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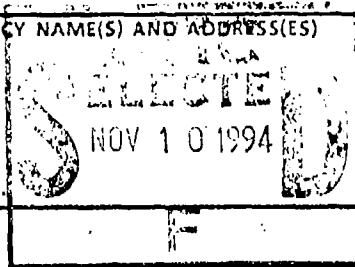
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13. ABSTRACT (Maximum 200 words)

THE OBJECT OF THIS STUDY WAS TO DETERMINE THE LEVELS OF TOXICITY OF THE LIQUIDS AND SOLIDS OF THE WASTE LAKES F AND F-1. THIS STUDY WAS REQUESTED FOLLOWING EVALUATION OF REPORTS RECEIVED THAT MALLARD DUCKS DIED AFTER ALIGHTING ON LAKES F AND F-1 AND THAT RABBITS HAD BEEN FOUND DEAD ALONG THEIR BANKS. THE STUDY FOUND THAT THE SOLIDS OF LAKE F ARE ONLY MODERATELY TOXIC, BUT THE SOLIDS OF LAKE F-1 ARE VERY TOXIC TO THE SPECIES TESTED. BASED ON THESE TESTS, LAKE F-1 MATERIAL WOULD PRESENT A HAZARD TO WILDLIFE AT LEAST 10 TIMES GREATER THAN THAT OF LAKE F MATERIAL.

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TOXICOLOGICAL EVALUATION OF THE
CONTENTS OF ROCKY MOUNTAIN
ARSENAL WASTE LAKES F AND F-1

by

Joseph W. Higgins
George F. Sell
Edward J. Owens

November 1965

Rocky Mountain Arsenal
Information Center
Commerce City, Colorado

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US Army Environmental Health Agency
CHEMICAL CENTER FOR HAZARDOUS WASTE
EVALUATION AND INVESTIGATION

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TOXICOLOGICAL EVALUATION OF THE CONTENTS OF ROCKY MOUNTAIN
ARSENAL WASTE LAKES F AND F-1

by

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Toxicology Division
Directorate of Medical Research

November 1965

US Army Edgewood Arsenal
CHEMICAL RESEARCH AND DEVELOPMENT LABORATORIES
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FOREWORD

The work described in this memorandum was authorized under Edgewood Arsenal Work Request Number 6-672-01, dated 15 December 1964 from the Directorate of Engineering and Industrial Services to the Director of Medical Research, and it was performed under Project No. LC522301A079, Non-Defense Medical Aspects of Chemical Agents (U). The work was started in December 1964 and completed in January 1965.

In conducting the research described in this report, the investigators adhered to the "Principles of Laboratory Animal Care" as established by the National Society for Medical Research.

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TOXICOLOGICAL EVALUATION OF THE CONTENTS OF ROCKY MOUNTAIN
ARSENAL WASTE LAKES F AND F-1

I. INTRODUCTION.

The object of this study was to determine the levels of toxicity of the liquids and solids of the Rocky Mountain Arsenal Waste Lakes F and F-1. This study was requested by the Directorate of Engineering and Industrial Services following evaluation of reports received from operating personnel of Rocky Mountain Arsenal that mallard ducks died after alighting on Lakes F and F-1, and rabbits had been found dead along their banks. These events indicated that a toxicity evaluation of the contents of the two lakes was required to determine their potential hazard to migrating wildfowl and local game.

II. EXPERIMENTAL PROCEDURE.

A. Materials.

Representative samples of the liquid and solid contents of both Lake F and Lake F-1 were received from Rocky Mountain Arsenal during the month of January 1965. The materials were shipped in polyethylene-lined steel drums to prevent reaction of the contents with the containers. Three hundred gallons of the liquid from each lake and 100 gallons of the solids from each lake were included in the shipment.

B. Laboratory Studies.

The ocular and cutaneous effects of the four test materials were determined in rabbits. The intragastric effects were determined in rabbits, rats, and mallard ducks that were deprived of food for 24 hr. before dosing. Selection of these routes of administration was based on the possible types of contamination that would be expected in wildlife entering the lake areas. The rat was included because extensive data are available on that species relative to organophosphate toxicity, and to provide a second rodent species for comparison with the rabbit.

The maximum ocular and cutaneous dosages that could be contained at the sites of administration were 0.5 and 5.0 gm, respectively, of the raw material. Intragastric doses of 75 gm/kg in the rabbit, 40 gm/kg in the rat, and 50 gm/kg in the mallard duck were found to be the maximum volumes of liquid that could be administered without exceeding the individual gut capacities. Under normal circumstances, these dosages are probably greater than a roving animal or transient duck might pick up during a single exposure to the lake materials.

The intragastric doses of the solids of Lakes F and F-1 were diluted with distilled water. The volume of the water and the washings of the injection apparatus and stomach tube was less than the volume of the intragastric doses of Lakes F and F-1 liquids administered to the three species tested.

All animals were held for 7 days after dosing and observed for any local or systemic toxic responses.

Gross pathological examinations were performed on a representative number of each species from every dose level at the end of the observation period and on all animals that died during the observation period. Brain cholinesterase activity of those ducks that died was also determined.

C. Field Studies.

This portion of the study was performed in three phases.

1. Phase One.

Outdoor holding pens, with adjacent tanks containing mixtures of Lake F or Lake F-1 liquids and solids, were constructed for the exposures of the test animals. These were so designed that the ducks would be held in a dry uncontaminated area until driven into the exposure tanks. For experimental purposes, the exposures were carried out side by side to compare the effects of the materials from both lakes on equal numbers of ducks of both sexes at the same air and liquid temperatures. Immediately before exposure, a group of three male and three female ducks was placed in each holding pen, the pen doors were opened, and the animals were driven into the tanks containing the two mixtures and pigeon feed. By this method, three groups were exposed to Lake F materials for 15, 45, and 120 min, and three groups to Lake F-1 materials for 15, 15, and 60 min. All animals were held for 7 days postexposure and observed for any local or systemic toxic responses. A representative number of survivors from each group were sacrificed for gross pathological examination at the end of the observation period. All animals that died during the observation period were also autopsied.

2. Phase Two.

In Phase One of the field studies, the ducks were forced into the exposure tanks. Phase Two tests were performed to ascertain whether ducks would enter the tanks without coercion. One male and one female duck were placed in each of the two holding areas, with the tank access doors open, and allowed to move about. The times to entry by the individual animals into the tanks were recorded.

3. Phase Three.

Two groups of one male and one female duck were exposed to the liquids only of Lakes F or F-1 in the same manner as was used in Phase One. Pigeon feed was thrown in the tanks. The exposure time for both groups was 2 hr; the tests were carried out simultaneously to compare the animals' responses under identical conditions of air and liquid temperatures. The same observation period and autopsy protocol were followed as in Phase One.

III. RESULTS.

A. Laboratory Studies.

The results of the ocular, cutaneous, and intragastric studies are shown in Table 1. These tests showed that doses of 0.1 and 0.5 gm/eye of Lakes F and F-1 waters and solids had no effect on the rabbit eye. There were no signs of systemic toxicity during the 7-day observation period, and no gross pathological changes were seen on autopsy.

Topical application of 1.0 and 5.0 gm of Lakes F and F-1 waters and solids to the backs of freshly clipped rabbits produced no skin irritation or any sign of systemic toxicity. Gross pathological examinations of the contaminated skin areas showed no remarkable changes. Skin texture and re-growth of the hair were normal.

Intragastric doses (50 and 75 gm/kg in the rabbit, 20 and 40 gm/kg in the rat, and 25 and 50 gm/kg in the duck) of Lake F liquids produced no deaths or any sign of systemic toxicity. Pathological examinations showed no changes from normal in the animals selected to be sacrificed on the seventh day.

The intragastric LD50 for Lake F solids in the rabbit was determined to be 1.8 (1.03-3.1) gm/kg. In the rat, doses of 2.0, 4.0, and 6.0 gm/kg produced one death in six animals at the 6.0 gm/kg dose. In the mallard duck, doses of 3.0, 4.0, 6.0, and 8.0 gm/kg produced one death in the four ducks receiving a dose of 4.0 gm/kg and in one of four ducks receiving a dose of 8.0 gm/kg. These data indicate that the intragastric LD50 for the mallard duck is more than 8.0 gm/kg and for the rat is more than 6.0 gm/kg.

Intragastric doses of 75 gm/kg of Lake F-1 liquids produced no deaths in the six rabbits tested, but one of six rabbits died after receiving a dose of 50 gm/kg. This death occurred within 1 hr after dosing. The animal exhibited no signs of systemic toxicity. Death could not be directly attributed to the test material and may have been caused by inadvertently placing a portion of the intragastric dose in the trachea. One of six rats given 40 gm/kg died. Dosages of 25 and 50 gm/kg in the mallard duck produced no deaths or signs of systemic toxicity.

TABLE 1
 RESULTS OF LABORATORY TOXICITY TESTS PERFORMED WITH LAKES F AND F-1 LIQUIDS AND SOLIDS IN THE RABBIT, RAT, AND MALLARD DUCK

Species	Average animal weight kg	Route of administration	Dose unit	Lake F				Lake F-1				
				Liquids		Solids		Liquids		Solids		
				Dose	Mortality fraction	Dose	Mortality fraction	Dose	Mortality fraction	Dose	Mortality fraction	
Rabbit	2.0	Ocular	0.1 µl/eye*	0/5	0.1	0/6	0.1	0/6	0.1	0/6	0.1	0/6
			0.5	0/6	0.5	0/6	0.5	0/6	0.5	0/6		
		Cutaneous	1.0 µl/animal	0/6	1.0	0/6	1.0	0/6	1.0	0/6	1.0	0/6
			5.0	0/6	5.0	0/6	5.0	0/6	5.0	0/6		
		Intragastric	50.0 µl/kg	0/6	50.0	0/6	50.0	1/6	50.0	0/6	0.170	0/6
			75.0	0/6	75.0	1/6	75.0	0/6	0.240	1/6	0.240	1/6
Rat	0.25	Intragastric	-	-	-	-	-	-	-	0.350	1/6	
			-	-	6.00	6/6	-	-	0.52	6/6		
Duck	1.0	Intragastric	20.0 µl/kg	0/6	2.0	0/6	20.0	0/6	0.06	0/6	0.06	0/6
			40.0	0/6	4.0	0/6	40.0	1/6	0.140	2/6		
			-	-	6.0	1/6	-	-	0.280	5/6		
			-	-	-	-	-	0.55	6/6	0.55	6/6	
			25.0 µl/kg	0/4	3.0	0/4	25.0	0/4	0.07	1/4	0.07	1/4
			50.0	0/4	4.0	1/4	50.0	0/4	0.110	2/4	0.110	2/4
			-	-	6.0	0/4	-	-	0.350	3/4	0.350	3/4
			-	-	8.0	1/4	-	-	0.700	4/4	0.700	4/4

*One eye for each animal

The intragastric LD50's for Lake F-1 solids in the rabbit, rat, and mallard duck were determined to be 0.34 (0.27-0.43), 0.17 (0.11-0.27), and 0.14 (0.08-0.25) gm/kg, respectively.

The rabbits, rats, and ducks dosed intragastrically with Lakes F and F-1 solids exhibited typical symptoms of anticholinesterase poisoning. All deaths occurred within 12 hr after dosing. Measurements of the brain cholinesterase activity in ducks that died showed decreases of between 85% and 90%. These results indicate that the cause of death in this species could be directly attributed to organophosphates or other cholinesterase inhibitors present in the solids. The duck was the only species tested for cholinesterase activity. No other pathological changes were seen in the three species tested.

A probit analysis (method of Bliss*) of the intragastric responses in the three species tested is shown in Table 2.

B. Field Studies.

1. Phase One.

The results of Phase One are shown in Table 3.

Ducks exposed to the mixture of Lake F liquids and solids appeared normal for 30 min after entering the tank. After this time, all animals appeared to be irritated and struggled violently. It was noted that their feathers had become wet and the increased weight was forcing the birds under the surface. Toxic signs exhibited postexposure were typical of those caused by anticholinesterase compounds, with vigorous convulsions occurring immediately before death. All birds in the groups exposed for 5 or 120 min died. The group exposed for 15 min did not become irritated nor struggle to leave the tank. The average time to death of the 5 ducks that died following this exposure was approximately three times longer than that recorded for the 45-min group.

Those ducks exposed to the Lake F-1 materials for 15 and 60 min showed signs of irritation after 2 min. Wetting of their feathers and severe irritation occurred at this time, causing them to struggle violently. The average times to death of the three exposure groups was 2 hr as compared with 24 hr for the Lake F exposure groups. Gross signs of poisoning were typical of those caused by anticholinesterase compounds.

* Bliss, C.I. The Statistics of Bioassay. Academic Press, Inc., New York. 1952.

TABLE 2

STATISTICAL ANALYSIS OF INTRAGASTRIC TOXICITY TESTS PERFORMED WITH LAKES F AND F-1 SOLIDS

Species	Materials	Dose unit	Mortality fraction	P	ED(P)	Statistical Analysis						
						Lower limit	Upper limit	Probit Y	Slope factors	Standard error of slope		
		gm/kg										
Rabbit	Lake F solids	.497 .994 1.988 3.964	0/6 1/6 3/6 6/6	1 16 30 50 84 99	.542 1.088 1.392 1.831 3.081 6.183	.067 .456 .808 1.076 .832 .473	4.383 2.596 2.396 3.118 11.403 80.707	-9.364	44.402	1.642		
Rabbit	Lake F-1 solids	.172 .241 .345 .518	0/6 1/6 2/6 6/6	1 16 30 50 84 99	.188 .265 .300 .344 .445 .629	.082 .180 .229 .273 .293 .256	.429 .392 .394 .432 .700 1.542	-17.533	+8.882	3.039		
Rat	Lake F-1 solids	0.55 .138 .273 .552	0/6 2/6 5/6 6/6	1 16 30 50 84 99	.061 .111 .137 .172 .268 .485	.003 .023 .057 .109 .091 .038	1.057 .431 .324 .272 .780 6.192	-6.619	+5.191	1.978		
Mallard duck	Lake F-1 solids	.069 .138 .345 .690	1/4 2/4 3/4 4/4	1 16 30 50 84 99	.015 .054 .085 .139 .356 1.253	.00002 .002 .014 .076 .054 .004	12.433 1.211 .504 .253 2.352 314.657	-.2340	+2.440	1.042		

TABLE 3

MORTALITY RESPONSE OF MALLARD DUCK EXPOSED TO MIXTURES OF LAKE F and F-1 WATERS AND SOLIDS

Material	Exposure time	Mortality fraction	Times to death
	min		hr*
Lake F Mix	120	6/6	2.5, 3.75, 4.5, 4.5, 8.25
	45	6/6	3, 4, 4.25, 7, 24, 28
	15	5/6	48, 51, 51, 74, 100
Lake F-1 Mix	60	6/6	1, 1, 1.5, 1.5, 2.25, 3.75
	15	6/6	1.5, 1.75, 2, 2, 1, 3.5
	15	6/6	2, 2.25, 2.5, 2.75, 3.75, 4.75

* From beginning of exposure

Necropsies conducted on a representative number of animals from the groups exposed to the two materials revealed no abnormalities. However, based on the symptoms of poisoning, the cause of death could be attributed to organophosphate or other anticholinesterase poisons. Brain cholinesterase levels were not determined.

2. Phase Two.

The average time to entry of the two ducks into the Lake F mixture was 5 min, and for the Lake F-1 mixture, 6 hr. The two animals in each group were removed from the tanks immediately after entry.

3. Phase Three.

Within 15 min, the feathers of the ducks exposed to Lake F-1 liquids alone had begun to wet; both animals had difficulty in staying afloat. After 30 min this same condition was seen in the ducks exposed to Lake F liquids. At the end of the 2-hr exposure period, both of the exposed animals in each group were thoroughly wet but showed no signs of irritation or toxicity. It was noted that the wetting of the feathers was much less in animals exposed to the liquids than to mixtures of the liquids and solids. It appeared doubtful, however, that the ducks from either group could have flown. After removal from the tanks, both groups appeared normal. No latent signs of toxicity, irritation, or damage to the plumage were observed during a 7-day observation period.

IV. DISCUSSION.

A. Laboratory Studies.

The waters and solids of Lakes F and F-1 were found to be innocuous to the rabbit by the ocular and percutaneous routes of administration. Doses used were the maximum quantities that could be contained at the site of administration and are considered to be at least as much as wild game would accidentally pick up along the lake shores.

Intragastric doses of the waters of Lakes F and F-1 produced no toxic responses in any of the three species tested that could be attributed to systemic poisoning.

The solids of Lake F can be considered to range from moderately to slightly toxic* by the intragastric route in the three species tested. These materials are in the toxicity category of lysol and most household soaps. By comparison, it is at least 1000, 2000, and 40,000 times less toxic, respectively, than hydrocyanic acid, GB, and VX. These values are based on average rabbit and rat intragastric LD50 values and do not include the duck.

* Gleason, M.N., Gosselin, R.E., and Hodge, H.C., Clinical Toxicology of Commercial Products, Acute Poisoning (Home and Farm). Williams and Wilkins Co., Baltimore. 1957.

The solids of Lake F-1 can be classed as being very toxic by the intragastric route in the three species tested. An average of these LD50 values, or 220 mg/kg, would place the material in the toxicity category of DDT and Dieldrin. It is approximately 50, 100, and 2000 times less toxic than hydrocyanic acid, GB, and VX, respectively, based on average rabbit and rat LD50 values.

B. Field Studies.

The waters of Lakes F and F-1 are not toxic to the three species tested. Contained in these materials, however, are wetting or degreasing agents that wet the feathers of wildfowl, resulting in their losing the ability to fly and the increased weight could cause them to drown. This situation may account for a portion of wildfowl mortalities in both lakes.

V. CONCLUSIONS.

1. The solids of Lake F are only moderately toxic, but the solids of Lake F-1 are very toxic to the three species tested. Either material would present a serious hazard to wildlife feeding along the lake shores or, in the case of ducks, from the lake bottoms. Either material may present a hazard to unprotected people not warned of the potential danger.

2. Based on these tests, Lake F-1 materials would present a hazard to wildlife at least ten times greater than that of Lake F materials.

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