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**EVALUATION OF THE ACUTE TOXICITY, IRRITATION,
SENSITIZATION, AND SUBCHRONIC DERMAL TOXICITY
OF ANTIMONY THIOANTIMONATE LUBRICANT**

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TECHNICAL REVIEW AND APPROVAL

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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

BRUCE O. STUART, PhD
Director Toxic Hazards Division
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19. ABSTRACT (Continue on reverse if necessary and identify by block number) Antimony thioantimonate (ATA) is used in a calcium cup grease (Ca grease) lubricant in Naval operations. Tests for acute intraperitoneal toxicity, eye irritation, sensitization and subchronic dermal toxicity were conducted. Single dose IP LD ₅₀ values for male and female Sprague-Dawley rats were 445 mg/kg and 652 mg/kg, respectively. ATA instilled in New Zealand White rabbit eyes produced reversible conjunctival erythema, chemosis and discharge. Superficial corneal injury was noted with ultraviolet light examination 7 days postapplication. Ca grease or 5% ATA in Ca grease (5% ATA/grease) produced a sensitization response in guinea pigs. Repeated dermal 6 hr/day, control 5 day/week for 90 days, with either Ca grease (vehicle control), 5% ATA/grease or 0.5% ATA in calcium cup grease (0.5% ATA/grease) produced epithelial keratosis in both male and female rabbits. Female rabbits treated with these materials also demonstrated mild hepatocellular fatty change. These lesions were attributable to the Ca grease lubricant with no apparent potentiation.												
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19. By ATA content. Unexposed control rabbits did not display these tissue changes. Food consumption, body weight, and organ weights were generally unaffected by ATA exposure. No changes were noted in the electrocardiograms of rabbits repeatedly exposed to ATA lubricant. In addition, no significant ATA exposure related cardiac tissue changes were observed microscopically. Keywords: Edema, Cardiotoxicity.

PREFACE

This document constitutes the final report on the Evaluation of the Acute Toxicity, Irritation, Sensitization, and Subchronic Dermal Toxicity of Antimony Thioantimonate Lubricant. The research covered a period from September 1982 through August 1984 and was performed under Contract No. F33615-80-C-0512. M.K. Pinkerton served as technical contract monitor for the Harry G. Armstrong Aerospace Medical Research Laboratory.

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J.D. MacEwen, Ph.D. served as the Laboratory Director for the THRU of the University of California, Irvine, and as co-principal investigator with T.T. Crocker, M.D., Department of Community and Environmental Medicine. Acknowledgement is made to D.E. Uddin, C.D. Flemming, and J.L. Monroe for their significant contributions and assistance in the preparation of this report.

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INTRODUCTION

Graphite based materials are used by the U.S. Navy on aircraft carriers as lubricants for airplane catch cables. These cables are stretched across the flight deck and are snagged by the arresting hood on the tail of incoming aircraft. Performance characteristics of the graphite lubricants are adequate; however, cleanup is often difficult and the slippery deck creates a safety hazard. Although the catch cables are mechanically lubricated, use conditions frequently result in dermal exposure of the crewmen. The Navy is evaluating a new lubricant which has a basic composition of 3-5% antimony thioantimonate (ATA) in calcium cup grease #3. Dermal toxicity studies are part of the evaluation.

The only effects noted after administration of 2000 mg/kg ATA to rabbit skin were varying degrees of erythema and edema (Wolfe, 1981). Acute irritation studies conducted with ATA indicate slight edema dermally and slight reversible eye irritation in non-washed rabbit eyes (Latven, 1981, 1981a). There were no effects noted in male and female rats after oral administration of 5000 mg/kg ATA (Wolfe, 1981a).

Cardiotoxicity has been the major effect associated with antimony compounds. Among the reported effects of these materials are electrocardiographic (ECG) changes in the T-wave configuration. Schroeder et al. (1984) studied 100 patients during treatment with tartar emetic and fuadin, two trivalent antimonials used in the treatment of human schistosomiasis. Varying degrees of decreased amplitude of T waves resulting in inversion in some cases, were found in 99% of the patients. Waye et al. (1982) also studied human patients undergoing antimonial compound therapy and found dose dependent elevations of SGOT and several instances of T-Wave effects, but no correlation could be established between the two observations.

Because of the reported toxic effects with antimony compounds, it was necessary to conduct routine toxicity screening tests with ATA. Acute tests performed included intraperitoneal toxicity, eye irritation, and skin sensitization. In addition to the acute tests, a repeated dose subchronic dermal test was conducted. This test method was selected since actual exposure of humans is expected to result via repeated skin contact. For the subchronic tests, rabbits were dermally exposed to either ATA, calcium cup grease # 3 (the vehicle employed during actual use), 0.5% ATA in calcium cup grease, or 5% ATA in calcium cup grease. The 5% ATA/grease represented the material as it would be used in normal operations, while the 0.5% ATA/grease employed in the toxicity tests was specially prepared as a comparative concentration.

MATERIALS AND METHODS

Test Materials

Antimony Thioantimonate (ATA)

Manufacturer: Pennwalt Corporation
Batch No.: 5363-93-1
Physical Appearance: Burnt orange powder
Chemical Formula: SbSbS_4

Calcium Cup Grease # 3 (Ca grease)

Manufacturer: Cook's Industrial Lubricants, Inc.
Boiling Point, °F: 545
Vapor Pressure (mmHg): < 0.01
Specific Gravity: 0.9
Physical Appearance: Light amber grease
Chemical Family: Calcium soap - thickened petroleum hydrocarbons

5% Antimony Thioantimonate/grease (5% ATA/grease)

Manufacturer: Pennwalt Corporation
Batch No.: 565D-5-1
Physical Appearance: Reddish-orange grease
Chemical Family: Calcium Soap - thickened petroleum hydrocarbons with 5% SbSbS_4

0.5% Antimony Thioantimonate/grease (0.5% ATA/grease)

Manufacturer: Pennwalt Corporation
Physical Appearance: Reddish-orange grease
Chemical Family: Calcium Soap - thickened petroleum hydrocarbons with 5% SbSbS_4

Animals

Male and female Sprague-Dawley rats were obtained from Charles River Breeding Labs., Wilmington, MA. Male and female New Zealand white rabbits used for subchronic dermal studies were purchased from Plummer Rabbitry, Peebles, Ohio. New Zealand White rabbits used for the irritation studies were bred inhouse. Female guinea pigs were purchased from Murphy Breeding Labs., Plainfield, Indiana. Commercial lab chow and water were available ad libitum.

Intraperitoneal Toxicity

Agglomerates of ATA were eliminated prior to use. Appropriate amounts of the test material were suspended in distilled water and injected intraperitoneally with a syringe and large gauge needle. Dose groups

consisted of 10 male and 10 female Sprague-Dawley rats weighing 200-300 grams and 150-250 grams, respectively. Dose volumes equivalent to 1% of the animal's body weight were administered.

Mortality was recorded for 14 days after dosing. Animals were observed frequently on the day of dosing and twice daily during the 14-day observation period for signs of toxicity. Body weights were obtained at the time of dosing and at 1, 2, 4, 7, 10, and 14 days posttreatment. All animals, whether dying by sacrifice or during the test, were subjected to gross necropsy following death.

At least 4 dose levels were tested, producing mortality sufficient to calculate at 14-day LD₅₀ with 95% confidence limits using the method of Finney (1971).

Eye Irritation

One-tenth gram of finely ground ATA was applied to one eye of each of twelve albino rabbits. The opposite eye was untreated and served as a control. The treated eye of six rabbits remained unwashed. The treated eyes of the remaining six rabbits were irrigated for one minute with lukewarm water starting no sooner than 20-30 seconds after instillation. Examination for gross signs of irritation were made at 1, 3, 7, 10, and 13 days postapplication. Scoring of irritative effects was according to the method of Draize (1959) in which corneal, iris, and conjunctival effects were scored separately.

Sensitization - Guinea Pigs

Ten female albino guinea pigs, Hartley strain, six to eight weeks of age, were used for each material (ATA and 5% ATA/grease).

ATA was suspended in liquid petrolatum to a final concentration of 10% while the 5% ATA grease formulation was applied undiluted. An area on the back of each animal directly above the forelegs was clipped with electric clippers and chemically depilated with a commercial depilatory ('Neet') on the morning of the first insult exposure. At each application, 0.1 g of test material was applied to this area on a 1/2" cotton gauze square, covered with dental dam, and held in place with adhesive tape. The first insult patch was allowed to remain in place for two days, then removed, and a second application of 0.1 g was made. Two days later, this patch was removed, a total of 0.2 mL per animal of Freund's^a 50% adjuvant was injected intradermally, using 2 or 3 points adjacent to the insult site, then a new patch of 0.1 g of the test material was applied. On the third day after this application, the patch was removed and a new patch of 0.1 g of the test material applied. The last patch was removed two days later, and the animals were allowed to rest for two weeks. Each time the insult patches were removed, the condition of the skin at the application site was evaluated and recorded. When the last patch was removed, the toes of the

^a Bacto Adjuvant Complete, Freund, Difco Laboratories, Detroit, Michigan.

hind feet of each animal were taped to prevent the animal from scratching the irritated area.

After the two-week rest period, both flanks of the animals were clipped and challenged on one side with the test material and the vehicle, if any, on the other flank. The challenge applications were not occluded. The skin responses at these sites were recorded at 24 and 48 hours after application. Any animal showing measureable erythema and/or edema at the test solution challenge site was rated as a positive responder.

Subchronic Dermal Toxicity

Groups of 10 male and 10 female New Zealand white rabbits weighing between 2-3 kg received occluded applications on week days (normal working days) for 13 consecutive weeks. Due to space limitations, male and female rabbits were tested at different time intervals. The male rabbits were tested first. Occlusion lasted for 6 hours daily. Hair was carefully clipped from the backs of the animals as necessary during the 13 week study. The test materials were weighed onto 4" square gauze patches which were then placed over the clipped areas. The entire trunk of the animal was then wrapped with polyethylene plastic wrap held in place by 2" surgical tape. After removal of the wrapping, the skin was wiped (not washed) with paper towels in order to remove excess material.

Dermal irritation scores (draize, 1959) were recorded daily for each animal prior to application of the test material. Initially, the exposed skin of 5 animals from each group was abraded weekly. However, this practice was terminated after 2 weeks when the animals began to exhibit skin irritation.

The treatment groups received Ca grease (vehicle control), 5% ATA/grease, or 0.5% ATA/grease. An untreated negative control group was also maintained. All materials were applied at a dose of 2 g test material/kg of body weight. The 2 g/kg dose level was selected since it is the upper level cut-off value specified in EPA acute dermal guidelines (EPA 560/6-82-001).

Male rabbit body weights were obtained daily just prior to dosing during the initial three weeks of exposure. Subsequently, body weights were measured twice weekly. Female rabbit body weights were obtained twice each week throughout the study. Individual male rabbit food consumption was measured daily during the 13 week study, while individual food consumption for female rabbits was measured during four randomly selected week long intervals.

Prior to the onset of the study, blood was drawn from the rabbits via cardiac puncture for hematologic and clinical chemistry preexposure determinations (Table 1). Blood was also sampled from five male rabbits from the unexposed control, 0.5% ATA/grease, and 5% ATA/grease groups after approximately 4 weeks of treatment. All male and female rabbits were again bled at exposure termination. Preexposure electrocardiograms (ECG) were obtained from all rabbits preexposure and at exposure termination. ECG's were also measured on male rabbits after approximately 3 and 7 weeks on test.

TABLE 1. BLOOD TESTS CONDUCTED ON RABBITS EXPOSED
DERMALLY TO ATA

Hematocrit	Total Protein
Hemoglobin	Albumin
RBC	BUN
WBC	Bilirubin
Differentials	Serum Alkaline Phosphatase
SGPT	Creatinine
SGOT	Glucose
Sodium	Calcium
Potassium	Cholesterol

In order to accommodate the necropsy of the rabbits at the termination of the 13-week study, it was necessary to initiate the exposure of the 5% ATA/grease and 0.5% ATA/grease groups one day later than the unexposed control and Ca' grease groups. This same staggered schedule was followed at termination with all groups receiving a full 13 week exposure at which time all surviving animals were sacrificed for necropsy. Rabbits were fasted approximately 12 hours prior to necropsy. Heart, liver, kidney, and brain weights were obtained at the scheduled necropsy. The tissues shown in Table 2 were fixed histopathologic evaluation.

TABLE 2. TISSUES SAMPLED FOR HISTOPATHOLOGIC EXAMINATION

Liver	Thyroid
Adrenals	Brain
Kidney	Skin
Heart	Gross lesions
Testes	

Body weights were analyzed by a repeated measure design using Scheffe's pairwise comparisons. Blood test results and organ weights were analyzed by a two factorial ANOVA. An overall chi-square test and linear trend test were performed on the histopathologic incidence data. Lesions demonstrating statistically significant values were further analyzed with a Yates corrected chi-square or a Fisher Exact test where appropriate. In all cases, 0.05 was accepted as the level of significance.

RESULTS

Intraperitoneal Toxicity

The calculated single dose intraperitoneal LD₅₀ values for male and female rats exposed to ATA are shown in Table 3. The values obtained for male and female rats were similar, with no significant sex difference indicated. Signs of toxicity exhibited by the rats receiving ATA included

lethargy and diarrhea. Body weight loss was also noted up to four days posttreatment in all groups exposed to ATA intraperitoneally (Table 4). Group body weights measured one week postexposure or later demonstrated gradual weight increase. Mortality generally resulted within the first two days following exposure. However, death as late as 7 to 10 days postexposure did occur at a few of the dose levels tested. Gross necropsy of the rats revealed adhesions between abdominal organs. Swollen livers with orange nodules were frequently noted.

TABLE 3. ATA SINGLE DOSE INTRAPERITONEAL LD₅₀ VALUES
FOR MALE AND FEMALE SPRAGUE-DAWLEY RATS

MALE RATS		FEMALE RATS	
Dose (mg/kg)	14-Day Mortality (No. Dead/No. Dosed)	Dose (mg/kg)	14-Day Mortality (No. Dead/No. Dosed)
125	1/10 ^a	400	0/10
250	0/10	500	7/10
350	4/10	630	6/10
500	5/10	794	7/10
750	10/10	1000	9/10
		1260	6/10

LD₅₀ = 445 (mg/kg)
95% CL = 383-539 (mg/kg)

LD₅₀ = 652 (mg/kg)
95% CL = 305-1707 (mg/kg)

^a Excluded from LD₅₀ calculation

Eye Irritation

The results of the eye irritation tests are shown in Table 5. ATA was found to be a reversible eye irritant. The irritation that was present 24 hours after instillation of ATA consisted primarily of conjunctival erythema, chemosis, and discharge. Mild vascular distension was also noted in many of the rabbits. The reactions to ATA tended to be slightly less severe in the group with eyes washed after instillation. Examination of the eyes at 7 days postexposure indicated very mild erythema still present in several of the rabbits. Corneal opacity was not observable with the unaided eye but superficial injury did appear in four rabbits when stained with fluorescein and examined with ultraviolet light at day 7. All signs of irritation were cleared by 13 days posttreatment.

TABLE 4. BODY WEIGHTS (G)^a OF MALE AND FEMALE SPRAGUE-DAWLEY RATS GIVEN A SINGLE INTRAPERITONEAL INJECTION OF ATA

Sex	DOSE (mg/kg)	Time (Day)						
		0	1	2	4	7	10	14
M	125	220 ± 9(10)	206 ± 10(10)	202 ± 13(9)	211 ± 16(9)	229 ± 16(9)	251 ± 16(9)	274 ± 17(9)
	250	227 ± 9(10)	211 ± 8(10)	205 ± 9(10)	211 ± 20(10)	228 ± 19(10)	249 ± 21(10)	275 ± 21(10)
	350	273 ± 12(10)	255 ± 14(9)	248 ± 22(7)	241 ± 34(7)	257 ± 47(7)	285 ± 27(6)	305 ± 42(6)
	500	243 ± 5(10)	226 ± 6(8)	218 ± 8(6)	215 ± 14(6)	223 ± 27(6)	232 ± 19(5)	253 ± 16(5)
	750	280 ± 14(10)	261 ± 12(7)	254 ± 13(3)	b	b	b	b
F	400	171 ± 11(10)	160 ± 8(10)	156 ± 10(10)	170 ± 13(10)	181 ± 11(10)	195 ± 12(10)	209 ± 14(10)
	500	178 ± 13(10)	c	153 ± 9(3)	165 ± 11(3)	181 ± 8(3)	187 ± 7(3)	202 ± 12(3)
	630	168 ± 9(10)	162 ± 7(5)	156 ± 3(4)	160 ± 6(4)	185 ± 10(4)	185 ± 4(4)	196 ± 11(4)
	794	175 ± 11(10)	c	156 ± 14(3)	155 ± 8(3)	181 ± 17(3)	198 ± 38(3)	201 ± 21(3)
	1000	163 ± 9(10)	159 ± 8(10)	154 ± 12(4)	134 ± 28(3)	173 ± -(1)	183 ± -(1)	198 ± -(1)
	1260	161 ± 14(10)	159 ± 14(9)	157 ± 16(7)	c	171 ± 21(4)	174 ± 33(4)	173 ± 44(4)

^a Mean ± SD (N).

^b Total mortality.

^c Not measured.

TABLE 5. PRIMARY EYE IRRITATION SCORES ^a OF RABBITS TREATED WITH ATA

Animal No.	Sex	Eye Washed	Examination Time (Day)				
			1	3	7	10	13
00	F	Yes	12	11	4	4	0
05	F	Yes	20	13	9	0	0
05	M	Yes	10	13	0	0	0
06	F	Yes	15	2	0	0	0
07	M	Yes	25	15	0	0	0
08	F	Yes	14	4	5 ^b	0	0
02	M	No	20	11	0	0	0
03	M	No	25	17	7	2	0
06	M	No	25	17	0 ^b	7	0
E96	M	No	20	25	7 ^b	2	0
E98	M	No	18	8	7 ^b	0	0
F00	M	No	25	12	4	0	0

^a Total score, maximum = 110.

^b Corneal injury.

Skin Sensitization

Results of the guinea pig skin sensitization tests with ATA and 5% ATA/grease are shown in Table 6. ATA demonstrated no sensitization

potential. The 5% ATA/grease material did produce mild to moderate erythema in all of the guinea pigs examined at 24 and 48 hours postchallenge. These results suggested the possibility that the calcium cup grease was responsible for the sensitization reaction noted in the animals treated with the 5% ATA/grease mixture. To examine this hypothesis, the sensitization test was reconducted with calcium cup grease and 5% ATA/grease as the test materials. Following the completion of the 48 hour evaluation at the normal termination of the study, the guinea pigs were cross-challenged with either ATA (19% ATA in petrolatum), 5% ATA/grease, or calcium cup grease (Ca grease). Examinations were then made at 24 and 48 hours following the rechallenge. The results of these tests are shown in Table 7. Similar to the results of the first study with 5% ATA/grease, all 10 guinea pigs rechallenged with 5% ATA/grease demonstrated sensitization response. Seven of the ten guinea pigs sensitized with Ca grease demonstrated a response at 24 hrs. postchallenge., while nine of these animals were considered positive responders at 48 hours postchallenge. Application of ATA to animals previously exposed to either Ca grease or 5% ATA/grease failed to elicit a response. However, cross challenging animals with either Ca grease or 5% ATA/grease produced reactions in all cases.

TABLE 6. SKIN SENSITIZATION TEST RESULTS OF GUINEA PIGS
TREATED WITH EITHER ATA OR 5% ATA/GREASE

Animal No.	ATA		Animal No.	5% ATA/grease	
	24 hrs.	48 hrs.		24 hrs.	48 hrs.
00	0 ^a	0	00	2	1
01	0	0	01	2	1
02	0	0	02	1	2
03	0	0	03	1	2
04	0	0	04	1	3
05	0	0	05	1	1
06	0	0	06	2	2
07	0	0	07	3	3
08	0	0	08	1	1
09	0	0	09	2	2

^a Erythema score based on a scale of 0-4, with 4 being most severe.

Subchronic Dermal Toxicity

There were no deaths in the male rabbits in the unexposed, 0.5% ATA/grease, or 5% ATA/grease groups. One male rabbit from the Ca/grease test group died after approximately one month of treatment. Gross necropsy of the rabbit did not establish a cause of death. A second male rabbit from the Ca grease group demonstrated weight loss and reduced food consumption after 6 weeks of treatment. Several large palpable subcutaneous masses were present on the rabbit. The rabbit was sacrificed in a moribund condition.

Upon necropsy the masses were found to be abscesses. Bacteriological cultures of the lesions revealed Pasturella Multocida.

TABLE 7. SKIN SENSITIZATION TEST RESULTS OF GUINEA PIGS TREATED WITH CA GREASE OR 5% ATA/GREASE AND RECHALLENGED WITH EITHER ATA, CA GREASE OR 5% ATA/GREASE

Animal Number	Sensitization Material	Reaction Score		Rechallenge Material	Reaction Score	
		24 hrs.	48 hrs.		24 hrs.	48 hrs.
01	Ca grease	1 ^a	1	ATA	0	0
02	Ca grease	0	0	ATA	0	0
03	Ca grease	0	1	ATA	0	0
04	Ca grease	1	1	5% ATA/grease	1	1
05	Ca grease	1	1	5% ATA/grease	1	1
06	Ca grease	1	1	5% ATA/grease	1	2
07	Ca grease	1	1	5% ATA/grease	1	2
08	Ca grease	1	1	5% ATA/grease	1	2
09	Ca grease	1	1	5% ATA/grease	2	2
10	Ca grease	0	1	5% ATA/grease	1	2
15	5% ATA/grease	1	2	ATA	0	0
16	5% ATA/grease	2	2	ATA	0	0
17	5% ATA/grease	1	1	ATA	0	0
18	5% ATA/grease	1	1	Ca grease	1	2
19	5% ATA/grease	1	1	Ca grease	2	2
20	5% ATA/grease	1	1	Ca grease	2	2
23	5% ATA/grease	1	1	Ca grease	2	2
24	5% ATA/grease	2	1	Ca grease	2	2
25	5% ATA/grease	2	2	Ca grease	1	2
26	5% ATA/grease	1	2	Ca grease	2	2

^a Erythema score based on a scale of 0-4, with 4 being most severe.

One female rabbit treated with 0.5% ATA/grease received an eye injury and was subsequently sacrificed in a moribund condition approximately mid-way through the exposure period.

Many of the rabbits from all treatment groups, including the Ca grease group, exhibited signs of skin irritation. The effects were noted during the second week of treatment and generally remained throughout the 13 weeks of exposure. The irritation appeared to be primarily superficial excoriation with mild subcutaneous edema. A general thickening of the skin with occasional minor erythema developed in most of the male or female rabbits treated with either of the two ATA formulations or Ca grease. The edema was considered to be mild to moderate in most cases.

Body weights of male and female rabbits are presented in Figures 1 and 2, respectively. Male rabbit body weight curves were parallel with respect to time, and no differences were detectable at the 0.5 level of significance. Female rabbit body weight curves were also parallel during the course of the study, and the only effect noted was that the Ca grease group weighed significantly less ($p < 0.05$) than the control group.

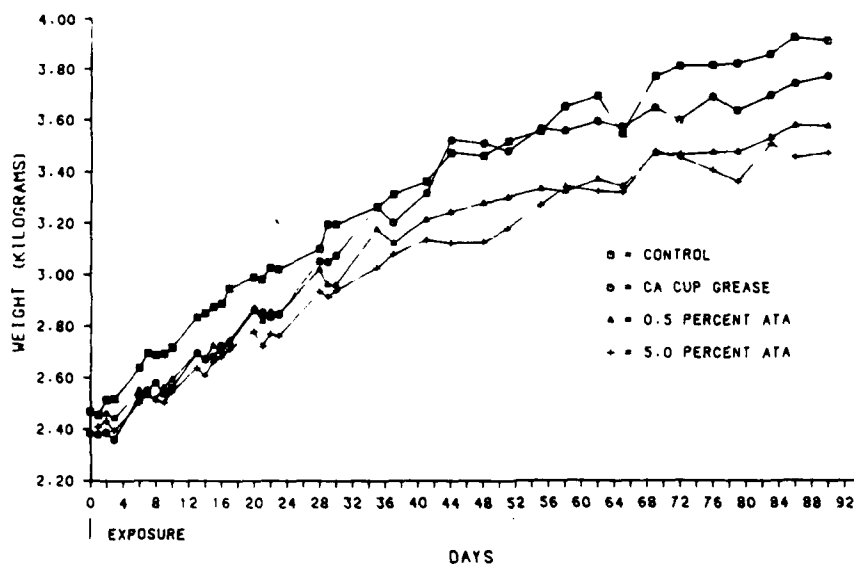


Figure 1. Effect of 90-day dermal exposure to ATA on male rabbit body weight.

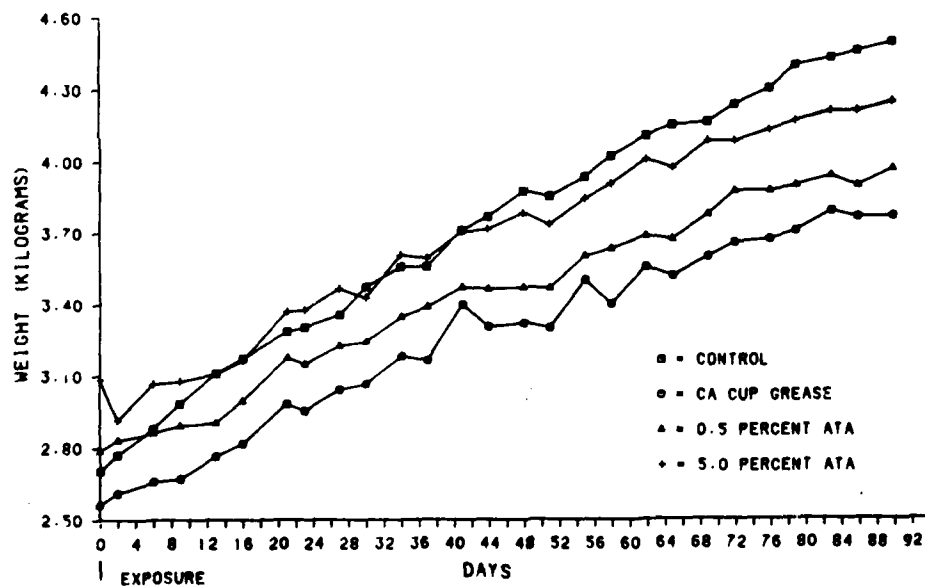


Figure 2. Effect of 90-day dermal exposure to ATA on female rabbit body weight.

Food consumption data for male and female rabbits are shown in Table 8. For purposes of statistical evaluation of the male rabbit food consumption data, four two week measurement periods were selected to correspond with the intervals measured during the female rabbit study. Male rabbits treated with ATA or Ca grease generally consumed more food than controls; however, no dose response relationship was apparent between the two ATA exposure groups. No statistically significant differences ($p < 0.05$) were noted in the food consumption of female rabbits compared to unexposed controls.

TABLE 8. FOOD CONSUMPTION (G/DAY)^a OF RABBITS EXPOSED DERMALLY TO ATA

Sex	Group	Time (Week)			
		2	6	9	13
M	Control	167 ± 11	161 ± 10	164 ± 8	146 ± 9
	Ca grease	157 ± 8	215 ± 17 ^b	194 ± 18	227 ± 14 ^b
	0.5% ATA/grease	169 ± 9	198 ± 14 ^b	194 ± 15	181 ± 19
	5% ATA/grease	153 ± 10	193 ± 11	178 ± 16	200 ± 17 ^b
F	Control	154 ± 6	154 ± 9	156 ± 7	161 ± 9
	Ca grease	150 ± 11	158 ± 8	147 ± 10	122 ± 11
	0.5% ATA/grease	164 ± 10	164 ± 10	160 ± 12	138 ± 13
	5% ATA/grease	167 ± 13	168 ± 12	158 ± 12	149 ± 18

^a Mean ± SE, N = 8 to 10 samples/group.

^b Different from control, $p < 0.05$.

The results of electrocardiogram tests with male and female rabbits did not indicate any exposure-related effects on the P interval, PR interval, QRS interval, QT interval, P amplitude, mean electrical axis, or heart rate. The T-wave amplitude values measured during the study are shown in Table 9. No significant alterations in T-wave amplitude were noted in male rabbits examined periodically during the study nor in female rabbits examined after 13 weeks of exposure.

TABLE 9. ELECTROCARDIOGRAM T-WAVE AMPLITUDE VALUES^a OF RABBITS EXPOSED DERMALLY TO ATA FOR 13 WEEKS

Sex	Time (Wk)	T-Wave Amplitude (mV)			
		Control	Ca Grease	0.5% ATA/grease	5% ATA/grease
M	Pre	0.25 ± 0.03 (10)	0.30 ± 0.04 (8)	0.26 ± 0.05 (9)	0.20 ± 0.03 (9)
	3	0.18 ± 0.03 (9)	0.27 ± 0.07 (7)	0.15 ± 0.03 (8)	0.13 ± 0.02 (8)
	7	0.14 ± 0.02 (9)	0.18 ± 0.03 (7)	0.16 ± 0.03 (9)	0.14 ± 0.02 (9)
	12	0.13 ± 0.01 (9)	0.11 ± 0.02 (6)	0.11 ± 0.02 (7)	0.11 ± 0.01 (9)
F	13	0.17 ± 0.02 (8)	0.16 ± 0.02 (10)	0.14 ± 0.02 (9)	0.15 ± 0.02 (10)

^a Mean ± SE (N).

Organ weights measured at exposure termination are shown in Table 10. Significantly ($p < 0.05$) increased liver/body weight ratios were found in the groups of female rabbits treated with Ca grease or either of the ATA formulations. Although not significant at $p < 0.05$, increased relative liver weight was also seen in the exposed male rabbits. There was no indication of any dose response relationship in this effect on male and female rabbits, and it was notable that the liver weights of the ATA treated groups were comparable to the liver weights of the Ca grease group. No significant weight effects were seen in the heart, kidney, or brain weights of rabbits treated with the ATA.

Hematology and clinical chemistry examinations conducted on male rabbits at 4 and 13 weeks of exposure and on female rabbits at 13 weeks of exposure did not indicate any definite exposure related changes. Tables 11 and 12 show results for clinical chemistry assays done on male and female rabbits after the 90-day study was completed. Values obtained for the various groups were within normal species variation and were generally consistent with the values established during the preexposure examination.

TABLE 10. ORGAN WEIGHTS^a OF RABBITS EXPOSED DERMALLY TO ATA FOR 13 WEEKS

Sex	Group	Fasted Body Wt. (Kg)	Organ Weight (gm)			
			Heart	Liver	Kidney	Brain
M	Control	3.95 ± 0.13	9.03 ± 0.87	105.53 ± 5.12	19.21 ± 0.41	9.27 ± 0.19
	Ca grease	3.69 ± 0.12	8.88 ± 0.88	110.22 ± 6.07	19.92 ± 1.39	9.68 ± 0.21
	0.5% ATA/grease	3.51 ± 0.12	9.59 ± 1.18	110.28 ± 4.75	19.08 ± 1.14	9.13 ± 0.55
	5% ATA/grease	3.41 ± 0.18	9.41 ± 1.02	105.47 ± 6.70	15.48 ± 1.01 ^b	9.07 ± 0.14
F	Control	4.49 ± 0.13	8.88 ± 1.02	103.78 ± 5.17	19.95 ± 1.03	9.03 ± 0.17
	Ca grease	3.76 ± 0.15 ^c	7.54 ± 0.23	110.42 ± 3.81	18.57 ± 0.89	9.11 ± 0.23
	0.5% ATA/grease	3.96 ± 0.14	7.48 ± 0.47	117.26 ± 6.48	19.09 ± 0.79	8.71 ± 0.17
	5% ATA/grease	4.24 ± 0.23	8.57 ± 0.60	119.63 ± 9.29	21.04 ± 1.35	9.38 ± 0.24

Sex	Group	Fasted Body Wt. (Kg)	Relative Organ Weight (% of Body)			
			Heart	Liver	Kidney	Brain
M	Control	3.95 ± 0.13	0.23 ± 0.03	2.68 ± 0.14	0.49 ± 0.02	0.24 ± 0.01
	Ca grease	3.69 ± 0.12	0.24 ± 0.02	2.99 ± 0.13	0.54 ± 0.03	0.26 ± 0.01
	0.5% ATA/grease	3.51 ± 0.12	0.27 ± 0.03	3.16 ± 0.14	0.55 ± 0.03	0.26 ± 0.02
	5% ATA/grease	3.41 ± 0.18	0.28 ± 0.03	3.10 ± 0.11	0.45 ± 0.01	0.27 ± 0.02
F	Control	4.49 ± 0.13	0.20 ± 0.02	2.31 ± 0.09	0.44 ± 0.01	0.20 ± 0.01
	Ca grease	3.76 ± 0.15 ^c	0.20 ± 0.01	2.95 ± 0.08 ^c	0.50 ± 0.02	0.25 ± 0.01 ^c
	0.5% ATA/grease	3.96 ± 0.14	0.19 ± 0.01	2.97 ± 0.14 ^c	0.48 ± 0.02	0.22 ± 0.01
	5% ATA/grease	4.24 ± 0.23	0.21 ± 0.01	2.81 ± 0.12 ^c	0.49 ± 0.01	0.23 ± 0.01

^a Mean ± SE, N = 7 to 10 samples/group.

^b Different from Ca grease group, $p < 0.05$.

^c Different from Control group, $p < 0.05$.

TABLE 11. MALE RABBIT HEMATOLOGY AND CLINICAL CHEMISTRY VALUES
AFTER 90-DAY DERMAL EXPOSURE TO ATA MATERIALS

	Control	Ca Cup Grease	0.5% ATA/Ca Cup Grease	5.0% ATA/Ca Cup Grease
WBC ($\times 10^3$ cells/mm ³)	7.9 $\pm$ 0.5 (10)	7.0 $\pm$ 0.7 (8)	8.9 $\pm$ 0.6 (8)	8.2 $\pm$ 0.6 (10)
RBC ($\times 10^6$ cells/mm ³)	6.5 $\pm$ 0.1 (10)	6.2 $\pm$ 0.2 (8)	6.5 $\pm$ 0.1 (8)	6.4 $\pm$ 0.1 (10)
HGB (gm/dl)	15.0 $\pm$ 0.2 (10)	13.9 $\pm$ 0.4 (8)	14.4 $\pm$ 0.2 (8)	14.3 $\pm$ 0.1 (10)
HCT (%)	42.3 $\pm$ 0.7 (10)	39.3 $\pm$ 1.2 (8)	41.4 $\pm$ 0.7 (8)	41.6 $\pm$ 0.6 (10)
MCV (μ^3)	64.8 $\pm$ 0.5 (10)	63.5 $\pm$ 0.3 (8)	64.1 $\pm$ 0.4 (8)	64.9 $\pm$ 0.8 (10)
MCH (pg)	23.0 $\pm$ 0.3 (10)	22.5 $\pm$ 0.2 (8)	22.3 $\pm$ 0.4 (8)	22.3 $\pm$ 0.3 (10)
MCHC (gm/dl)	35.5 $\pm$ 0.2 (10)	35.4 $\pm$ 0.2 (8)	34.8 $\pm$ 0.4 (8)	34.4 $\pm$ 0.3 (10)
Glucose (mg/dl)	119.3 $\pm$ 2.0 (10)	121.5 $\pm$ 2.9 (8)	131.5 $\pm$ 3.4 (10)	133.2 $\pm$ 3.4 (10)
BUN (mg/dl)	20.0 $\pm$ 1.0 (10)	18.9 $\pm$ 1.2 (8)	23.2 $\pm$ 1.7 (10)	24.4 $\pm$ 1.4 (10) ^c
Creatinine (mg/dl)	1.4 $\pm$ 0.06 (10)	1.2 $\pm$ 0.08 (8)	1.5 $\pm$ 0.10 (10)	1.7 $\pm$ 0.08 (10) ^{bc}
Total Protein (gm/dl)	6.3 $\pm$ 0.1 (10)	6.1 $\pm$ 0.1 (8)	6.3 $\pm$ 0.1 (10)	6.2 $\pm$ 0.1 (10)
Albumin (gm/dl)	1.9 $\pm$ 0.06 (10)	1.9 $\pm$ 0.03 (8)	1.9 $\pm$ 0.06 (10)	1.9 $\pm$ 0.02 (10)
Globulin (gm/dl)	4.4 $\pm$ 0.1 (10)	4.2 $\pm$ 0.1 (8)	4.4 $\pm$ 0.1 (10) ^b	4.3 $\pm$ 0.1 (10)
A/G Ratio	0.4 $\pm$ 0.01 (10)	0.4 $\pm$ 0.01 (8)	0.4 $\pm$ 0.01 (10)	0.4 $\pm$ 0.004 (10)
SGOT (IU/L)	47.3 $\pm$ 6.1 (10)	56.0 $\pm$ 11.2 (8)	88.7 $\pm$ 12.1 (10) ^b	85.0 $\pm$ 16.7 (10)
SGPT (IU/L)	50.6 $\pm$ 4.7 (10)	68.3 $\pm$ 19.4 (8)	80.0 $\pm$ 14.4 (10)	55.2 $\pm$ 4.4 (10)
Alk. Phos. (IU/L)	121.6 $\pm$ 12.0 (10)	112.3 $\pm$ 15.0 (8)	134.6 $\pm$ 19.8 (10)	123.6 $\pm$ 18.9 (10)
Bilirubin (mg/dl)	0.10 $\pm$ 0.00 (10)	0.10 $\pm$ 0.00 (8)	0.13 $\pm$ 0.015 (10)	0.14 $\pm$ 0.016 (10)

^a Values expressed as mean $\pm$ SE (N)

^b Different from negative control, 0.01 < p < 0.05

^c Different from grease control, 0.01 < p < 0.05

TABLE 12. FEMALE RABBIT HEMATOLOGY AND CLINICAL CHEMISTRY VALUES
AFTER 90-DAY DERMAL EXPOSURE TO ATA MATERIALS

	Negative Control	Ca Cup Grease Vehicle Control	0.5% ATA/Ca Cup Grease	5% ATA Ca1 Cup Grease
WBC ($\times 10^3$ cells/mm ³)	7.4 $\pm$ 0.6 (9)	6.9 $\pm$ 0.6 (10)	8.1 $\pm$ 0.9 (8)	6.7 $\pm$ 0.5 (10)
RBC ($\times 10^6$ cells/mm ³)	5.9 $\pm$ 0.2 (9)	5.5 $\pm$ 0.2 (10)	5.6 $\pm$ 0.2 (8)	5.7 $\pm$ 0.1 (10)
HGB (gm/dl)	13.1 $\pm$ 0.3 (9)	12.1 $\pm$ 0.4 (10)	12.5 $\pm$ 0.3 (8)	12.4 $\pm$ 0.3 (10)
HCT (%)	39.0 $\pm$ 1.0 (9)	36.0 $\pm$ 1.2 (10)	37.0 $\pm$ 1.0 (8)	37.3 $\pm$ 0.9 (10)
MCV (μ^3)	66.0 $\pm$ 1.0 (9)	66.2 $\pm$ 0.8 (10)	65.5 $\pm$ 0.8 (8)	65.3 $\pm$ 0.7 (10)
MCH (pg)	22.2 $\pm$ 0.3 (9)	22.1 $\pm$ 0.3 (10)	22.2 $\pm$ 0.3 (8)	21.8 $\pm$ 0.3 (10)
MCHC (gm/dl)	33.7 $\pm$ 0.1 (9)	33.5 $\pm$ 0.2 (10)	33.8 $\pm$ 0.2 (8)	33.3 $\pm$ 0.2 (10)
Glucose (mg/dl)	126.8 $\pm$ 4.0 (9)	128.7 $\pm$ 3.6 (9)	124.0 $\pm$ 2.2 (9)	140.9 $\pm$ 14.8 (10)
BUN (mg/dl)	26.8 $\pm$ 1.3 (9)	20.4 $\pm$ 1.4 (9) ^b	22.2 $\pm$ 1.3 (9) ^{bc}	20.6 $\pm$ 1.0 (10) ^b
Creatinine (mg/dl)	1.69 $\pm$ 0.07 (9)	1.48 $\pm$ 0.06 (9)	1.51 $\pm$ 0.06 (9)	1.41 $\pm$ 0.08 (10) ^b
Total Protein (gm/dl)	6.0 $\pm$ 0.1 (9)	5.8 $\pm$ 0.2 (9)	6.1 $\pm$ 0.1 (9)	5.7 $\pm$ 0.1 (10)
Albumin (gm/dl)	2.0 $\pm$ 0.04 (9)	2.0 $\pm$ 0.03 (9)	2.0 $\pm$ 0.03 (9)	2.0 $\pm$ 0.04 (10)
Globulin (gm/dl)	4.0 $\pm$ 0.1 (9)	3.8 $\pm$ 0.2 (9)	4.1 $\pm$ 0.2 (9)	3.7 $\pm$ 0.1 (10)
A/G Ratio	0.5 $\pm$ 0.01 (9)	0.5 $\pm$ 0.02 (9)	0.5 $\pm$ 0.02 (9)	0.5 $\pm$ 0.02 (10)
SGOT (IU/L)	38.2 $\pm$ 4.4 (9)	52.0 $\pm$ 11.7 (9)	39.2 $\pm$ 3.7 (9)	37.2 $\pm$ 3.9 (10)
SGPT (IU/L)	38.9 $\pm$ 5.7 (9)	34.9 $\pm$ 3.4 (9)	35.1 $\pm$ 6.5 (9)	25.6 $\pm$ 2.7 (10)
Alk. Phos. (IU/L)	99.1 $\pm$ 14.5 (9)	105.8 $\pm$ 12.8 (9)	96.7 $\pm$ 8.9 (9)	85.2 $\pm$ 6.3 (10)
Bilirubin (mg/dl)	0.11 $\pm$ 0.01 (9)	0.10 $\pm$ 0.02 (9)	0.14 $\pm$ 0.02 (9)	0.14 $\pm$ 0.02 (10)

^b Different from negative control 0.01 < p < 0.05

^c Different from grease control 0.01 < p < 0.05

The majority of the tissue lesions observed in the rabbits were considered to be common incidental changes unassociated with ATA exposure. The most frequently occurring lesions are shown in Table 13. Rabbits receiving either ATA/grease material or Ca grease exhibited mild to moderate epidermal hyperkeratosis at the treatment site. These changes were not observed in unexposed controls or in untreated skin. Mild hepatocellular fatty change was observed in several rabbits exposed to both ATA/grease materials as well as the Ca grease alone, but no dose response relationship was apparent. No ATA-induced cardiomyopathy was observed. However, several rabbits displayed evidence of trauma associated with the cardiac puncture blood collection procedures. Granulomatous inflammation was noted in the brain tissue of five rabbits and was considered highly compatible with infection by the protozoan, *Encephalitozoon cuniculi*. This pathogen was also considered to be the causative factor for several renal lesions recorded in this study.

TABLE 13. SELECTED HISTOPATHOLOGIC LESIONS OBSERVED IN RABBITS EXPOSED DERMALLY TO ATA

Treated Skin	Male				Female			
	Control	Ca Grease	0.5% ATA/Grease	5% ATA Grease	Control	Ca Grease	0.5% ATA/Grease	5% ATA Grease
Hyperkeratosis	0/9 ^a	8/10 ^b	9/10 ^b	8/10 ^b	0/8	10/10 ^b	10/10 ^b	9/10 ^b
Hyperplasia	0/9	1/10	1/10	0/10	0/9	0/10	0/10	0/10
<u>Untreated Skin</u>								
Hyperkeratosis	0/8	0/9	0/10	0/9	0/8	0/10	0/10	0/10
<u>Liver</u>								
Fatty Change	0/9	0/10	5/10 ^b	0/10	0/9	3/10	4/10	3/10
<u>Heart</u>								
Inflammation	2/9	1/10	2/10	2/10	3/8	0/10 ^c	4/10	0/8 ^c
Fibrosis	0/9	0/10	0/10	0/10	0/8	0/10	0/10	0/8
Mineralization	0/9	0/10	0/10	0/10	0/8	1/10	0/10	1/8
<u>Brain</u>								
Granulomatous Inflammation	1/9	1/10	0/10	0/10	2/9	0/10	1/10	0/10
<u>Kidney</u>								
Inflammation	2/9	1/10	0/10	0/10	0/9	1/10	1/10	0/10
Fatty Change	2/9	0/10	1/10	0/10	0/9	0/10	0/10	0/10
Tubular Degeneration	0/9	0/10	0/10	0/10	0/9	0/10	3/10	1/10

^a Number observed/number examined.

^b Different from Control, $p < .01$.

^c Different from Control, $p < .05$.

DISCUSSION

Acute irritation tests with ATA indicated mild reversible eye irritation. These results are generally consistent with those reported by Latvin (1981); however, in the present study the recovery period was longer and corneal effects were seen.

Since the ATA was administered as a dry powder, there is a possibility that the irritation was related to mechanical abrasions of corneal surfaces as the eyelid closed and opened. The presence of corneal lesions at 7 days posttreatment, visible only with a stained preparation, suggests that surface damage resulted.

ATA produced mortality when administered intraperitoneally, with death generally resulting within the first 2 days following exposure. Sensitization tests with ATA were negative. However, it was demonstrated that there was a potential hazard related to the calcium cup grease used as the carrier for ATA. This is important since normal human contact would be to both materials in the ATA lubricant, and skin sensitization responses may develop.

Repeated dermal exposure test results also suggested that calcium cup grease exposure may present a dermal irritation hazard. All groups treated with pure calcium cup grease or ATA formulation developed persistent skin irritation. Microscopically this consisted of epidermal hyperkeratosis hyperplasia. In some instances, exfoliation of the epidermal skin layer occurred during exposure. Despite the continued presence of skin irritation, the rabbits in the treated groups demonstrated normal weight gains compared to controls. Food consumption was also unaffected by repeated exposure. Deaths occurring in the rabbits treated for 90 days were considered unrelated to ATA exposure. There was a trend toward increased liver weight, particularly evident in female rabbits, where it was associated with microscopically observable fatty change. The absence of a dose response in the liver weights as well as the equal presence of fatty change in the calcium cup grease group and ATA groups suggests the liver effects were related to calcium cup grease exposure rather than ATA. Weights of hearts, kidneys, and brains of treated animals were unaffected by exposure, and all hematology and clinical chemistry parameters examined were within normal species variation.

One of the primary effects of antimony exposure is reported to be cardiac toxicity (Goodman and Gelman, 1965; Schroeder et al., 1946; Waye et al., 1962). Principally, these effects are seen as alterations in the T-wave configuration. Repeated examination of electrocardiograms of the male rabbits during the course of the 90-day exposure failed to demonstrate any exposure-related alterations in the heart. Similar results were obtained for female rabbits examined at the conclusion of the study. Furthermore, microscopic examination of heart tissue did not identify any lesion attributable to ATA exposure.

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