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Explosive Compounds Detected in Tissue of Freshwater Mussels from Selected Streams near or on the Fort Polk Military Reservation, Louisiana, May and June 2002

INTRODUCTION

Combat training at the Joint Readiness Training Center and Fort Polk (Reservation) often involves using materials such as ammunition, propellants, projectiles, explosives, and pyrotechnics. Residues from these materials could affect the quality of surface water that drains training areas of the Reservation. Transport of these typically hydrophobic compounds can occur when contaminants are dispersed in the water or when they are adsorbed to suspended solids. Freshwater mussels are ideal integrators of stream water quality and may be useful in determining whether residues from explosive compounds are transported into surface water downstream from training areas. The mussels (1) have limited mobility in a stream, (2) filter several gallons of water daily, (3) are exposed to suspended and dissolved chemicals in a stream, and (4) have life spans that range from 10 to 100 years.

In 2002, the U.S. Geological Survey, in cooperation with the U.S. Army Joint Readiness Training Center and Fort Polk, collected freshwater mussels as part of an ongoing water-quality monitoring program. This report presents data for selected explosive compounds (table 1) analyzed in tissue from mussels collected from 16 selected streams that drain the two major training areas, the Main Post and the Peason Ridge training area, of the Reservation. This report also presents a comparison of results between a previous study (1994) and the current study (2002).

Table 1. Explosive compounds analyzed and laboratory reporting limits
[Concentrations are in milligrams per kilogram]

Explosive compound	Method 8330	Method 8321A
4-Amino-2, 6-dinitrotoluene	0.25	0.30
2-Amino-4, 6-dinitrotoluene	.25	.30
1,3-Dinitrobenzene	.25	.30
2,4-Dinitrotoluene	.25	.30
2,6-Dinitrotoluene	.25	.30
HMX	.25	.30
Nitrobenzene	.25	.30
Nitroglycerin	.50	1.20
2-Nitrotoluene	.25	.30
3-Nitrotoluene	.25	.30
4-Nitrotoluene	.25	.30
PETN	.50	1.20
RDX	.25	.30
Tetryl	.25	.30
1,3,5-Trinitrobenzene	.25	.30
2,4,6-Trinitrotoluene	.25	.30
Perchlorate	.25	.30

DESCRIPTION OF STUDY AREAS

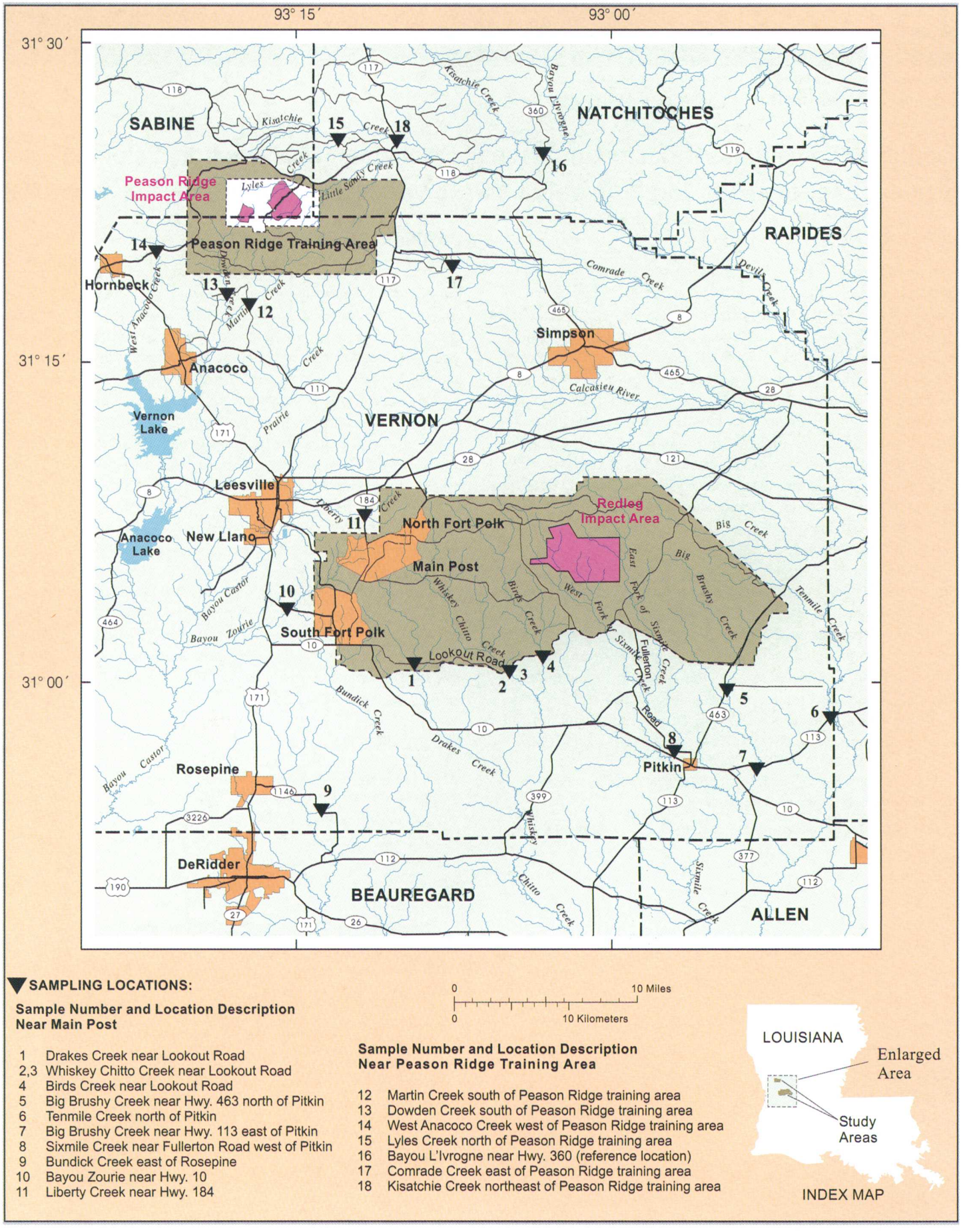
The two study areas, the Main Post and the Peason Ridge training area, are located in west-central Louisiana (fig. 1) and encompass about 199,000 acres in these two areas, 165,500 acres at the Main Post and 33,500 acres at the Peason Ridge training area. The western part of the Main Post includes a southern and northern cantonment area (Fort Polk and North Fort Polk), the east-central part includes the Redleg Impact area, and the remainder is used for military training. The northern part of the Peason Ridge training area includes two impact areas, and the remaining part is used for military training purposes.

The study areas are characterized by rolling hills and second growth timber. They are located on topographic highs and are near the headwaters of the Calcasieu River. The streams in the study areas are classified as first and second order streams that drain hilly, densely forested, piney uplands. Drainage basins are characterized by loamy soils, high runoff and infiltration, and rapid changes in stream stage during heavy rainfall. The climate in west-central Louisiana is humid subtropical, with an average annual rainfall of 58 inches and temperature of 66 degrees Fahrenheit (Elizabeth Mons, Louisiana Office of State Climatology, written commun., 2000).

PREVIOUS STUDY

In 1994, seven explosive compounds were detected in freshwater mussel tissue samples collected at 17 locations from streams draining the Main Post and the Peason Ridge training area (Hixson and others, 1994). Mussels were gut-purged in deionized water for about 48 hours and analyzed using Method 8330. Explosive compounds detected include RDX,

Mussel tissue is scraped into a collection jar for further processing and analysis.



▼ SAMPLING LOCATIONS:

Sample Number and Location Description Near Main Post

1 Drakes Creek near Lookout Road
2,3 Whiskey Chitto Creek near Lookout Road
4 Birds Creek near Lookout Road
5 Big Brushy Creek near Hwy. 463 north of Pitkin
6 Tenmile Creek north of Pitkin
7 Big Brushy Creek near Hwy. 113 east of Pitkin
8 Sixmile Creek near Fullerton Road west of Pitkin
9 Bundick Creek east of Rosepine
10 Bayou Zourie near Hwy. 10
11 Liberty Creek near Hwy. 184

0 10 Miles
0 10 Kilometers

Sample Number and Location Description Near Peason Ridge Training Area

12 Martin Creek south of Peason Ridge training area
13 Dowden Creek south of Peason Ridge training area
14 West Anacoco Creek west of Peason Ridge training area
15 Lyles Creek north of Peason Ridge training area
16 Bayou L'Ivrogne near Hwy. 360 (reference location)
17 Comrade Creek east of Peason Ridge training area
18 Kisatchie Creek northeast of Peason Ridge training area

Figure 1. Location of 18 samples collected near the Fort Polk Military Reservation, Vernon and Natchitoches Parishes, Louisiana, 1994 and 2002.

Table 3. Explosive compounds detected in freshwater mussel tissue in 1994 and 2002
[Concentrations are in milligrams per kilogram]

Sample	Compound detected	Method 8330 mg/kg	Compound detected	Method 8321A mg/kg	Method 8330 mg/kg
	1994			2002	
1	Dinitrobenzene	0.008			
	Nitrobenzene	0.030			
	(1,3,5-)Trinitrobenzene	0.975			
2	Nitrobenzene	0.080			
	3-Nitrotoluene	0.070			
	RDX	0.229			
	(1,3,5-)Trinitrobenzene	0.537			
3	2,6-Dinitrotoluene	0.112			
4	RDX	0.303	PETN	1.3	
	(1,3,5-)Trinitrobenzene	2.063			
5	Dinitrobenzene	0.109			
	(1,3,5-)Trinitrobenzene	1.160			
6	RDX	0.259	PETN	E 0.92[a]	
	3-Nitrotoluene	0.092			
	(1,3,5-)Trinitrobenzene	0.178			
8	RDX	0.148	PETN	E 0.50 [a]	
9	RDX	0.049	PETN	E 0.78[a]	
	(1,3,5-)Trinitrobenzene	1.386			
10	Nitrobenzene	0.124	PETN	E 0.47[a]	
	RDX	0.034			
11	RDX	0.212			
	(1,3,5-)Trinitrobenzene	0.346			
13	(1,3,5-)Trinitrobenzene	2.997			
14			HMX		E 0.18[a]
15	Nitrobenzene	0.021			
	2-Nitrotoluene	0.038			
16	(1,3,5-)Trinitrobenzene	1.160			
18	Dinitrobenzene	0.004			
	Nitrobenzene	0.039			
	(1,3,5-)Trinitrobenzene	0.252			

[a]Estimated concentration (<1.2 mg/kg laboratory reporting level).

the report, nor was there an explanation to support the reliability of the extremely low reported values.

Bayou L'Ivrogne was selected as the reference location for both studies because this stream receives no drainage from either the Main Post or Peason Ridge training area, and, therefore, the stream should be unaffected by military training activities (Hixson and others, 1994). There were no detected concentrations of explosive compounds at this location in 2002. In contrast, TNB was detected in mussel tissue from Bayou L'Ivrogne (sample 16) at a concentration of 1.160 mg/kg in 1994. This concentration is much higher than the LRL for TNB and greater than the reported concentrations of explosives in most of the other 1994 samples. The source of the explosive compounds at the reference location was suggested as effects from military activities during the mid- to late-1940's. However, it is unlikely that concentrations would persist until the mid-1990's, then be reduced through natural processes over the last 8 years. Therefore, contamination of mussels during handling and purging or false positives from laboratory analysis could have produced the concentrations of explosives at Bayou L'Ivrogne in 1994.

SELECTED REFERENCES

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Table 2. Mussel species and number of each species collected in 1994 and 2002 for samples collected near the Fort Polk Military Reservation, Louisiana

Samples	Species name, year sampled, and number of each species sampled																											
	Corbicula fluminea		*Fuscanaia askewi*		*Fusconaia flava*		*Lampsilis hydiana*		*Obovaria jacksonian*		*Potamilus purpuratus*		*Pyganodon gradis*		*Strophitus subvexus*		*Toxolasmus parvus*		*Trigogonia verrucosa*		*Uniomerus declivus*		*Uniomerus tetralasmus*		*Utterbackia imbecillus*		*Villosa lienosa*	
	1994	2002	1994	2002	1994	2002	1994	2002	1994	2002	1994	2002	1994	2002	1994	2002	1994	2002	1994	2002	1994	2002	1994	2002	1994	2002	1994	2002
1			31	70			24	12									1				4	2			5		5	
2			13	46			33	14	12	8						1	8	7									13	5
3	147	55																										
4			4	25			52	20									8	1									12	2
4a				22				25										1										2
5							6	44									3	1									15	5
6			4	22			26	16									10	7			6	1					12	10
7			21	17			22	30		1						2	2	1									34	23
8			80	39			12	19									3	1									2	1
9			12	32			70	28												4							6	3
10							12	4													8	6					6	
11							32	43						4			5	3			1	4					18	4
12							46	42										1									6	11
13							40	37																			4	4
14							46	43										2									15	3
15					1		10	17																			3	
16					2	1	41	46	4	13		1					1	10						3			9	3
17							38	72									5	3			1	1					11	6
18					29	23	9	22	20	13								11				1					9	18

EXPLOSIVE COMPOUNDS DETECTED, MAY AND JUNE 2002

Only two explosive compounds, PETN and HMX, were detected in six (table 3) of the 18 samples. PETN was the only explosive compound detected by Method 8321A. PETN was detected in five samples, and concentrations were expressed as estimated values (<1.2 mg/kg LRL) in four of the five samples. The concentration of PETN in sample 4 was 1.3 mg/kg, which is greater than the LRL of 1.2 mg/kg. PETN was not analyzed in the previous study. HMX was the only explosive compound detected by Method 8330. HMX was detected in one sample, sample 14, at an estimated concentration of 0.18 mg/kg, which is less than the LRL of 0.25 mg/kg. Explosive compounds were not detected in a reference sample (sample 16) collected from Bayou L'Ivrogne. The frequency and concentration of explosive compounds detected in mussel tissue indicate that low levels of explosive compounds can be found off the Main Post and the Peason Ridge training area.

Based on the results of the 2002 samples, Method 8321A appears to be more appropriate than Method 8330 for the detection of PETN in freshwater mussel tissue. PETN was detected in five samples using Method 8321A. The LRL for PETN using Method 8330 is 0.50 mg/kg. However, using Method 8330, PETN was not detected in samples 4, 6, 8, 9, and 10. The concentration of PETN in those five samples was estimated or identified by Method 8321A, and concentrations ranged from an estimated value of 0.47 to 1.3 mg/kg.

Method 8330 appears to be more appropriate than Method 8321A for the detection of HMX. HMX was detected in sample 14 using Method 8330 but not detected using Method 8321A. HMX may be more sensitive to the 8330 method, or, because of the low concentration of 0.18 mg/kg, may have been a false positive detection.

COMPARISON BETWEEN 1994 AND 2002 STUDIES

Sampling locations were identical for both the 1994 and 2002 studies (fig. 1), but differences exist between the 1994 and 2002 laboratory results (table 3). The differences might be attributed to (1) the ultra-clean sample collection and handling protocol used in the 2002 study, (2) false positives reported in the 1994 study, or (3) natural processes removing explosive compounds from the environment. Applying ultra-clean sampling techniques in the 2002 study reduced the potential for sample contamination. In 1994, 18 of the 29 detected concentrations were less than Method 8330 LRL's (Hixson and others, 1994); however, no distinction of estimated values was made in

nitrobenzene, dinitrobenzene, trinitrobenzene (TNB), 2-nitrotoluene, 3-nitrotoluene, and 2,6-dinitrotoluene. The explosive compounds were detected in 14 of the 18 freshwater mussel tissue samples with as many as four compounds detected in a sample. Eighteen of the 29 detected concentrations were less than laboratory reporting limits (LRL's) for Method 8330 (U.S. Environmental Protection Agency, 1994); however, there was no discussion of how detections below the LRL were quantified. Samples 2 and 3 were collected from the same location at Whiskey Chitto Creek. Sample 2 consisted of a composite of native mussel species, while sample 3 consisted of a composite of one nonindigenous mussel species, *Corbicula fluminea*. One explosive compound was detected in the tissue of the nonindigenous mussels, but the compound detected was different from the compounds detected in the native mussels at the same location. Explosives also were detected in a reference sample (sample 16) collected from Bayou L'Ivrogne, where no detectable concentrations were anticipated.

SAMPLE COLLECTION AND ANALYSIS

For the current study (2002), eleven samples were collected near the Main Post, six samples were collected near the Peason Ridge training area, and a reference sample was collected northeast of the Peason Ridge training area. The reference sample was collected from Bayou L'Ivrogne, which drains neither the Main Post nor the Peason Ridge training area. To avoid possible contamination by compounds washed off adjacent roads, mussels were collected upstream from bridges. If adequate numbers of mussels for analysis were not found upstream from a bridge, mussels were collected at least 600 feet downstream from that bridge.

In 2002, ultra-clean sample collection and handling protocols were used to ensure to the greatest extent possible that, if present, explosives were retained in the mussel tissues for laboratory analysis and to reduce the potential for sample contamination. Explosive compounds in surface water and bed sediments typically are low (less than 0.14 micrograms per liter in surface water and less than 0.20 mg/kg, milligrams per kilogram, in bed sediments) or not detected in streams on the Reservation (Tollett and Fendick, 1998). The mussels were not gut-purged to minimize the possibility of sample contamination during the purging process.

Ultra-clean techniques were used throughout sample preparation to collect approximately 500 grams of mussel tissue (wet weight) needed for each sample to meet the laboratory requirement of 50 grams of dry weight per sample. Depending on the species, 10 to 88 mussels were collected for each sample. Mussels were placed in methanol-rinsed, vinyl-coated steel cages during collection. Mussels were identified, weighed, and counted by species (by Malcolm F. Vidrine, Environmental Consultant, Eunice, Louisiana) for each sample (table 2). Mussels were opened with a stainless steel oyster knife inside a clear, plastic bag sample processing chamber. The soft tissue was scraped with a stainless-steel oyster knife (photograph on p. 1) and placed in a clean, labeled jar provided by the Severn Trent Laboratory in Denver, Colorado. The jars of mussel tissue were chilled and shipped to the laboratory for further processing and analysis of explosive compounds. A field-replicate sample was collected at Birds Creek (sample 4a). A laboratory split-replicate was performed on the sample from Bayou L'lvrogne (sample 16).

Dennis Jeffrey (right) and Jared Fontenot collect mussel tissue in processing chamber.

Although established standard laboratory testing methodology has been developed for the analysis of explosives in soil and water, currently (2002) no methodology exists specifically for the analysis of explosives in animal tissue. Laboratory results must be derived from the mussel tissue with a reliable and sensitive analytical method, thereby yielding concentrations of explosive compounds at the lowest reliable LRL's. Samples were analyzed by two methods, U.S. Environmental Protection Agency (USEPA) Methods 8330 and 8321A, for comparative purposes. LRL's ranged from 0.25 to 0.50 mg/kg for Method 8330 and ranged from 0.30 to 1.20 mg/kg for Method 8321A (table 1). Concentrations reported less than the LRL's for each method are estimated.

Method 8330 was developed specifically for the analysis of explosive compounds in soil and water. Method 8330 produces interference effects that can mask actual concentrations of explosives in the samples, resulting in false negative data (U.S. Environmental Protection Agency, 1994). These interference effects also lead to difficulties in distinguishing between detected compounds, resulting in false positive data. Interference effects are compounded when analyzing matrices such as animal tissue, for which the test methodology was not intended. Results from tissue analyses using Method 8330 are uncertain for concentrations at or less than the LRL's. Method 8321A was developed specifically for the analysis of dyes, organophosphates, and herbicides in soil and water (U.S. Environmental Protection Agency, 1996). Its methodology and scope are adaptable to the analysis of other non-volatile and semi-volatile compounds such as explosives. Interference effects are reduced and sensitivity is increased by the mass spectrometer detector in the instrumentation used with Method 8321A (U.S. Environmental Protection Agency, 1996).

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