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EXPERIMENTAL PATHOLOGY LABORATORIES, INC.

PATHOLOGY REPORT ON THE BRAINS FROM
MICE, HAMSTERS, AND RATS
EXPOSED TO VINYL CHLORIDE IN
IBT STUDY 663-03222

Submitted to:

Chemical Manufacturers Association
Washington, D. C. 20009

May 14, 1980

CMA 116798

PATHOLOGY REPORT ON THE BRAINS FROM MICE, HAMSTERS, AND RATS
EXPOSED TO VINYL CHLORIDE IN IBT STUDY 663-03222

I. INTRODUCTION

At the request of the Chemical Manufacturers Association, Experimental Pathology Laboratories, Inc. conducted a pathology evaluation of the available brains from mice, hamsters, and rats either exposed to vinyl chloride or used as controls in Industrial Bio-Test (IBT) Study 663-03222. This inhalation toxicology study was conducted in the IBT facilities at Decatur, Illinois, and was initiated on September 10, 1973.

The experimental design for this study is as follows:

Group	Number of Animals						Exposure Levels
	Mice		Rats		Hamsters		
	M	F	M	F	M	F	
Control	100	100	100	100	100	100	-
T1*	100	100	100	100	100	100	50 ppm Vinyl Chloride
T2*	100	100	100	100	100	100	200 ppm Vinyl Chloride
T3*	100	100	100	100	100	100	2500 ppm Vinyl Chloride
T4**	-	-	100#		-	-	2500 ppm Vinyl Chloride

*No food, water, or bedding in cages during exposure

**Food, water, and bedding to remain in cages during exposure

#All rats of same sex, either sex permitted

The protocol as amended called for the inhalation exposure of the above groups six hours per day, five days per week. Mice were to be exposed for nine months; rats and hamsters for twelve months. These groups were originally scheduled to be held for equal additional periods of time (nine or twelve months); but, subsequently it was agreed that

they would be kept for their lifetime or sacrificed if moribund. All animals were to be necropsied either at the time of death or sacrifice, and a relatively complete set of tissues was to be preserved in 10% neutral buffered formalin. These tissues were to be examined from all animals in the control, T2, and T3 groups. If exposure related lesions were detected in the organs of the T2 or T3 groups, these same organs from the T1 group were to be examined.

An EPL pathologist (Dr. William Busey) and four histology/pathology technicians traveled to IBT at Northbrook, Illinois, where the material from the study is archived. At IBT, facilities were provided for examination of the residual wet tissue, paraffin blocks, and microscope slides. In addition, facilities were provided for the histologic processing of brains recovered from the residual wet tissue.

II. MATERIALS AND METHODS

A. Residual Wet Tissue

All of the bags of residual fixed wet tissue from the mice, hamsters, and rats were examined for brain material. If brain material was discovered in the bag, it was recovered, examined grossly for any alterations, and trimmed into three to five sections for histologic processing.

B. Paraffin Blocks

All of the paraffin blocks from the mice, hamsters, and rats were examined for the presence of embedded brain material. If brains were discovered, the blocks were set aside for microtoming and staining.

C. Microscope Slides (IBT Prepared)

All of the microscope slides from the mice, hamsters, and rats were examined for brains. All brain slides were examined microscopically at IBT by the EPL pathologist.

D. Pathology Sheets

The original pathology sheets from the mice, hamsters, and rats were examined for gross descriptions of brain abnormalities. The descriptions of any brain abnormalities were recorded along with the date of death of any animal with a recorded gross or microscopic brain lesion. In addition to the gross description of any brain abnormality, the microscopic findings of the IBT pathologist were also recorded.

In the animals for which brain material was recovered from either the residual wet tissue, paraffin blocks, or microscope slides, an attempt was made to verify the animal identification. This was done by comparing the animal number and date of death recorded on the pathology sheet with the animal numbers and date of death recorded on the bags of residual wet tissue and with the animal numbers embedded in the paraffin blocks or recorded on the microscope slides.

The brains recovered from the residual wet tissue were processed, embedded, microtomed, and mounted on microscope slides at IBT. The paraffin blocks containing brain material, previously embedded by IBT, were microtomed and mounted on microscope slides. Duplicate slides were prepared from each paraffin block containing brain. One set of slides was left at IBT and the other set was returned to EPL, stained with hematoxylin and eosin, and evaluated by an EPL pathologist.

III. RESULTS

A. Number of Recovered Brains

The following table details the number of brains recovered from either the residual wet tissue or paraffin blocks previously prepared by IBT:

Number of Brains Recovered from Residual Wet Tissue
or IBT Paraffin Embedded Blocks

<u>Group</u>	<u>Mice</u>		<u>Hamsters</u>		<u>Rats</u>	
	M	F	M	F	M	F
Untreated Control	5	4	4	3	18	20
T1	13	8	8	4	15	13
T2	3	7	14	10	23	11
T3	12	14	13	6	23	17
T4	-	-	-	-	-	12

B. Pathology1. Mice and Hamsters

With the exception of focal encephalitis in Group T3 male hamster No. 2208, no gross or microscopic alterations were seen in any of the brains examined from the mice and hamsters.

2. Rats

Grossly detected changes in the brain were described at the time of necropsy by IBT in four male rats from the T3 group. These gross observations are as follows:

<u>Animal No.</u>	<u>Gross Observation</u>
641	1 cm. mass located in front of brain in skull leaving a compression in brain.
664	A gelatinous-like focus beginning on the anterior frontal half of the brain beneath the meninges compressing the adjacent brain tissue. Mid sagittal section of the brain reveals an 8 mm white firm focus involving olfactory region and compression of adjacent cerebrum.
667	A soft tan mass was found in the right frontal region in the skull cap compressing the front quarter of the brain. Mass located between the meninges and the skull cap 1 cm. in diameter.
672	Front portion of brain was gelatinous and very soft.

In addition to the above four gross brain abnormalities, the following brain with a gross abnormality was detected by EPL personnel:

<u>Animal No.</u>	<u>Gross Observation</u>
649	0.2 x 0.5 tan mass attached to the left olfactory bulb.

The detailed histologic findings for the microscopic evaluation of the brains are presented in Table II. A summary of the findings is presented in Table I.

Microscopic evaluation of the brains from the control and vinyl chloride exposed male and female rats revealed olfactory neuroblastomas (esthesioneuroblastoma, esthesioneuroepithelioma, medulloepithelioma) in five T3 males (641, 649, 664, 667, and 672), one T3 female rat (761), and one T4 female rat (2413). A single glioblastoma multiforme was detected in the brain from T2 male rat No. 412, and a single astrocytoma was detected in the brain of T2 female rat No. 566.

The histological features of the seven olfactory neuroblastomas were remarkably similar. They were characterized by a proliferation of deeply basophilic cylindrical cells with abundant cytoplasm supported by small blood vessels. Characteristic pseudo-rosettes were present along with numerous mitotic figures. In most instances these neoplasms compressed the anterior portion of the cerebrum; and, in some cases, infiltration under the meninges was evident. The glioblastoma multiforme was characterized by a proliferation of a mixture of highly pleomorphic cells containing large nuclei. Numerous giant cells and mitotic figures were also evident. The astrocytoma was characterized by a proliferation of well differentiated astrocytes infiltrating the neuropile, forming no particular structural pattern. A few mitotic figures were evident.

Scattered instances of meningoencephalitis, suppurative meningitis, encephalomalacia, and focal encephalitis were detected in a few rats from both the control and vinyl chloride exposed groups. The incidence of these inflammatory lesions was low and not related to any treatment group.

IV. DISCUSSION AND CONCLUSION

The presence of seven olfactory neuroblastomas in the brains from vinyl chloride exposed rats indicates a causal relationship between the exposure and the development of these neoplasms. Olfactory neuroblastomas rarely, if ever, occur spontaneously in the rat. Three of these neoplasms were initially diagnosed by IBT as neuroepitheliomas. Subsequent review of the slides indicates the classification of olfactory neuroblastoma to be appropriate for these neoplasms. The glioblastoma multiforme detected in T2 male rat No. 412 was also initially diagnosed as a neuroepithelioma by IBT. Subsequent evaluation of this neoplasm indicates that this lesion is more properly classified as a mixed glioma or glioblastoma multiforme.

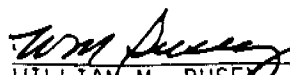
The olfactory neuroblastomas detected in these vinyl chloride exposed rats are apparently identical to the neuroblastomas reported by Maltoni in 1975,¹ and again in 1977.² In 1975, Maltoni reported at the New York Academy of Sciences Symposium on the toxicity of vinyl chloride-

¹Carcinogenicity Bioassays of Vinyl Chloride: Current Results, Maltoni, C. and Lefemine, G., Annual New York Academy of Science, Vol. 246 (1975), 195-218.

²Vinyl Chloride Carcinogenicity: An Experimental Model for Carcinogenesis Studies, Maltoni, C., Origins of Human Cancer, Cold Spring Harbor Conferences on Cell Proliferation, Vol 4, 1977.

poly vinyl chloride. The results of his BT1 experiment revealed seven neuroblastomas in rats exposed to 10,000 ppm of vinyl chloride, three neuroblastomas in rats exposed to 6,000 ppm, and five neuroblastomas in rats exposed to 2,500 ppm of vinyl chloride. In 1977, at the Cold Spring Harbor Conference on Cell Proliferation, he again reported the results of his BT1 experiment in addition to the results of his BT3 and BT7 experiments where neuroblastomas were induced in rats exposed to vinyl chloride at 10,000, 6,000, and 2,500 ppm. The findings of olfactory neuroblastomas in the T3 male and female rats and a T4 female rat confirms the findings of Maltoni of an oncogenic effect on the rat brain from vinyl chloride exposure.

The shortcomings of this study have been previously documented. Regardless of these, the rarity of this neurological neoplasm coupled with the fact that it has only been reported in rats exposed to known carcinogens indicates a relationship between their presence in this study and exposure to vinyl chloride. As mentioned previously, the finding of these neoplasms confirms previously reported results from vinyl chloride exposures by Maltoni. Since the exposure conditions in this study have not been confirmed, the levels of vinyl chloride inducing these neoplasms cannot be stated at this time. Similarly, because of the large number of missing brains from the control and exposed rats, the true incidence of these neoplasms in IBT Study 663-03222, resulting from vinyl chloride exposure, cannot be determined.


WILLIAM M. BUSER, D.V.M., Ph.D.
Pathologist

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IBT STUDY 663-03222

TABLE I

SUMMARY INCIDENCE TABLES

CMA 116807

IBT Study 663-03222
Vinyl Chloride
Male Mice

[illegible]

BT Study 663-03222
Vinyl Chloride
Female Mice

[illegible]

IBT Study 663-03222
Vinyl Chloride
Male Hamsters

[illegible]

SUMMARY INCIDENCE TABLE

IBT Study 663-03222
Vinyl Chloride
Female Hamsters

[illegible]

BT Study 663-03222
Vinyl Chloride
Male Rats

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BT Study 663-03222
Vinyl Chloride
Female Rats

[illegible]

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IBT STUDY 663-03222

TABLE II

HISTOPATHOLOGY INCIDENCE TABLES

CMA 116814

Figure 1

Untreated Control

[illegible]

-15-

E P L

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Key **P** = Present **N** = No Section
 1 = Minimal **2** = Slight
 5 = Severe/High **I** = Incomplete Section

A = Autolysis X = Not Remarkable
3 = Moderate 4 = Moderately Severe/High

CMA 116815

T1

ANIMAL
NUMBER[illegible]

-16-

E P L

Key: P = Present N = No Section
1 = Minimal 2 = Slight
5 = Severe/High I = Incomplete Section

A = Autolysis X = Not Remarkable
3 = Moderate 4 = Moderately Severe/High

CMA 116816

Figure 1

ANIMAL
NUMBER[illegible]

Key: P = Present N = No Section
1 = Minimal 2 = Slight
5 = Severe/High I = Incomplete Section

A = Autolysis X = Not Remarkable
3 = Moderate 4 = Moderately Severe/High

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CMA 116817

Figure 1

T3

[illegible]

*Missexed

-18-

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Key P = Present N = No Section
1 = Minimal 2 = Slight
5 = Severe/High I = Incomplete Section

A = Autolysis X = Not Remarkable
3 = Moderate 4 = Moderately Severe/High

Fig. 1

T1

[illegible]

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Experimental Pathology Laboratories, Inc.

Key: P = Present N = No Section
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A = Autolysis X = Not Remarkable
3 = Moderate 4 = Moderately Severe/High

Figure 1

T2

[illegible]

-21-

EPL

Key: P = Present
1 = Minimal
5 = Severe/High

N = No Section
2 = Slight
I = Incomplete Section

A = Autolysis X = Not Remarkable
3 = Moderate 4 = Moderately Severe/High

CMA 116821

Figure 1 *Effect of the concentration of the polymer on the swelling ratio of the hydrogel.*

IBT Study 663-03222
Vinyl Chloride
Male Hamsters

Untreated Control

[illegible]

-23-

E P L

Experimental Pathology Laboratories, Inc.

Key. P = Present N = No Section
1 = Minimal 2 = Slight
5 = Severe/High I = Incomplete Section

A = Autolysis X = Not Remarkable
3 = Moderate 4 = Moderately Severe/High

Fig. 1.

T3

[illegible]

IBT Study 663-03222
Vinyl Chloride
Female Hamsters

5. 1. 1

ANIMAL
NUMBER[illegible]

-27-

E P L

Experimental Pathology Laboratories, Inc

Key P = Present N = No Section
t = Minimal 2 = Slight
5 = Severe/High I = Incomplete Section

A = Autolysis X = Not Remarkable
3 = Moderate 4 = Moderately Severe/High

CMA 116827

5.1.1

Female Hamsters

T1

[illegible]

-28-

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Experimental Pathology Laboratories, Inc.

Key P = Present N = No Section
1 = Minimal 2 = Slight
5 = Severe/High I = Incomplete Section

A = Autolysis X = Not Remarkable
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CMA 116828

4. $\frac{1}{2} \times 100 = 50$

T2

[illegible]

-29-

Experimental Pathology Laboratories, Inc

A = Autolysis X = Not Remarkable
3 = Moderate 4 = Moderately Severe/High

HISTOPATHOLOGY INCIDENCE TABLE

IBT Study 663-03222
Vinyl Chloride
Female Hamsters

T3

[illegible]

-30-

E P L

Experimental Pathology Laboratories, Inc

Key **P** = Present **N** = No Section
 1 = Minimal **2** = Slight
 5 = Severe/High **I** = Incomplete Section

A = Autolysis X = Not Remarkable
3 = Moderate 4 = Moderately Severe/High

5. 10. 1.

Untreated Control

[illegible]

Key: P = Present N = No Section
1 = Minimal 2 = Slight
5 = Severe/High I = Incomplete Section

A = Autolysis X = Not Remarkable
3 = Moderate 4 = Moderately Severe/High

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CMA 116831

T1

ANIMAL
NUMBER[illegible]

-32-

EPL

Experimental Pathology Laboratories, Inc.

Key: P = Present N = No Section
1 = Minimal 2 = Slight
5 = Severe/High I = Incomplete Section

A = Autolysis X = Not Remarkable
3 = Moderate 4 = Moderately Severe/High

1. 1. 1.

T2

*Missexed

-33-

Experimental Pathology Laboratories, Inc

A = Autolysis X = Not Remarkable
3 = Moderate 4 = Moderately Severe/High

CMA 116833

IBT Study 663-03222
Vinyl Chloride
Male Rats

ANIMAL
NUMBER[illegible]

-34-

CMA 116834

EPL

Experimental Pathology Laboratories, Inc.

Key: P = Present N = No Section
1 = Minimal 2 = Slight
5 = Severe/High I = Incomplete Section

A = Autolysis X = Not Remarkable
3 = Moderate 4 = Moderately Severe/High

HISTOPATHOLOGY INCIDENCE TABLE

IBT Study 663-03222
Vinyl Chloride
Female Rats

Untreated Control

[illegible]

-35-

EPL

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Key: P = Present N = No Section
1 = Minimal 2 = Slight
5 = Severe/High I = Incomplete Section

A = Autolysis X = Not Remarkable
3 = Moderate 4 = Moderately Severe/High

CMA 116835

Keywords: *Self-esteem, self-esteem threat, self-esteem threat sensitivity, self-esteem threat sensitivity scale, self-esteem threat sensitivity scale-2*

Untreated Control - Continued

[illegible]

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Key P = Present N = No Section
 1 = Minimal 2 = Slight
 5 = Severe/High I = Incomplete Section

A = Autolysis X = Not Remarkable
3 = Moderate 4 = Moderately Severe/High

CMA 116836

Figure 1

T1

[illegible]

-37-

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Key P = Present N = No Section
1 = Minimal 2 = Slight
5 = Severe/High I = Incomplete Section

A = Autolysis X = Not Remarkable
3 = Moderate 4 = Moderately Severe/High

CMA 116837

1994, 1995, 1996, 1997, 1998, 1999, 2000, 2001, 2002, 2003, 2004, 2005, 2006, 2007, 2008, 2009, 2010, 2011, 2012, 2013, 2014, 2015, 2016, 2017, 2018, 2019, 2020, 2021, 2022, 2023, 2024, 2025, 2026, 2027, 2028, 2029, 2030, 2031, 2032, 2033, 2034, 2035, 2036, 2037, 2038, 2039, 2040, 2041, 2042, 2043, 2044, 2045, 2046, 2047, 2048, 2049, 2050, 2051, 2052, 2053, 2054, 2055, 2056, 2057, 2058, 2059, 2060, 2061, 2062, 2063, 2064, 2065, 2066, 2067, 2068, 2069, 2070, 2071, 2072, 2073, 2074, 2075, 2076, 2077, 2078, 2079, 2080, 2081, 2082, 2083, 2084, 2085, 2086, 2087, 2088, 2089, 2090, 2091, 2092, 2093, 2094, 2095, 2096, 2097, 2098, 2099, 2100, 2101, 2102, 2103, 2104, 2105, 2106, 2107, 2108, 2109, 2110, 2111, 2112, 2113, 2114, 2115, 2116, 2117, 2118, 2119, 2120, 2121, 2122, 2123, 2124, 2125, 2126, 2127, 2128, 2129, 2130, 2131, 2132, 2133, 2134, 2135, 2136, 2137, 2138, 2139, 2140, 2141, 2142, 2143, 2144, 2145, 2146, 2147, 2148, 2149, 2150, 2151, 2152, 2153, 2154, 2155, 2156, 2157, 2158, 2159, 2160, 2161, 2162, 2163, 2164, 2165, 2166, 2167, 2168, 2169, 2170, 2171, 2172, 2173, 2174, 2175, 2176, 2177, 2178, 2179, 2180, 2181, 2182, 2183, 2184, 2185, 2186, 2187, 2188, 2189, 2190, 2191, 2192, 2193, 2194, 2195, 2196, 2197, 2198, 2199, 2200, 2201, 2202, 2203, 2204, 2205, 2206, 2207, 2208, 2209, 2210, 2211, 2212, 2213, 2214, 2215, 2216, 2217, 2218, 2219, 2220, 2221, 2222, 2223, 2224, 2225, 2226, 2227, 2228, 2229, 2230, 2231, 2232, 2233, 2234, 2235, 2236, 2237, 2238, 2239, 2240, 2241, 2242, 2243, 2244, 2245, 2246, 2247, 2248, 2249, 2250, 2251, 2252, 2253, 2254, 2255, 2256, 2257, 2258, 2259, 2260, 2261, 2262, 2263, 2264, 2265, 2266, 2267, 2268, 2269, 2270, 2271, 2272, 2273, 2274, 2275, 2276, 2277, 2278, 2279, 2280, 2281, 2282, 2283, 2284, 2285, 2286, 2287, 2288, 2289, 2290, 2291, 2292, 2293, 2294, 2295, 2296, 2297, 2298, 2299, 2300, 2301, 2302, 2303, 2304, 2305, 2306, 2307, 2308, 2309, 2310, 2311, 2312, 2313, 2314, 2315, 2316, 2317, 2318, 2319, 2320, 2321, 2322, 2323, 2324, 2325, 2326, 2327, 2328, 2329, 2330, 2331, 2332, 2333, 2334, 2335, 2336, 2337, 2338, 2339, 2340, 2341, 2342, 2343, 2344, 2345, 2346, 2347, 2348, 2349, 2350, 2351, 2352, 2353, 2354, 2355, 2356, 2357, 2358, 2359, 2360, 2361, 2362, 2363, 2364, 2365, 2366, 2367, 2368, 2369, 2370, 2371, 2372, 2373, 2374, 2375, 2376, 2377, 2378, 2379, 2380, 2381, 2382, 2383, 2384, 2385, 2386, 2387, 2388, 2389, 2390, 2391, 2392, 2393, 2394, 2395, 2396, 2397, 2398, 2399, 2400, 2401, 2402, 2403, 2404, 2405, 2406, 2407, 2408, 2409, 2410, 2411, 2412, 2413, 2414, 2415, 2416, 2417, 2418, 2419, 2420, 2421, 2422, 2423, 2424, 2425, 2426, 2427, 2428, 2429, 2430, 2431, 2432, 2433, 2434, 2435, 2436, 2437, 2438, 2439, 2440, 2441, 2442, 2443, 2444, 2445, 2446, 2447, 2448, 2449, 2450, 2451, 2452, 2453, 2454, 2455, 2456, 2457, 2458, 2459, 2460, 2461, 2462, 2463, 2464, 2465, 2466, 2467, 2468, 2469, 2470, 2471, 2472, 2473, 2474, 2475, 2476, 2477, 2478, 2479, 2480, 2481, 2482, 2483, 2484, 2485, 2486, 2487, 2488, 2489, 2490, 2491, 2492, 2493, 2494, 2495, 2496, 2497, 2498, 2499, 2500, 2501, 2502, 2503, 2504, 2505, 2506, 2507, 2508, 2509, 2510, 2511, 2512, 2513, 2514, 2515, 2516, 2517, 2518, 2519, 2520, 2521, 2522, 2523, 2524, 2525, 2526, 2527, 2528, 2529, 2530, 2531, 2532, 2533, 2534, 2535, 2536, 2537, 2538, 2539, 2540, 2541, 2542, 2543, 2544, 2545, 2546, 2547, 2548, 2549, 2550, 2551, 2552, 2553, 2554, 2555, 2556, 2557, 2558, 2559, 2560, 2561, 2562, 2563, 2564, 2565, 2566, 2567, 2568, 2569, 2570, 2571, 2572, 2573, 2574, 2575, 2576, 2577, 2578, 2579, 2580, 2581, 2582, 2583, 2584, 2585, 2586, 2587, 2588, 2589, 2590, 2591, 2592, 2593, 2594, 2595, 2596, 2597, 2598, 2599, 2600, 2601, 2602, 2603, 2604, 2605, 2606, 2607, 2608, 2609, 2610, 2611, 2612, 2613, 2614, 2615, 2616, 2617, 2618, 2619, 2620, 2621, 2622, 2623, 2624, 2625, 2626, 2627, 2628, 2629, 2630, 2631, 2632, 2633, 2634, 2635, 2636, 2637, 2638, 2639, 2640, 2641, 2642, 2643, 2644, 2645, 2646, 2647, 2648, 2649, 2650, 2651, 2652, 2653, 2654, 2655, 2656, 2657, 2658, 2659, 2660, 2661, 2662, 2663, 2664, 2665, 2666, 2667, 2668, 2669, 2670, 2671, 2672, 2673, 2674, 2675, 26

T2

[illegible]

-38-

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Key: P = Present N = No Section
1 = Minimal 2 = Slight
5 = Severe/High I = Incomplete Section

A = Autolysis X = Not Remarkable
3 = Moderate 4 = Moderately Severe/High

CMA 116838

[illegible]

T3

[illegible]

4. 1. 1

T4

[illegible]

-40-

EPL

Experimental Pathology Laboratories, Inc.

Key: P = Present
1 = Minimal
5 = Severe/High

N = No Section
2 = Slight
I = Incomplete Section

A = Autolysis X = Not Remarkable
3 = Moderate 4 = Moderately Severe/High

CMA 116840