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IRRITATION AND SENSITIZATION STUDIES ON TRIAZINE T17-2

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TECHNICAL REVIEW AND APPROVAL

AAMRL-TR-90-002

The experiments reported herein were conducted according to the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council.

This report has been reviewed by the Office of Public Affairs (PA) and is releasable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public, including foreign nations.

This technical report has been reviewed and is approved for publication.

FOR THE COMMANDER

MICHAEL B. BALLINGER, Lt Col, USAF, BSC
Chief, Toxic Hazards Division
Harry G. Armstrong Aerospace Medical Research Laboratory

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This study was designed to evaluate the toxic effects associated with accidental skin and eye contact. A single neat dose of 0.1 mL T17-2 into NZW rabbit eyes resulted in slight conjunctivae irritation one hour after treatment. Single treatment of 0.5 mL neat T17-2 to rabbit skin produced no irritation. Guinea pigs failed to elicit a sensitization response following repeated application of T17-2. T17-2 does not demonstrate an irritation or sensitization hazard under the conditions tested

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PREFACE

This is one of a series of technical reports describing results of the experimental laboratory programs conducted at the Toxic Hazards Research Unit, NSI Technology Services Corporation - Environmental Sciences. This document serves as a final report on the in-life toxicity of triazine T17-2. The research described in this report began in August 1989 and was completed in November 1989 under U.S. Air Force Contract No. F33615-85-C-0532. Lt Col Michael B. Ballinger served as Contract Technical Monitor for the U.S. Air Force, Harry G. Armstrong Aerospace Medical Research Laboratory.

The animals used in this study were handled in accordance with the principles stated in the *Guide for the Care and Use of Laboratory Animals*, prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council, Department of Health and Human Services, National Institute of Health Publication #86-23, 1985, and the Animal Welfare Act of 1966, as amended.

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ABBREVIATIONS

cm	Centimeter
EPA	Environmental Protection Agency
g	Gram
h	Hour
IR	Infrared
kg	Kilogram
mg	Milligram
mL	Milliliter
min	Minute
NZW	New Zealand White
sec	Second

SECTION I

INTRODUCTION

The compound 2,4-Bis(2,4,6,7-tetrachloro-1,1,2,3,3,4,5,5,6,7,7-undecafluoroheptyl)-6-(2-bromo-3-chloro-1,1,2,3,3-pentafluoropropyl)-1,3,5-triazine (trade name T17-2) is presently in use as a missile gyro damping fluid. A literature review on this compound indicated no reference, nor does a CAS number exist for the chemical formula $C_{20}BrCl_9F_{27}N_3$. The basic chemical structure is similar to triazine herbicides which are used in large quantities worldwide. The triazine compounds are generally mildly toxic requiring an oral dose greater than 1000 mg/kg (Murphy, 1980) or a dermal LD_{50} dose ranging from 5 to 10 g/kg (Reinhardt and Brittelli, 1981) to cause an effect. Generally, the triazines are at most mild skin and eye irritants.

The primary routes of exposure expected with this compound would be by dermal absorption or accidental eye contact. Therefore, acute eye and skin irritation tests as well as a sensitization study were conducted. Species and sex of animals selected for these acute studies were in conformance with the requirements of the Environmental Protection Agency (EPA, 1982). Existing alternative methods to animal testing were inadequate for use in this study.

SECTION 2

MATERIALS

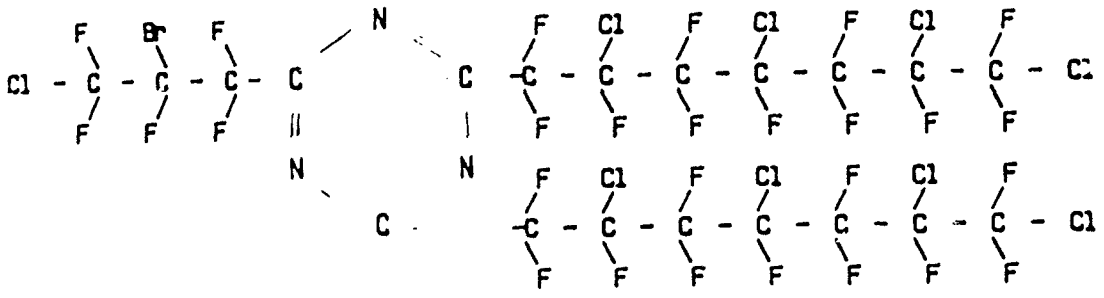
TEST AGENT

The test material used in this study was supplied by the Air Force. Pertinent physical and chemical characteristics are listed below:

Chemical family:	Triazine
Formula:	$C_{20}BrCl_9F_{27}N_3$
Trade name:	T17-2
Chemical name:	2,4-bis(2,4,6,7-tetrachloro-1,1,2,3,3,4,5,5,6,7,7-undecafluoroheptyl)-6-(2-bromo-3-chloro-1,1,2,3,3-pentafluoropropyl)-1,3,5-triazine
Molecular weight:	1194.1
Sp. gravity @ 135°C:	1.9273
Appearance:	Clear, colorless liquid
Lot number:	16-7.1

TEST AGENT QUALITY CONTROL

An infrared (IR) spectrum of triazine T17-2 was generated using a Beckman Acculab 4 (Beckman Instruments, Inc., Fullerton, CA) infrared spectrophotometer. Figure 1 is a typical IR spectrum of the supplied triazine T17-2 fluid. The triazine material exceeds the molecular weight range required for analysis by mass spectrometry. Because of this and the fact that the compound does not have the volatility required for this analysis, mass spectrometry was not done. The structure of the material is given below:



ANIMALS

Male Hartley albino guinea pigs weighing between 200 and 250 g were purchased from Murphy Breeding Labs, Plainfield, IN. Female New Zealand White (NZW) rabbits weighing between 2 and 3 kg were purchased from Clerco Research Farms, Cincinnati, OH. All animals were subjected to a two-week quarantine period. The guinea pigs and rabbits were housed individually; the guinea pigs in plastic cages with wood chip bedding, and the rabbits in wire-bottom, stainless-steel cages. Water and feed (Purina Rabbit Chow #5320 and Purina Formulab #5025 for guinea pigs) were available *ad libitum*. Animal room temperatures were maintained at 21° to 25°C and the light/dark cycle was set at 12-h intervals.

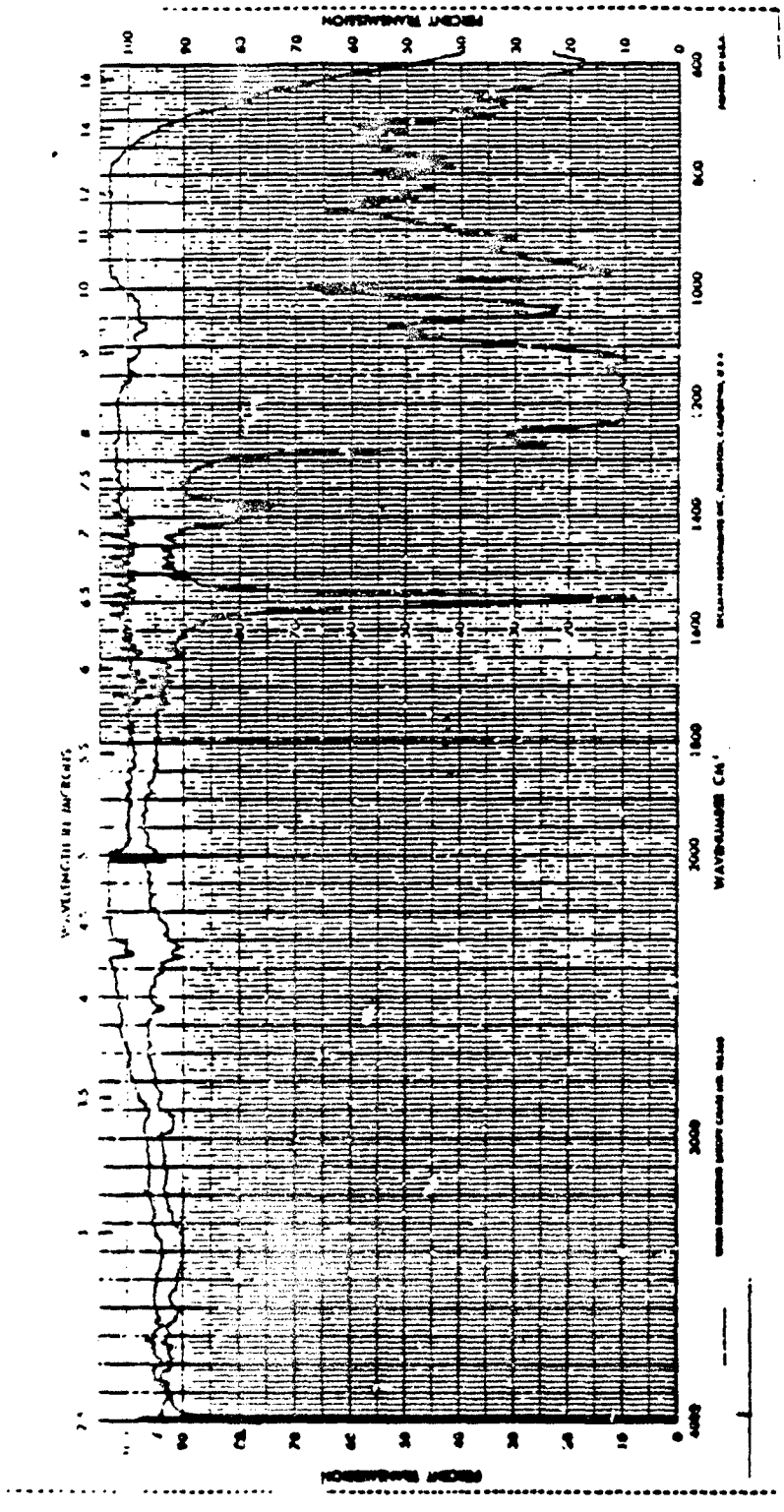


Figure 1. Infrared spectrum of T17-2 (Lot #16-7.1) generated using a Beckman Acculab 4 infrared spectrophotometer.

SECTION 3

EXPERIMENTAL APPROACH

EYE IRRITATION

Nine NZW female rabbits, weighing between 2 and 3 kg, were examined with fluorescein stain prior to use to ensure absence of lesions or injury. A topical anesthetic (Ophthetic, Allergan Pharmaceuticals, Inc., Irvine, CA; Proparacaine HCl 0.5%) was instilled in the eyes of all rabbits, treated and control, approximately 2 min prior to application of the test material. Approximately one tenth of a mL of the test material was applied to one eye of each of the nine rabbits. The opposite eye was left untreated and served as the control. The treated eye of three rabbits was flushed with lukewarm deionized water for 1 min starting 30 sec after instillation. The eyes of the remaining six were not flushed. Examinations for gross signs of eye irritation were made at 1, 24, 48, and 72 h following treatment. Irritation was scored according to the method of Draize(1944)(**Appendix A**), in which the total score for the eye was the sum of all scores obtained for the cornea, iris, and conjunctivae.

SKIN IRRITATION

Six NZW female rabbits were clipped on the back and sides 24 h prior to dosing to allow for recovery of the skin from any abrasion resulting from the clipping. The test agent (approximately 0.5 mL) was applied to a designated patch area and was covered by a 3 cm square of surgical gauze two single layers thick. Strips of surgical adhesive tape held the gauze patch in place and the entire shaved area was covered with dental dam and secured with Vetrap (3M Corp., Minneapolis, MN) and adhesive tape. The patches remained in place for 4 h, then all wrappings were removed and the residual test agent wiped from the skin. Test areas were evaluated for irritation using the Draize Table (Draize, 1959; **Appendix B**) as a reference standard at 4, 24, 48, and 72 h. Total scores of the four observations for all rabbits were divided by 24 to yield a primary irritation rating which was interpreted using the National Institute for Occupational Safety and Health skin test rating (**Appendix C**).

SENSITIZATION

Prior to the start of the study, ten male guinea pigs were treated on the clipped left flank with 0.1 mL of the undiluted test material to determine the baseline irritation response. The site of the sensitization test was an area just behind the shoulder girdle. This site was clipped with an Oster^{R} animal clipper and depilated with a commercial depilatory (Surgex Hair Remover Cream, Sparta

Instrument Corp , Hayward, CA) 4 h prior to treatment. A Vetrap frame with a 1.5 x 1.5 cm opening was affixed to the guinea pig at the site of the depilated area. Approximately one-tenth of a mL of the test material was topically applied to the test area and covered with gauze, dental dam, and adhesive tape. This was done on Mondays, Wednesdays, and Fridays until a total of four sensitizing treatments were applied and evaluated. At the time of the third sensitizing treatment, 0.2 mL of a 50% aqueous dilution of Freund's adjuvant (Bacto Adjuvant Complete, Freund, Difco Laboratories, Detroit, MI) per animal was injected intradermally using two or three sites next to the test site. Following the fourth sensitizing treatment, the animals were rested for two weeks. Both flanks were then clipped and one served as control while the other was challenged with 0.1 mL of the test material. The challenge application was not occluded. The skin response at these sites was recorded at 24 and 48 h after application (scoring method in **Appendix D**). Any animal eliciting a score of two or more at the test solution challenge site at the 48-h scoring interval was rated a positive responder. The percentage of animals responding was the important factor in determining sensitization potential (**Appendix E**)

SECTION 4

RESULTS

EYE IRRITATION

The instillation of 0.1 mL of triazine T17-2 in rabbit eyes produced no corneal opacity or congestion, swelling, or discharge of the iris when test animals were observed at 1, 24, 48, or 72 h postinstillation. However, slight irritation of the conjunctivae was noted in four rabbit eyes 1 h after treatment. All signs of irritation had dissipated by 24 h (**Table 1**).

SKIN IRRITATION

Six rabbits were treated dermally with 0.5 mL of triazine T17-2. No erythema, edema, or necrosis was observed in any of the rabbits upon examination immediately following 4-h dermal contact with the test agent (**Table 2**). Subsequent observations at 24, 48, and 72 h were also negative.

SENSITIZATION

No test animals exhibited erythema or edema following the baseline response treatment of 0.1 mL test material to the shaved flank. Following 10 days of sensitization dosing and two weeks of rest, the test animals were challenged with 0.1 mL of the test material. The triazine T17-2 produced no erythema or edema at 24 or 48 h after this challenge treatment (**Table 3**).

TABLE 1. PRIMARY EYE IRRITATION SCORES FOLLOWING INSTILLATION OF TRIAZINE T17-2

Rabbit No.	Eyes Washed	Examination Time (Hours Post-treatment) 1	24	48	72
Z07	No	2	0	0	0
Z09	No	2	0	0	0
Z13	No	2	0	0	0
Z15	No	2	0	0	0
Z17	No	0	0	0	0
Z19	No	0	0	0	0
Z21	Yes	0	0	0	0
Z23	Yes	0	0	0	0
Z25	Yes	0	0	0	0

TABLE 2. PRIMARY SKIN IRRITATION SCORES FOLLOWING DERMAL CONTACT WITH TRIAZINE T17-2

Rabbit No.	Examination Time (Hours Post-treatment) 4	24	48	72
Z09	0	0	0	0
Z11	0	0	0	0
Z13	0	0	0	0
Z17	0	0	0	0
Z21	0	0	0	0
Z23	0	0	0	0

TABLE 3. SKIN SENSITIZATION TEST SCORES FOR CHALLENGE APPLICATION OF TRIAZINE T17-2

Guinea Pig No	Erythema/Eschar 24 h Post-treatment	48 h Post-treatment
01160001	0/0	0/0
01160003	0/0	0/0
01160004	0/0	0/0
01160005	0/0	0/0
01160008	0/0	0/0
01160009	0/0	0/0
01160010	0/0	0/0
01160011	0/0	0/0
01160014	0/0	0/0
01160015	0/0	0/0

POSITIVE RESPONDERS = 0%

CLASSIFICATION = Non-Responder

SECTION 5

DISCUSSION

Triazine T17-2 exhibited slight irritating effects to conjunctival tissue of rabbit eyes. Remarkable irritating effects were not observed as a result of exposure to intact skin of rabbits, nor did the repeat application elicit a sensitization reaction in guinea pigs.

Table 4 is a summary of these acute test results with Triazine T17-2. Under conditions of these tests, Triazine T17-2 did not demonstrate an irritation or sensitization hazard.

TABLE 4. SUMMARY OF ACUTE TEST RESULTS FOR TRIAZINE T17-2

Eye Irritation	Skin Irritation	Sensitization
Slight	Negative	Negative

SECTION 6

REFERENCES

Draize, J.H., G. Woodard, and H.O. Calvery. 1944. Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes. *J. Pharm. Exp. Therap.* 32:377-390.

Draize, J.H. 1959. *Dermal Toxicity, Appraisal of the Safety of Chemicals in Food, Drugs, and Cosmetics.* The Staff of the Division of Pharmacology of the Federal Food and Drug Administration, Austin, Texas. The Editorial Committee of the Associates of Food and Drug Officials of the United States.

Murphy, Sheldon D. 1980. Pesticides In Toxicology. *The Basic Sciences of Poisons* (J. Doull, C. Klaassen and M. Amdur, Eds.). 2nd Ed., p. 392.

Reinhardt, Charles F. and Mavis R. Brittelli. 1981 Heterocyclic and Miscellaneous Compounds. *Patty's Industrial Hygiene and Toxicology,* 3rd Ed., Vol. II A, pp. 2772-2776.

U.S. Department of Health and Human Services. 1985. *Guide for the Care and Use of Laboratory Animals* (Publication #86-23). Bethesda, MD: National Institute of Health.

U.S. Environmental Protection Agency. 1982. *Health Effects Test Guidelines* (Report No. EPA 560/682-001). Washington, DC: Office of Pesticides and Toxic Substances.

APPENDIX A

DRAIZE[a] SCALE FOR SCORING OCULAR LESIONS

Parameter		Score
1. CORNEA		
A. Opacity-degree of density (area most dense taken for reading)		
No opacity		0
Scattered or diffuse areas, 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 one-half		2
Greater than one-half, but less than three-quarters		3
Greater than three quarters, up to whole area		4
Score = A × B × 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 reaction is positive)		1
No reaction to light, hemorrhage, gross destruction (any or all of these)		2
Score = A × 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

continued

APPENDIX A (continued)

Parameter	Score
B. Chemosis	
No swelling	0
Any swelling above normal (including nictitating membrane)	1
Obvious swelling with partial eversion of lids	2
Swelling with lids about half closed	3
Swelling with lids above 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 = (A + B + C) x 2 Total Maximum =	20

The TOTAL MAXIMUM SCORE is the sum of all scores obtained for the cornea, iris, and conjunctivae.

Total Maximum Score Possible =	110

[4] Draize, J.H., G. Woodard, and H.O. Calvery. 1944. Methods for the Study of Irritation and Toxicity of Substances Applied Topically to the Skin and Mucous Membranes. *J. Pharm. Exp. Therap.* 32:377-390.

APPENDIX B

DRAIZE[a] SCALE FOR EVALUATION AND SCORING OF SKIN REACTIONS

Parameter	Score
1. ERYTHEMA	
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness)	4
2. EDEMA	
No edema	0
Very slight edema (barely perceptible)	1
Slight edema (edges of area well defined by definited raising)	2
Moderate edema (raising approx. 1 mm)	3
Severe edema (raising more than 1 mm and extending beyond area of exposure)	4
3. NECROSIS[b]	
No necrosis	0
Slight necrosis (less than one-fourth exposed area)	5
Moderate necrosis (one-fourth to one-half exposed area)	10
Severe necrosis (more than one-half exposed area)	15

[a] Draize, J.H., G. Woodard, and H.O. Calvery. 1944. Methods for the Study of Irritation and Toxicity of Substances Applied Topically to the Skin and Mucous Membranes. *J. Pharm. Exp. Therap.* 32:377-390.

[b] Necrosis, for the purpose of this scoring system, is defined as a chemical denaturation of tissue sufficiently severe to result in fibrotic replacement (scar tissue). Superficial eschar that heals without scar is not classified as necrosis.

APPENDIX C

NATIONAL INSTITUTE FOR OCCUPATIONAL SAFETY AND HEALTH INTERPRETATION OF SKIN RATINGS[a]

	Rating	Interpretation
Intact Skin	0 – 0.9	Nonirritant; probably safe for human skin contact
	1 – 1.9	Mild irritant; may be safe for use, but appropriate protective measures are recommended during contact
	2 – 4	Too irritating for human skin contact; avoid contact

[a] Campbell, K.I., E.L. George, L.L. Hale, and J.F. Stara. 1975. Dermal Irritancy of Metal Compounds. *Arch. Environ. Health.* 30:168-170.

APPENDIX D

GRADING SYSTEM[a] FOR SENSITIZATION TEST

Erythema	Edema
0 – None	0 – None
1 – Very Slight Pink	1 – Very Slight
2 – Slight Pink	2 – Slight
3 – Moderate Red	3 – Moderate
4 – Very Red	4 – Marked

[a] Toxic Hazards Research Unit grading system for sensitization test.

APPENDIX E

SCALE[a] FOR DETERMINING SENSITIZATION POTENTIAL

Sensitization Rate (%)	Grade
10	Weak
20 – 30	Mild
40 – 60	Moderate
70 – 80	Strong
90 – 100	Extreme

[a] Toxic Hazards Research Unit grading system for sensitization potential.

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QUALITY ASSURANCE

The study, 'Irritation and Sensitization Studies on T17-2,' was conducted by the NSI Technology Services Corporation, Toxic Hazards Research Unit under the guidance of the Environmental Protection Agency's Good Laboratory Practices Guidelines, 40CFR PART 792. No claim will be made that this was a 'GLP' study as no attempt was made to adhere to the strict requirements of these guidelines. The various phases of this study were inspected by members of the Quality Assurance Unit. Results of these inspections were reported directly to the Study Director at the close of each inspection.

DATE OF INSPECTION:	ITEM INSPECTED:
September 26, 1989	Animal Receipt Process
October 16-20, 1989	Skin Irritation Phase

The Quality Assurance Unit has determined by review process that this report accurately describes those methods and standard operating procedures required by the protocol and that the reported results accurately reflect the raw data obtained during the course of the study. No discrepancies were found that would alter the interpretation presented in this Final Report.

M G Schneider

M. G. Schneider
QA Coordinator
Toxic Hazards Research Unit

Date December 11, 1989