual rabbit stocks during insult and for a four-hour period subsequent to exposure. Occlusion gauze patches were removed from the treated skin sites of those animals so covered, one hour subsequent to exposure.

Skin responses to the material tested were recorded 1, 24, 48, 72 and 96 hours subsequent to application.

Twelve rabbits, exposed to a one-second burst from an inert training unit served as controls for the study.

Ocular Irritation (monkeys)

Twenty-four young-adult rhesus monkeys (Macaca mulatta) of unknown ages, obtained from a commercial importer were used. Following a brief holding period at the supplier's facilities, during which time all monkeys were adjudged healthy, free from parasites and acute infectious disease, the animals were transhipped to the Biological Science Laboratory in Elizabeth, New Jersey. All monkeys were again deparasitized (Thiabendazole and Niclosamide) at the Elizabeth, New Jersey facility.

All monkeys were held in isolation for one week during which time they were tuberculin tested, using Tuberculin Purified Protein Derivative, First Test Strength, intracutaneously. Seventy-two hours after all animals were declared negative, each monkey was retested using Tuberculin P.P.D., Second Test Strength. No positive reactors were found in the group.

Twelve monkeys were used in assessing the ocular irritation potential of the three products (three experimental groups of three monkeys each plus one control group). The remaining twelve

animals were utilized in the repeated exposure study.

Three monkeys weighing approximately three kilograms and judged free of ocular defects were used for testing each of the three products. Each monkey in turn was placed on a restraining board with limbs rigidly held in place with ties. The head of the animal was hand-held and one eye was held open with a lid-retractor. Exposure insult to the face and eyes was of one-second duration from a distance of one foot. Ocular responses were determined 1 and 10 minutes after exposure and again after 1, 24, 48, 72 and 96 hours.

Fluorescein staining was determined 1, 24 and 72 hours subsequent to exposure. One group of three monkeys, exposed in an identical manner to an inert training unit, was similarly studied, to determine the ocular responses to the propellant system.

Ocular irritation scores, based upon numerical values assigned to the observed responses, were calculated for each of the materials studied.

Replicate Exposure Study (monkey)

In order to determine the effects of repeated exposures of each material submitted, three monkeys were subjected to a facial exposure, once weekly, for a total of four exposures. Each animal was rigidly restrained on a suitable animal board and subjected

to a direct facial burst of material from the dispensing package from a distance of one foot for a one-second period. Animals were exposed individually and remained on the animal board for a minimum of 30 minutes after receiving the exposure. Exposures were performed on successive monkeys in order to permit close observation of the animals during the work-week.

Following each of the exposure periods, animals were observed for dermal and ocular effects. Gross behavioral changes indicative of discomfort and pain elicited by the exposures were also noted.

During the fourth exposure, heart and respiratory rate changes were monitored. Heart rates were determined from an electrocardiogram segment (Lead II). Respiration rates were obtained using an impedence pneumograph (Physiograph MK-IV). Parameters were monitored using a Desk Model Type DMP-4A Physiograph manufactured by E & M Instrument Company, Inc., Houston, Texas.

During each exposure, an attempt was made to determine whether the animal was temporarily incapacitated as a result of exposure to the test material.

One hour following the fourth exposure, one monkey was sacrificed under light anaesthesia by exsanguination and a complete necropsy examination was performed. A second monkey was sacrificed four weeks following the fourth exposure. The remaining monkey was sacrificed eight weeks following the last exposure.

At necropsy, the contents of the calvarium, thoracic and abdominal cavities were examined. Attention was also given to the appearance of the eyes, skin, nasal and oral mucosa.

Representative sections of the following tissues were collected into 10% neutral, buffered formalin for subsequent microscopic review:

- adrenals
- bone marrow
- brain
- bronchi
- eyes
- heart
- intestine
- trachea
- kidney
- liver
- lung
- pituitary
- skin
- stomach
- spinal cord

A group consisting of three monkeys was exposed to an inert training unit following the same exposure schedule used for the evaluation of the active units. Except for the content of the dispensing package, all experimental procedures previously described were rigorously followed with this group of animals.

RESULTS

Acute Inhalation Toxicity (rats)

Exposure concentrations estimated for each of the materials investigated were as follows:

Product	Quantity Used (grams)	Max. Conc. (mg/liter)
Chemical Mace MK-IV	23.2	77.3
Federal Streamer	22.6	75.3
Chemical Weapon	24.0	80.0
Training Unit	30.5	101.7

No mortalities among animals exposed to any of the three active products were recorded. One female rat exposed to the inert trainer was found dead on day 7 of the post-exposure observation period. The death was not considered to be related to the exposure.

Ocular and nasal discharge through the initial 30 minutes of exposure were readily observable in all animals exposed to each of the CN-containing formulations. Upon removal of animals from the chamber at the conclusion of the one-hour exposure, lacrimination and rhinorrhea persisted for approximately 15 minutes, after which time, all animals appeared grossly normal. Conjunctival

erythema was not apparent.

The only observable effect noted in animals subjected to the content of the training unit was an immediate and transitory preening of the face. Upon removal from the chamber all animals were normal.

No untoward behavioral effects attributable to the exposure were seen in any animals during the 14-day observation period. Food and fluid intake were normal. Growth, as indicated by increased body weight, was normal.

No gross lesions, attributable to the exposures, were seen in any of the animals autopsied at the conclusion of the observation period. Careful examination of the bronchi, and lungs revealed no differences between control and test animals.

Pertinent data are presented in Table 1.

Primary Dermal Irritation (rabbit)

Results of the dermal irritation studies are summarized in Tables 2-5. Irritation was graded and scored using the procedure suggested by J. H. Draize in the Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics, Association of Food and Drug Officials of the U.S. (1959).

Examination of these data indicates that observed dermal

irritation was less than that normally seen when the FHSA procedure is employed. In those animals with skin sites covered with a gauze pad (equivalent to loose clothing), observed irritation values were higher than those seen in animals without occlusive patches.

Observed irritation was generally more pronounced during the initial 24-hour post-exposure period after which time values rapidly decreased. In only one instance (Chemical Mace - Occluded) was a score greater than one recorded.

No retardation of healing of abraded skin sites was noted with the formulations tested.

The results of these studies suggest that a test paralleling this design would be useful in comparing the safety and efficacy of products currently marketed since it takes into account:

- application mimicking a "use" situation
- exposure of both clothed and unprotected skin surfaces
- anticipated vaporization of the material subsequent to application

Formulations containing excessive quantities of CN would be expected to induce more irritation than that observed with formulations containing the recommended concentration. Formulations with an insufficient quantity of chloroacetophenone should be without effect under the conditions of this test.

Ocular Irritation (monkey)

Immediate objectives to be served by the ocular irritation studies in monkeys included:

- a differentiation of effects presented by eyes protected and unprotected by eyelids during the exposure period
- observation of ocular effects occurring during the immediate (60-minute) post-exposure period
- a determination of any irreversible ocular effects resulting from exposure

At the outset of the study, it quickly became apparent that, under the conditions established, it was impossible to observe any perceptible difference in response of the eyes to a single discharge from a canister. Responses of the eye with the lids held open during the exposure were indistinguishable from those seen in the eyes "protected" by the unrestricted lids. Thus, the data presented in Tables 6-9 are representative of the observations made on either or both eyes.

Notable ocular irritation scores were obtained during the 60-minute post-exposure period for each formulation containing CN, with the highest score seen at the 10-minute observation time. In all instances, the 24-hour score was considerably less than that seen at the 1-, 10- or 60-minute observation time.

Lingering or irreversible effects, reflected by persistent irritation, disturbance of the corneal epithelium or frank opacities

were not observed. All eyes were essentially normal at the 48-hour reading.

Although at and subsequent to the 24-hour reading, eyes of monkeys exposed to the training unit were normal, demonstrable irritation was seen at the 10-minute reading. It was concluded, however, that the ocular irritation potential of the propellent system was extremely low.

Persistent irritation of the facial dermis, equivalent to a pronounced sunburn (first degree) was seen in all monkeys exposed to the three formulations of chloroacetophenone. Redness was perceptible within minutes of exposure. Desquamation followed within 3-4 days. The folds of skin at the palpebral angles were particularly affected.

Limited experience with this experimental system strongly suggests its utility in assessing the efficacy to risk ratio of existing or newly developed products. Responses (excluding corneal and iridal involvement) seen during the initial 60-minute post-exposure period can be viewed as desirable. In contrast, the degree and duration of responses noted at and after the 24-hour observation are indicative of undesirable effects. One approach to reducing these responses to a value indicative of the effectiveness of the lacrimogenic agent and at the same time taking into account the undesirable effects is to average the scores obtained at the 1-, 10- and 60-minute observation times. This combined score can

then be adjusted by subtracting the average of the scores obtained at the 24-, 48,-, 72- and 96-hour readings. Adjusted scores for the materials used in establishing the procedure were as follows:

Chemical Mace MK-IV	.....5.6
Federal Streamer	.....6.1
Chemical Weapon	.....6.4

Combined averages for the 1-, 10- and 60-minute scores were 6.3, 6.5 and 7.3 for Chemical Mace, Federal Streamer and Chemical Weapon respectively. Since responses observed at and persisting beyond 24 hours were minimal, scores observed during the initial 60 minutes changed very little. In the event one of the materials tested had produced any severe, persistent effect, the adjusted score would have been markedly reduced.

No attempt was or should be made to define the superiority or inferiority of the three products studied since the differences among the adjusted scores were negligible.

Multiple Exposure Study

The effects of four exposures to each formulation studied, spaced at weekly intervals, were evaluated in groups of three monkeys. Ocular and dermal responses seen subsequent to each weekly exposure were similar to those seen in the animals subjects of the ocular irritation study.

Measurements of heart and respiration rates during the fourth exposure revealed transient marked increases in both parameters. Respiratory rates obtained for approximately 20 seconds subsequent to exposure were so rapid that no absolute values could be obtained. However, a similar response was obtained with monkeys exposed to the inert training unit. Thus, it was concluded that the changes in the parameters studied reflected exposure to a non-specific stress situation rather than an effect elicitied by the CN-containing formulations.

No clear-cut indication of total incapacitation of monkeys exposed, when restrained, was visible. However, in an additional series of monkeys similarly exposed but allowed freedom of the cage subsequent to insult, several indications of disorientation and incapacitation were observed. These included:

- immediate return to the rear of the cage
- profuse lacrimination and facial discomfort as evidenced by rubbing of the face and eyes
- blepherospasm
- assuming of prone or otherwise abnormal position in the cage
- phonation

Based upon these observations, it was concluded that any estimate of incapacitating potential must be made using monkeys permitted freedom of the cage. Behavioral responses to exposure would appear to be better indicators of the distress and disorientation

than alterations of normal physiologic values.

No gross evidence of any local or systemic pathology was seen in monkeys sacrificed 1 hour, 4 and 8 weeks after the last exposure. The results of the microscopic review of several tissues taken at autopsy are not yet available.

From the results obtained during this phase of the investigation, we have suggested a test system which may, as more experience is gained and corresponding refinements made, prove valuable in predicting the potential for temporary incapacitation and/or disorientation of a non-lethal chemical weapon.

Table 1. Summary of Results of Acute Inhalation Toxicity Studies.

Unit	Sex	Mortality 24-hour	Mortality 14-day	Body Weight (grams) Initial	Body Weight (grams) Final	Body Weight (grams) Change
Chemical Mace MK-IV	M	0/5	0/5	189	282	+93
	F	0/5	0/5	220	245	+25
Federal Streamer	M	0/5	0/5	221	218	+97
	F	0/5	0/5	245	270	+25
Chemical Weapon	M	0/5	0/5	200	265	+65
	F	0/5	0/5	221	242	+21
Trainer	M	0/5	0/5	215	291	+76
	F	0/5	1/5	190	236	+46
Air Control	M	0/5	0/5	201	263	+62
	F	0/5	0/5	194	217	+23

Primary Dermal Irritation

Evaluation of Skin Reactions	Value*
Erythema and eschar formation:	
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to servere erythema	3
Severe erythema (beet redness) to slight eschar formation (injuries in depth)	4
Edema formation:	
No edema	0
Very slight edema (barely perceptible)	1
Slight edema (edges of area well defined by definite raising)	2
Moderate edema (raised approximately 1.0 mm.)	3
Severe edema (raised more than 1.0 mm. extending beyond the area of exposure)	4

*The "value" recorded for each reading is the average value of the three animals subjected to the test.

Table 2. Observed Dermal Responses in Rabbits Following Topical Application of Chemical Mace MK-IV (Non-occluded)

Erythema and Eschar	Time*	Score**
Intact Skin	1	0.7
Intact Skin	24	0
Intact Skin	48	0
Intact Skin	72	0
Intact Skin	96	0
Abraded Skin	1	1.0
Abraded Skin	24	0
Abraded Skin	48	0
Abraded Skin	72	0
Abraded Skin	96	0
Edema		
Intact Skin	1	0
Intact Skin	24	0
Intact Skin	48	0
Intact Skin	72	0
Intact Skin	96	0
Abraded Skin	1	0
Abraded Skin	24	0
Abraded Skin	48	0
Abraded Skin	72	0
Abraded Skin	96	0

* hours after application

**average score for three rabbits

Table 2 (cont'd). Observed Dermal Responses in Rabbits Following Topical Application of Chemical Mace MK-IV (Occluded)

Erythema and Eschar	Time*	Score**
Intact Skin	1	1.0
Intact Skin	24	0.3
Intact Skin	48	0
Intact Skin	72	0
Intact Skin	96	0
Abraded Skin	1	1.7
Abraded Skin	24	0.3
Abraded Skin	48	0
Abraded Skin	72	0
Abraded Skin	96	0
Edema		
Intact Skin	1	0.3
Intact Skin	24	0.3
Intact Skin	48	0
Intact Skin	72	0
Intact Skin	96	0
Abraded Skin	1	0.7
Abraded Skin	24	0.3
Abraded Skin	48	0
Abraded Skin	72	0
Abraded Skin	96	0

* hours after application

**average score for three rabbits

Table 3. Observed Dermal Responses in Rabbits Following Topical Application of Federal Streamer (Non-occluded)

Erythema and Eschar	Time*	Score**
Intact Skin	1	0.3
Intact Skin	24	0.3
Intact Skin	48	0
Intact Skin	72	0
Intact Skin	96	0
Abraded Skin	1	0.7
Abraded Skin	24	0.7
Abraded Skin	48	0
Abraded Skin	72	0
Abraded Skin	96	0
Edema		
Intact Skin	1	0
Intact Skin	24	0
Intact Skin	48	0
Intact Skin	72	0
Intact Skin	96	0
Abraded Skin	1	0
Abraded Skin	24	0
Abraded Skin	48	0
Abraded Skin	72	0
Abraded Skin	96	0

* hours after application

**average score for three rabbits

Table 3 (cont'd). Observed Dermal Responses in Rabbits Following Topical Application of Federal Streamer (Occluded)

Erythema and Eschar	Time*	Score**
Intact Skin	1	0.7
Intact Skin	24	0.3
Intact Skin	48	0
Intact Skin	72	0
Intact Skin	96	0
Abraded Skin	1	1.0
Abraded Skin	24	0.3
Abraded Skin	48	0
Abraded Skin	72	0
Abraded Skin	96	0
Edema		
Intact Skin	1	0.3
Intact Skin	24	0
Intact Skin	48	0
Intact Skin	72	0
Intact Skin	96	0
Abraded Skin	1	0.3
Abraded Skin	24	0.3
Abraded Skin	48	0
Abraded Skin	72	0
Abraded Skin	96	0

* hours after application

**average score for three rabbits

Table 4. Observed Dermal Responses in Rabbits Following Topical Application of Chemical Weapon (Non-occluded)

Erythema and Eschar	Time*	Score**
Intact Skin	1	0.3
Intact Skin	24	0.3
Intact Skin	48	0
Intact Skin	72	0
Intact Skin	96	0
Abraded Skin	1	0.7
Abraded Skin	24	0
Abraded Skin	48	0
Abraded Skin	72	0
Abraded Skin	96	0
Edema		
Intact Skin	1	0.3
Intact Skin	24	0
Intact Skin	48	0
Intact Skin	72	0
Intact Skin	96	0
Abraded Skin	1	0.3
Abraded Skin	24	0
Abraded Skin	48	0
Abraded Skin	72	0
Abraded Skin	96	0

* hours after application

**average score for three rabbits

Table 4 (cont'd). Observed Dermal Responses in Rabbits Following Topical Application of Chemical Weapon (Occluded)

Erythema and Eschar	Time*	Score**
Intact Skin	1	1.0
Intact Skin	24	0.3
Intact Skin	48	0
Intact Skin	72	0
Intact Skin	96	0
Abraded Skin	1	1.0
Abraded Skin	24	0.3
Abraded Skin	48	0
Abraded Skin	72	0
Abraded Skin	96	0
Edema		
Intact Skin	1	0.3
Intact Skin	24	0
Intact Skin	48	0
Intact Skin	72	0
Intact Skin	96	0
Abraded Skin	1	0.7
Abraded Skin	24	0
Abraded Skin	48	0
Abraded Skin	72	0
Abraded Skin	96	0

* hours after application

**average score for three rabbits

Table 5. Observed Dermal Responses in Rabbits Following Topical Application of Inert Trainer (Non-occluded)

Erythema and Eschar	Time*	Score**
Intact Skin	1	0
Intact Skin	24	0
Intact Skin	48	0
Intact Skin	72	0
Intact Skin	96	0
Abraded Skin	1	0
Abraded Skin	24	0
Abraded Skin	48	0
Abraded Skin	72	0
Abraded Skin	96	0
Edema		
Intact Skin	1	0
Intact Skin	24	0
Intact Skin	48	0
Intact Skin	72	0
Intact Skin	96	0
Abraded Skin	1	0
Abraded Skin	24	0
Abraded Skin	48	0
Abraded Skin	72	0
Abraded Skin	96	0

* hours after application

**average score for three rabbits

Table 5 (cont'd). Observed Dermal Responses in Rabbits Following Topical Application of Inert Trainer (Occluded)

Erythema and Eschar	Time*	Score**
Intact Skin	1	0
Intact Skin	24	0
Intact Skin	48	0
Intact Skin	72	0
Intact Skin	96	0
Abraded Skin	1	0.3
Abraded Skin	24	0
Abraded Skin	48	0
Abraded Skin	72	0
Abraded Skin	96	0
Edema		
Intact Skin	1	0
Intact Skin	24	0
Intact Skin	48	0
Intact Skin	72	0
Intact Skin	96	0
Abraded Skin	1	0
Abraded Skin	24	0
Abraded Skin	48	0
Abraded Skin	72	0
Abraded Skin	96	0

* hours after application

**average score for three rabbits

Scale for Scoring Ocular Lesions*

(1) Cornea

(A) Opacity-degree of density (area most dense taken for reading)

- No Opacity 0
- Scattered or diffuse area, details of iris clearly visible 1
- Easily discernible translucent areas, details of iris slightly obscured . . 2
- Opalescent areas, no details of iris visible, size of pupil barely discernible 3
- Opaque, iris invisible 4

(B) Area of cornea involved

- One quarter (or less) but not zero 1
- Greater than one quarter, but less than half 2
- Greater than half, but less than three quarters 3
- Greater than three quarters, up to whole area 4

Score equals A x B x 5 Total maximum = 80

(2) Iris

(A) Values

- Normal 0
- Folds above normal, congestion, swelling, circumcorneal injection (any or all of these or combination of any thereof) iris still reacting to light (sluggish reactions is positive) 1
- No reaction to light, hemorrhage, gross destruction (any or all of these) 2

Score equals A x 5 Total maximum = 10

(3) Conjunctivae

(A) Redness (refers to palpebral and bulbar conjunctivae excluding cornea and iris)

- Vessels normal 0
- Vessels definitely injected above normal 1
- More diffuse, deeper crimson red, individual vessels not easily discernible 2
- Diffuse beefy red 3

(B) 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 about half closed to completely closed 4

(C) Discharge

- No discharge 0
- Any amount different from normal (does not include small amounts observed in inner canthus of normal animals) 1
- Discharge with moistening of the lids and hairs just adjacent to lids . . . 2
- Discharge with moistening of the lids and hairs, and considerable area around the eye 3

Score equals (A + B + C) x 2 Total maximum = 20

The maximum total score is the sum of all scores obtained for the cornea, iris, and conjunctivae. Total maximum score possible = 110

*Lehman, A. J., et al., Appraisal of the Safety of Chemicals in Foods, Drugs, and Cosmetics, Assoc. Food and Drug Officials of the U. S., Austin, Texas, 1959.

Table 6. Observed Responses* of the Monkey Eye to Chemical Mace MK-IV

	Observation Time						
	Minutes			Hours			
Observation	1	10	60	24	48	72	96
Cornea	0	0	0	0	0	0	0
Iris	0	0	0	0	0	0	0
Conjunctivae							
Redness	1.0	2.0	1.7	1.3	0	0	0
Chemosis	0	0	0	0	0	0	0
Discharge	1.0	3.0	0.7	0	0	0	0
Score	4.0	10.0	4.8	2.6	0	0	0
Fluorescein Staining	-	-	N(3)	N(3)	-	N(3)	-

N=negative
P=positive
Number in parenthesis=number of animals

*scores averaged for three monkeys

Table 7. Observed Responses* of the Monkey Eye to Federal Streamer

	Observation Time						
	Minutes			Hours			
Observation	1	10	60	24	48	72	96
Cornea	0	0	0	0	0	0	0
Iris	0	0	0	0	0	0	0
Conjunctivae							
Redness	1.3	2.0	2.0	0.7	0	0	0
Chemosis	0	0	0.3	0	0	0	0
Discharge	0.7	2.7	0.7	0	0	0	0
Score	4.0	9.4	6.0	1.4	0	0	0
Fluorescein Staining	-	-	N(3)	N(3)	-	N(3)	-

N=negative
P=positive
Number in parenthesis=number of animals

*scores averaged for three monkeys

Table 8. Observed Responses* of the Monkey Eye to Chemical Weapon

	Observation Time						
	Minutes			Hours			
Observation	1	10	60	24	48	72	96
Cornea	0	0	0	0	0	0	0
Iris	0	0	0	0	0	0	0
Conjunctivae							
Redness	1.3	2.0	1.7	1.0	0.3	0	0
Chemosis	0	0.3	0.3	0	0	0	0
Discharge	1.0	2.7	1.3	0	0	0	0
Score	4.6	10.0	6.6	2.0	0.6	0	0
Fluorescein Staining	-	-	N(3)	N(3)	-	N(3)	-

N=negative
P=positive
Number in parenthesis=number of animals

*scores averaged for three monkeys

Table 9. Observed Responses* of the Monkey Eye to Inert Trainer

	Observation Time						
	Minutes			Hours			
Observation	1	10	60	24	48	72	96
Cornea	0	0	0	0	0	0	0
Iris	0	0	0	0	0	0	0
Conjunctivae							
Redness	0	0.7	0.3	0	0	0	0
Chemosis	0	0	0	0	0	0	0
Discharge	0	0.3	0	0	0	0	0
Score	0	2.0	0.6	0	0	0	0
Fluorescein Staining	-	-	N(3)	N(3)	-	N(3)	-

N=negative
P=positive
Number in parenthesis=number of animals

*scores averaged for three monkeys

In the section to follow, we are suggesting three additional test procedures for the evaluation of irritant formulations. Before considering them for inclusion in the POLICE CHEMICAL AGENTS MANUAL, the test systems must be validated with several products in several laboratories.

The procedures we have suggested for inclusion in the manual are proposed to provide an indication of:

- minimal dermal irritation under "use" conditions
- efficacy and safety of lacrimogens
- potential for incapacitation and/or disorientation

We also suggest the tests currently described in the Manual no longer be designated as "interim" tests. To emphasize this recommendation, we have reproduced the test of the animal studies currently employed, in this section.

We have not made any recommendations for altering the human evaluation studies since we have no experience in this area.

TEST RECOMMENDATIONS

A. Injury Potential

1. Eye Irritation

The FHSA eye irritant test, when properly conducted has proved to be a valuable screening test and it is recommended as a test for police aerosol irritant formulations.

Eye Irritation Test

191.12 Test for eye irritants.

(a)(1) Six albino rabbits are used for each test substance. Animal facilities for such procedures shall be so designed and maintained as to exclude sawdust, wood chips, or other extraneous materials that might produce eye irritation. Both eyes of each animal in the test group shall be examined before testing, and only those animals without eye defects or irritation shall be used. The animal is held firmly but gently until quiet. The test material is placed in one eye of each animal by gently pulling the lower lid away from the eyeball to form a cup into which the test substance is dropped. The lids are then gently held together for one second and the animal is released. The other eye, remaining untreated, serves as the control. For testing liquids, 0.1 milliliter is used. For Solids or pastes, 100 milligrams of the test substance is used, except that for substances in flake, granule, powder, or other particulate form the amount that has a volume of 0.1 milliliter (after compacting as much as possible without crushing or altering the individual particles, such as by tapping the measuring container) shall be used wherever this volume weighs less than 100 milligrams. In such a case, the weight of the 0.1 milliliter test dose should be recorded. The eyes are not washed following instillation of test material except as noted below.

(2) The eyes are examined and the grade of ocular reaction is recorded at 24, 48 and 72 hours. Reading of reactions is facilitated by use of a binocular loupe, hand slit-lamp, or other expert means. After recording of observation at 24 hours, any or all eyes may be further examined after applying fluorescein. For this optional test, one drop of fluorescein sodium ophthalmic solution U.S.P. or equivalent is dropped directly on the cornea. After flushing out the excess fluorescein with

sodium chloride solution U.S.P. or equivalent, injured areas of the cornea appear yellow; this is best visualized in a darkened room under ultraviolet illumination. Any or all eyes may be washed with sodium chloride solution U.S.P. or equivalent after the 24 hour reading.

(b)(1) An animal shall be considered as exhibiting a positive reaction if the test substance produces at any of the readings ulceration of the cornea (other than a fine stippling), or opacity of the cornea (other than a slight dulling of the normal luster), or inflammation of the iris (other than a slight deepening of the folds (or rugae) or a slight circumcorneal injection of the blood vessels), or if such substance produces in the conjunctivae (excluding the cornea and iris) an obvious swelling with partial eversion of the lids or a diffuse crimson-red with individual vessels not easily discernible.

(2) The test shall be considered positive if four or more of the animals in the test group exhibit a positive reaction. If only one animal exhibits a positive reaction, the test shall regarded as negative. If two or three animals exhibit a positive reaction, the test is repeated using a different group of six animals. The second test shall be considered positive if three or more of the animals exhibit a positive reaction. If only one or two animals in the second test exhibit a positive reaction, the test shall be repeated with a different group of six animals. Should a third test be needed, the substance will be regarded as an irritant if any animal exhibits a positive response.

This test is graded by the scale for scoring ocular lesions illustrated below. Positive values are indicated by a check (✓)

SCALE FOR SCORING OCULAR LESIONS*

(1) Cornea
  (A) Opacity-degree of density (area most dense taken for reading)
    No Opacity 0
    Scattered or diffuse area, details of iris clearly visible.....1
    Easily discernible translucent areas, details of iris slightly obscured 2
    Opalescent areas, no details of iris visible, size of pupil barely discernible 3
    Opaque, iris invisible 4

(B) Area of cornea involved
One quarter (or less) but not zero 1
Greater than one quarter, but less than half 2
Greater than half, but less than three quarters 3
Greater than three quarters, up to whole area 4

Score equals A x B x 5 Total maximum = 80

Iris
(A) Values
Normal 0
Folds above normal, congestion, swelling, circumcorneal injection (any or all 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

Score equals A x 5 Total maximum = 10

Conjunctivae
(A) Redness (refers to palpebral and bulbar conjunctivae excluding cornea and iris)
Vessels normal.....0
Vessels definitely injected above normal.....1
More diffuse, deeper crimson red, individual vessels not easily discernible 2
Diffuse beefy red 3

(B) 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 about half closed to completely closed 4

Score equals (A+B) x 2 Total maximum = 14

The maximum total score is the sum of all scores obtained for the cornea, iris, and conjunctivae. Total maximum score possible = 104

*Adapted from Lehman, A.J. et al., Appraisal of the Safety of Chemicals in Foods, Drugs, and Cosmetics, Assoc. Food and Drug Officials of the U.S., Austin, Texas, 1959.

2. Skin Irritation

While the FHSA 191.11 test for primary skin irritation is too stringent for the evaluation of irritant formulations, it has been modified to serve as a meaningful skin test. The modified test as outlined below is recommended for police aerosol irritant formulations.

Skin Irritation Test

Primary irritation to the skin is measured by a patch-test technique on the abraded and intact skin of the albino rabbit, clipped free of hair. A minimum of six rabbits are used in abraded and intact skin tests. Introduced under a square patch such as surgical gauze measuring 1 inch x 1 inch, two single layers thick, 0.5 milliliter (in case of liquids) or 0.5 gram (in case of solids and semisoldis) of the test substance. Dissolve solids in an appropriate solvent and apply the solution as for liquids. The animals are immobilized with patches secured in place by adhesive tape.

An equal number of exposures are made on areas of skin that have been previously abraded. The abrasions are minor incisions through the stratum corneum, but not sufficiently deep to disturb the derma or to produce bleeding. After 24 hours of exposure, the patches are removed and the resulting reactions are evaluated on the basis of the designated values in the following table:

Evaluation of skin reactions	Value
Erythema and eschar formation:	
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) to slight eschar formation (injuries in depth)	4
Edema formation:	
No edema	0
Very slight edema (barely perceptible)	1
Slight edema (edges of area well defined by definite raising)	2
Moderate edema (raised approximately 1 millimeter)	3
Severe edema (raised more than 1 millimeter and extending beyond the area of exposure)	4

The "value" recorded for each reading is the average value of the six or more animals subject to the test.

Patches are not replaced and readings are again made at the end of a total of 72 hours (48 hours after the first reading), 96 hours, 120 hours, and 240 hours.

Add the values for erythema and eschar formation at each reading for both intact and abraded skin. Similarly, add the values for edema formation at each reading. The total of the twenty values is divided by 10 to give the irritation score.

The skin irritation test shall be considered positive if four or more of the animals in the test group exhibit a positive reaction at 240 hours. If only one animal in the test group exhibits a positive reaction at 240 hours the test shall be regarded as negative. If two or three animals exhibit a positve reaction at 240 hours the test is repeated using a different group of six animals. The second test shall be considered positive if three or more of the animals exhibit a positive reaction at 240 hours. If only one or two of the animals in the second test exhibit a positive reaction, the test shall be repeated with a different group of six animals. Should a third test be needed, the substance will be regarded as an unacceptable irritant if any animal exhibits a postive response.

A positive response is represented by any value above zero.

The present purpose of the skin irritation test is to screen out formulations that may produce permanent after-effects or systemic toxicity. Once this test procedure has been applied to a wide variety of formulations it may be possible to establish a numerical value cut-off point for acceptability. In the meantime, the product of choice will be that formulation which is effective, negative in the eye test, and which has the lowest skin irritation value.

2. Skin Irritation

The preceding test is useful in screening out formulations capable of producing severe or permanent dermal toxicity under maximized conditions of application. A second test for evaluating the safety of a formulation under "use" condition is suggested below.

Skin Irritation Test

In the evaluation of each irritant formulation, twelve adult albino New Zealand White rabbits, weighing approximately 2.5 kilograms are used. Animals are randomly placed into two groups of six animals each. The backs of all animals are clipped free of hair (intact skin sites). Three animals in each of the two groups are further prepared by making minor incisions through the stratum corneum (abraded skin sites).

To one group of six rabbits, three with intact and three with abraded skin sites, the test material is applied directly to the prepared skin sites by spraying for one second from a distance of one foot. Treated sites are not covered subsequent to exposure. However, the eyes of the animals are protected by covering patches during the exposure.

Intact and abraded skin sites of animals of the second group are covered with a gauze pad, fixed to the skin with a non-irritating adhesive tape. Eyes of the animals are covered with protective patches. A one-second burst from the canister of test material is applied from a distance of one foot to each of the gauze pads.

All animals are restrained in individual rabbit stocks during insult and for a four-hour period subsequent to exposure. Occlusive gauze patches are removed from the treated skin sites of those animals so covered, one hour subsequent to exposure. Skin responses are recorded 1, 24, 48, 72 and 96 hours subsequent to application.

Each of the two groups of rabbits are considered a separate test. "Values" recorded for each reading are the average values of the six animals subject to the test.

Add the values for erythema and eschar formation at each reading for both intact and abraded skin. Similarly, add the values for edema formation at each reading. The total of the twenty values is divided by 10 to give the irritation score.

The skin irritation test shall be considered positive if four or more of the animals in the test group exhibit a positive reaction at 96 hours.

A positive response is represented by any value greater than two.

B. Effectiveness

1. Lacrimination

Two tests, employing animal models, are suggested for the evaluation of irritant formulations as agents capable of safely and rapidly inducing lacrimination and membrane irritation to the point of temporary disorientation and/or incapacitation of an individual affected. Obviously, the ultimate test subject is man. However, before human exposure is undertaken, an evaluation in animals should be completed.

Lacrimination Test

Three monkeys (Macaca mulatta) weighing approximately 2.5-5.0 kilograms are used in this test. Monkeys are judged free of pre-existing ocular lesions using ophthalmic fluorescein and slit-lamp examination. Each monkey in turn is placed on a restraining board with limbs rigidly held in place with ties. Exposure insult to the face and eyes is of one-second duration from a distance of one foot. Animals are released from the board one hour subsequent to exposure. Ocular responses using the Draize scoring system are determined 1, 10, and 60 minutes after exposure and again after 24, 48, 72 and 96 hours. Fluorescein staining is determined 1, 24 and 72 hours subsequent to exposure.

Ocular irritation scores are calculated for each observation time. Scores for the 1-, 10- and 60-minute observations are combined and averaged. The combined score is adjusted by subtracting the average of the scores obtained at the 24-, 48-, 72- and 96-hour readings.

The average score obtained for the initial three observations is indicative of a desired effect. The presence of persistent effects is undesirable and thus detracts from the efficacy of the product.

A product with an adjusted average score falling between 4 and 8, should be efficacious and at the same time be free of severe and persistent effects. However, considerably more experience with this

test will be required to establish the final range of values to define both the safety and efficacy of an irritant formulation.

2. Incapacitation

Although no completely adequate animal test system for evaluating incapacitation and disorientation is as yet available, the proposed test described below is suggested for initial indications of behavioral responses following exposure to irritant formulations.

Behavioral Test

Three monkeys (Macaca mulatta) are used in this test. Animals are housed individually in wire cages. Each monkey is provided with a suitable collar with chain approximately 4 feet in length attached. This arrangement provides the animal freedom of the cage and at the same time permits control of the animal by the investigator. A distance marker is placed on the chain, 14 inches from point of attachment to the collar.

At the time of the test each monkey (in turn) is drawn to the door of the cage and the face is fully-exposed. The canister of test material is held at the chain-marker and the animal is given a one-second burst. At this distance, it is nearly impossible for the animal to avoid the stream.

Coincident with the end of the exposure, the chain is released and the monkey is free to respond to the exposure. Responses to the exposure should include:

Score	Local Effects	Time to Onset	Duration
3	Profuse lacrimination	2-10 seconds	60 minutes
2	Facial erythema	1-60 minutes	72 hours
1	Blepherospasm	1-10 seconds	30 minutes
1	Scratching and rubbing of face	1-10 seconds	1 hour

Score	Behavioral Effects	Time to Onset	Duration
1	Retreating to rear of cage	1-10 seconds	-
2	Phonation	2-30 seconds	variable
3	Lying prone in cage	10-60 seconds	variable
3	Assuming a static position	10-60 seconds	variable

An efficacious product is one demonstrating a high numerical score. A scoring base of 7 should be obtainable with an effective product tested. Observations can be scored once during the one-hour post-exposure period. The total scores recorded for each monkey are averaged. This number reflects the activity of the product.

As experience is gained with the procedure, refinements in the observations and assigned scores can be made. It is anticipated that the information generated in this test can be correlated with effects seen in humans.

END