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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

DATE: November 18, 1981

SUBJECT: Addendum to Memo of June 24, 1981 - Review of Permethrin
Chronic/Oncogenicity Rat Study Submitted by the FMC Corporation

FROM: Gary J. Burin, Toxicologist *Gary J Burin*
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TO: William Burnam, Acting Chief
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JDC 11/24/81

Background Information

On June 15, 1977, the FMC Corporation submitted a combined chronic/oncogenicity study of Permethrin conducted with Long-Evans rats to the EPA. This study was originally reviewed by Martha Panitch on March 30, 1978. Dr. Panitch noted an increase in alveolar tumors in treated animals compared to controls and stated that "An oncogenic effect appears to be present, but of low potency". However, she classified the study as "Supplementary Data" based on her finding that an unacceptable number of tissue masses were not accounted for by gross or histologic examination.

Subsequent additional pathologic evaluations were performed and submitted by FMC Corporation:

1. Additional tissues were examined from masses and gross lesions that were not originally examined histologically. Slides were read by Dr. Billups of Environmental Pathology Services (Acc. No. 097421).
2. Lung tissue slides were re-examined by Dr. William Busey of Experimental Pathology Laboratories (Acc. No. 097419).

3. Remaining wet tissues and paraffin blocks of the lung were step sectioned and slides of the step-sectioned lungs were evaluated by Dr. Billups (Acc. No. 097418).

Thus, three separate examinations of the lung occurred - Drs. Busey and Billups each examined the same set of tissues and submitted separate reports and Dr. Billups examined step-sectioned lungs and submitted an additional (third) report.

In addition, FMC Corporation independently compiled and submitted the following:

4. Gross-Histological correlation of findings based on the updated histological report from Dr. Billups (Acc. No. 099964).

In May of this year, this reviewer transmitted to you a review of those submissions.

As you will recall, my findings at that time were the following;

1. The incidence of alveogenic lung tumors is significantly elevated in males treated with Permethrin compared to untreated males ($p = .016$). No tumor type other than alveogenic lung tumors is significantly elevated in either sex, although a trend was noted for pheochromocytomas in males.
2. The procedure used in the step-sectioning of lung involves a serious bias which precludes its use in the assessment of the incidence of alveogenic lung tumors. This bias probably leads to an underestimation of the number of tumors found in the step-sectioned lungs of Permethrin treated animals.
3. The confirmation of tissue masses found at the final in-life palpation through gross necropsy and histologic observation, although neither complete nor thorough, is minimally satisfactory for the purposes of this study. The follow-up of gross lesions through histologic examination can be considered to be satisfactory. This study should thus be considered "Core-Minimum".

Since that review was written, additional information has come to light which is relevant to the interpretation of the study. On August 13, 1981, FMC submitted a document which purportedly explains the bias which had been noted by this reviewer in the step-sectioning submission. The document acknowledged a serious discrepancy in the processing of lung tissues both in the original sectioning and in the step-sectioning process. As a follow-up to that submission, a joint EPA-FDA audit was conducted. During the course of the audit and the subsequent EPA investigation, this reviewer has;

1. Examined all slides of lung tissue; male and female, original and step-sectioned.
2. Interviewed a number of the histology technicians originally involved with the study and their managers.
3. Examined all available documentation and raw data relating to tissue processing. and;
4. Examined and analyzed measurements of the area of lung tissue for all male animals in this study.

Recommendation:

As a result of a thorough investigation of the processing of lung tissue in this study, it is concluded that a serious inconsistency exists in the histological processing of this study which renders both the original and step-sectioning assessments of tumor incidences inadequate. However, if an attempt to correct the inherent biases is performed, both the original and step-sectioning findings suggest an increase in tumor incidence in mid and high dose treated male animals (See Section III of Discussion below).

Discussion:

I. How the Bias Occurred

The in-life portion of this study was conducted at Biodynamics, Inc. of East Millstone, N.J. After the animals were necropsied at Biodynamics, Inc. their tissues were shipped in individual glass jars to American Histolabs of Rockville, Md. for slide preparation and it was there that the inadvertent bias was introduced into this study.

The bias was introduced due to one technician, who processed only treated animals, preparing lung tissue in a manner which was markedly different than the manner of other technicians involved in the tissue trimming, embedding and slide preparation. Although this technician has not yet been identified by name, his (or her) style of recording the number of paraffin blocks and other relevant information on each animals necropsy sheet can be readily distinguished from that of the

other 4 technicians involved in the histological phase of this study (See Attachment C, compare to Attachment D). Lung slides of animals prepared by this technician invariably correspond to those animals in which the entire lung was embedded; lung slides of animals prepared by other technicians invariably correspond to those animals which only had a small portion of lung tissue sectioned on the original slides. The animals processed by this technician are shown in Table I.

TABLE I

Male Animals Processed By An Unknown Technician*

(Animal Number)	<u>Control</u>	<u>20 ppm</u>	<u>100 ppm</u>	<u>500 ppm</u>	
	None	732-734	310-312	511-517	546-548
		739	316-317	519	550-551
		741-745	323	521	553
		747	325-328	524-525	555
		749-754	331-334	529	557-559
		756-757	336-343	532	562-565
		762-764	345	534-535	567
		766-769	347-348	537	569
			351	542	
			353	539	
			356-363	542	
			366-368	544	

*As identified by a characteristic handwriting and method of recording data on necropsy sheets (See Attachment C).

By matching these animal numbers with the lung slides shown in Attachment A, it can be seen that this technician was indeed responsible for processing all male animals which had the entire lung embedded.

The technicians identified as having worked on this study at American Histolabs have been identified as:

- Dr. Mohammed Asif
- Dr. Mohammed A. Ansari
- Dr. Khalid M. Shariff
- Dr. Ghulam Murtaza Malik
- Ms. Margie Henning

Mr. Richard Verfuert, who was identified in the letter of July 3, 1981 to FMC as having "been totally in charge and responsible for the histology portion of this study", was in fact not involved with the processing of tissues in this study. This conclusion is supported by signed affidavits from Mr. Richard Verfuert, Dr. Mohammed Asif and Mr. Kenneth Rawley (a supervisor at American Histolabs) (See Attachments E, F and G). All three of these parties agree that Dr. Mohammed Asif had primary responsibility for the trimming, microtoming and staining of tissues in this study. The contents of the letter of July 3, 1981 from Mr. Richard Verfuert must therefore be considered speculative rather than factual.

Dr. Mohammed Asif is currently employed as Chief Histologist at the International Research and Development Corporation of Matawan, Michigan. A joint EPA-FDA visit was made to IRDC on October 23, 1981 for the purpose of discussing this study with Dr. Asif. A signed affidavit obtained from Dr. Asif states that he recognized his own handwriting on several of the necropsy sheets. Furthermore, the type of handwriting which Dr. Asif identified as his own is that which is found on the vast majority of necropsy sheets (including almost every control animal). Lungs from animals processed by Dr. Asif were consistently trimmed in a manner which he described in his affidavit (See Attachment E). He could not identify the technician who processed the entire lung rather than a trimmed lung.

Discussions with the individuals involved this study and a review of all raw data and correspondence associated with the study suggests the inconsistency in tissue processing in this study was inadvertent. Evidence for this conclusion is as follows:

1. Instructions from FMC to American Histolabs regarding tissue processing were almost nil. For lung tissue, the only requirements were that two slides be made and that major bronchi be demonstrated. Thus trimming of lung tissue was neither required nor forbidden.
2. American Histolabs was not familiar with the processing of a study of this type. The Permethrin rat and mouse studies were the first oncogenicity studies which American Histolabs had received, although they have since had extensive experience with studies of this nature.
3. Technicians functioned under very little, if any, direct supervision at the time of this study.

Thus it is likely that the inconsistency in tissue processing was the result of a lack of guidance by the sponsor, poor quality control and inexperience in processing studies of this nature at the American Histolabs.

II. The Problem With The Step-Sectioning Results

The step-sectioning of remaining lung tissue in this study did not, and could not, correct the inherent bias that was created in the initial sectioning of tissue. To understand why step-sectioning of all remaining tissue could not correct the bias it is necessary to understand how the bias originated. Lung tissue trimming and slide preparation were consistent among the technicians at the time of the initial sectioning with the exception of a single technician who processed only those animals that had received test compound. This technician, who processed most of the terminal sacrifice treated animals, embedded and sectioned the entire lung of each animal while other technicians embedded and sectioned only a small portion (less than one-half) of each lung.

Of the lungs that were embedded intact, a far greater amount of tissue was necessarily lost as a result of the initial sectioning of tissue when compared to those animals which only had a small portion of lung tissue embedded. The loss of tissue is inevitable in the routine process of selecting a single slice of tissue due to the need to cut thorough a large portion of embedded tissue to obtain the optimum cross-section. All tissue cut prior to the optimum section is routinely discarded by the histology technician. This was noted by Mr. Richard J. Verfuert, past President of American/Histolabs and the manager during the step-sectioning procedure, in his letter of August 13, 1981 to Dr. Donald Nye of FMC Corporation;

"Some concern has been made by the smaller number of Treated Terminal slides. This can be explained by the fact, that originally these lungs were embedded flat, the entire right and left sides intact, thus greater amounts of tissue were lost in the original sectioning (In sectioning, a technician routinely cuts deep into a paraffin block in order to obtain the best section, containing the most surface area). Whereas, the lungs that were trimmed, far less tissues were lost in sectioning, because the samples were smaller. Also, when we returned to the wet tissues for the remaining lung tissues, all of these tissues were step-sectioned, none of which were lost due to routine sectioning."

Furthermore, not only are the number of treated terminal slides smaller than the number of control terminal slides after step-sectioning (1041 slides in the control group compared to 589, 480 and 479 slides in the 20, 100 and 500 ppm groups), but the areas available for histological examination also are consistently less in treated terminal animals compared to control terminal animals after step-sectioning (2358.4 mm²/animal in the control group compared to 1755.1, 1788.5 and 1560.2 mm²/animal in the 20, 100 and 500 ppm groups, respectively).

On June 24, 1981, this reviewer stated that "The procedure used in the step-sectioning of lung tissue involves a serious bias which precludes its use in the assessment of the incidence of alveogenic lung tumors. This bias probably leads to an underestimation of the number of tumors found in the step-sectioned lungs of Permethrin treated animals."

This conclusion has been subsequently reinforced by the following:

1. As noted above, the recently submitted area measurements clearly indicate that a great deal less tissue was examined in treated animals compared to controls after the step-sectioning process.
 2. The clear and consistent bias in the original tissue processing is illustrated in Attachment A which is a reproduction of all of original slides still available. The bias was carried through to the step-sectioning as illustrated by Attachment B.
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In summary, the original conclusion of this reviewer, that the step-sectioning procedure was seriously biased, is still valid and has, in fact, been substantially reinforced by a thorough investigation.

It must also be acknowledged, however, that the original sectioning of lung tissue was biased as well. Substantially less tissue per animal was examined in the control group than was examined in each of the treated groups.

III. An Attempt to Correct for the Bias

As noted above, the lung tissues in this study were processed in a manner which was not consistent between treated and control animals. FMC has stated in their submission of August 13, 1981 that lungs of certain animals "had peripheral tumor bearing lung tissues trimmed away" while other animals were embedded intact. They also noted that "the plane in which the tissues were embedded resulted in a smaller cross sectioned area" in control animals compared to treated animals.

The statement regarding the trimming of peripheral tumor bearing tissue was based solely on the letter of July 3, 1981 from Mr. Richard Verfuert of American Histolabs to Dr. Nye of FMC Corp. However, Mr. Verfuert has since acknowledged, in a signed affidavit (Attachment F), that;

"My opinions (in the letter of July 3, 1981) were largely conjecture based on past experience that I have had trimming tissue over the years, but what appears in the letter as a statement of fact is in fact speculation. For this study, to my recollection, I never personally observed anyone actually trimming away peripheral lung tissue of the rats as I described in my letter and illustrated in the accompanying diagrams to the letter. I wrote this letter without having the benefit of consulting with Dr. Asif or anyone of the persons who actually performed the routine tissue trimming..."

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During the course of this investigation, Dr. Asif was contacted and his statement is submitted as Attachment E. His statement does not indicate a trimming away of all peripheral tissue as originally indicated by Mr. Verfuert. Rather, his manner of collecting sections of lung tissue to be embedded required the taking of transverse (or oblique) cross section of the left lung and a longitudinal cross section of the right lung. It was his opinion, as a histologist with many years of experience, that such sectioning "provide(d) a representative section of each of the lobes."

The other method of tissue embedding and sectioning was that of not trimming away any tissue but rather embedding and sectioning all lung tissue. Neither method of tissue processing is considered incorrect and both can yield representative cross sections of lung tissue. However, they do differ in at least one important way other than plane of sectioning - that being the area of tissue which eventually is sectioned is much smaller using the method of Dr. Asif.

It must be noted that few of the tumors in this study were associated with grossly observable lesions. Dr. William Busey, one of two pathologists who examined lung tissues from this study, found that "the bronchio-alveolar adenomas were characterized by proliferation of the bronchiolar/alveolar epithelial cells forming a lesion involving the terminal bronchioles, alveolar ducts and proximal alveoli. In general, these adenomas were small... The bronchio-alveolar carcinomas were characterized by a relatively undifferentiated proliferation of bronchiolar/alveolar epithelial cells and were most frequently located deep in the lung parenchyma...". In either case, it is likely that the tumors would not be observed grossly, especially if the gross necropsy was only of minimally satisfactory quality, as was the case in this study. The lack of gross observation of the tumors in this study is borne out by an examination of the gross necropsy sheets.

Thus, the area of tissue examined can be expected to be an important variable in this study. Although not all of the treated animals had larger areas of tissue examined than the controls, the vast majority of treated terminal sacrifice animals did have substantially more tissue examined than the control terminal sacrifice animals.

Fortunately, this variable has been quantified for all male lung slides, original and step-sectioned, in this study by the study sponsor, FMC Corporation. On August 13, 1981, area measurements for lung slides of all male animals in this study were submitted to the Agency. The measurements were performed at Structure Probe™ of Metuchen, N.J. On October 1, 1981, all raw data pertaining to the area measurements

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were also submitted. (It is noted that a number of inaccuracies in the reported data have been identified by FMC based on a review of the raw data. It is also noted that the material submitted as raw data cannot be clearly identified as original data due to a lack of dates and technicians signature or initials. However, for the purpose of this review it will be assumed that area measurements of male lung tissue reported by Structure Probe, and corrected by FMC, are accurate.)

The total areas of tissue originally examined for each test group, based on the measurements performed at Structure Probe™, are as shown in Table 2.

TABLE II

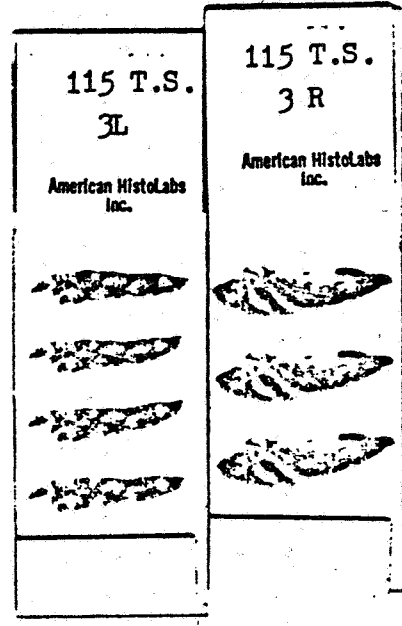
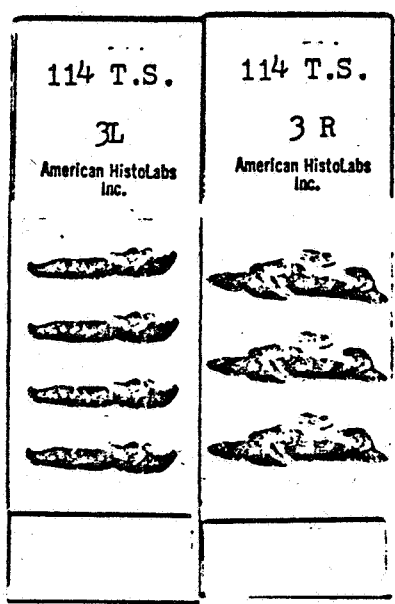
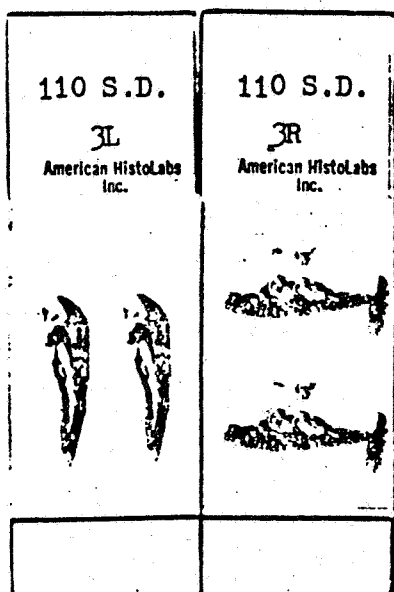
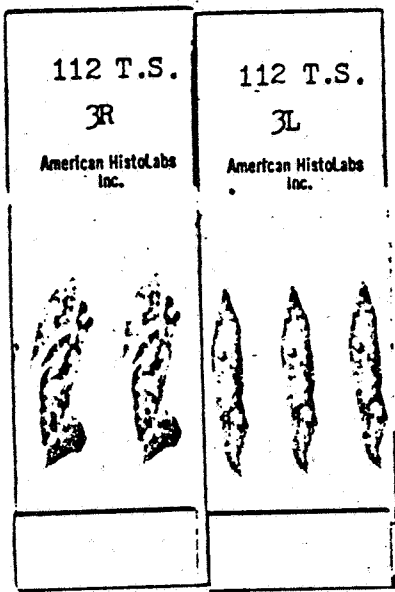
Total Area of Lung Tissue Available for Histopathological Evaluation of Male Lungs (Both original and step-sectioned)

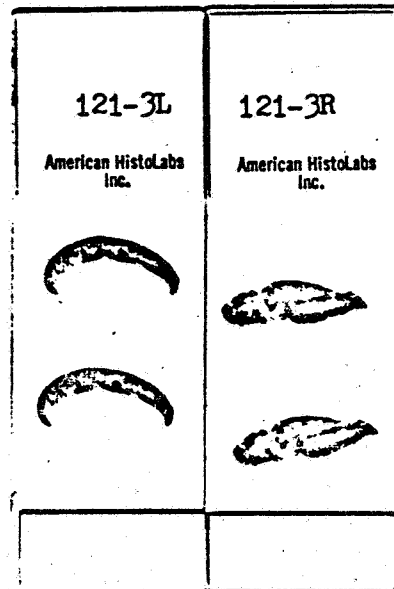
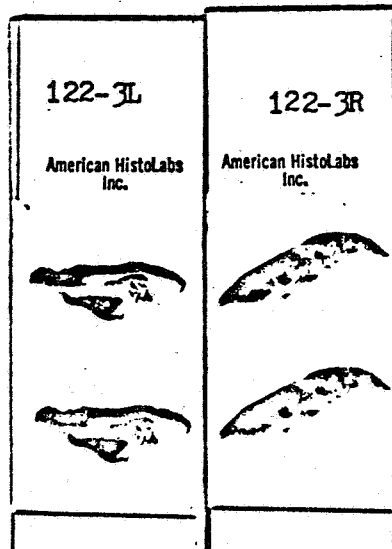
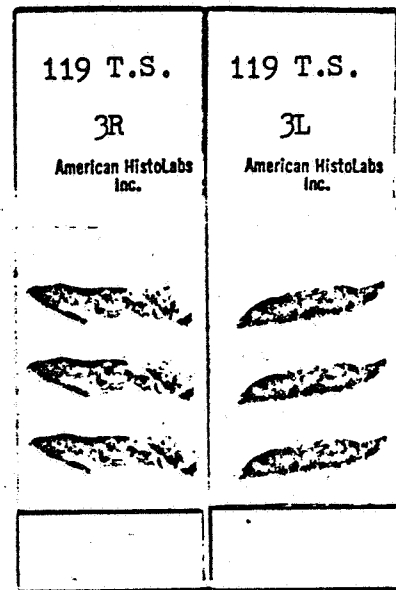
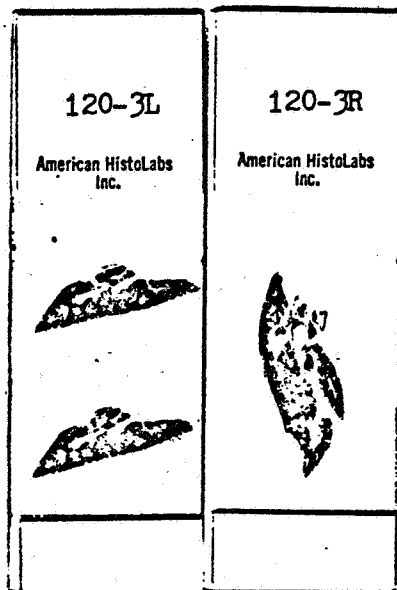
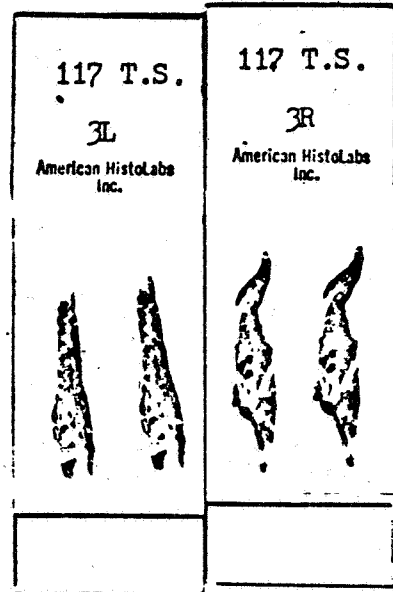
	<u>Control</u>	<u>Area (mm²)/Group</u> <u>20 ppm</u>	<u>100 ppm</u>	<u>500 ppm</u>
Original Slides	8882.5	12168.3	14940.61	14800.67
Step-Sectioned Slides	149202	106461.6	115173.9	109763.3
Total	158084.5	118629.9	130114.5	124564

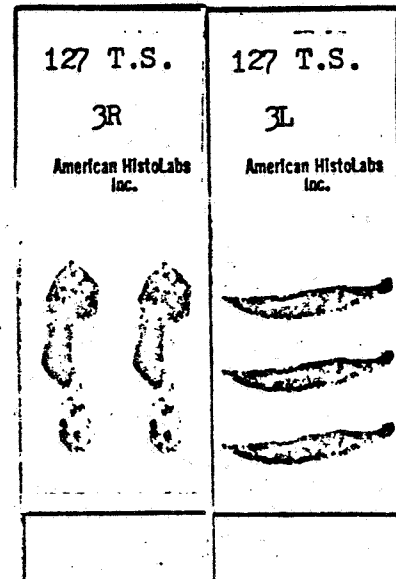
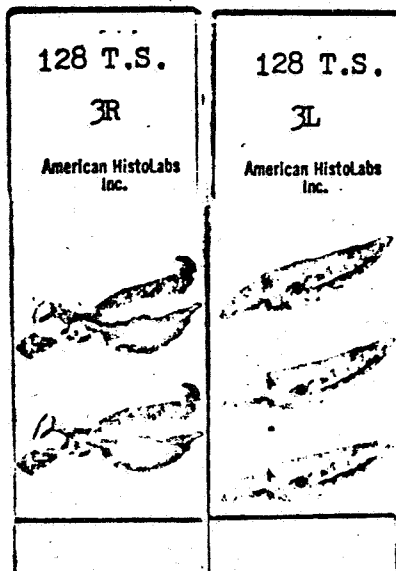
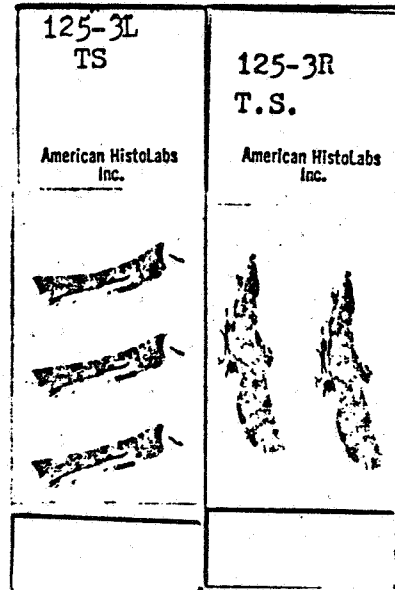
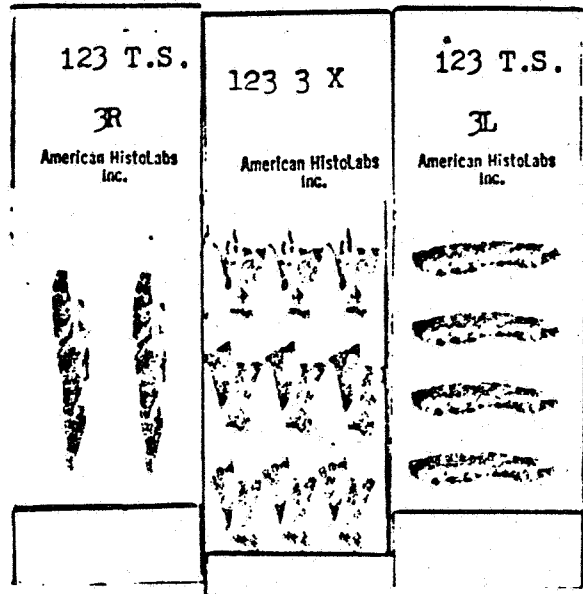
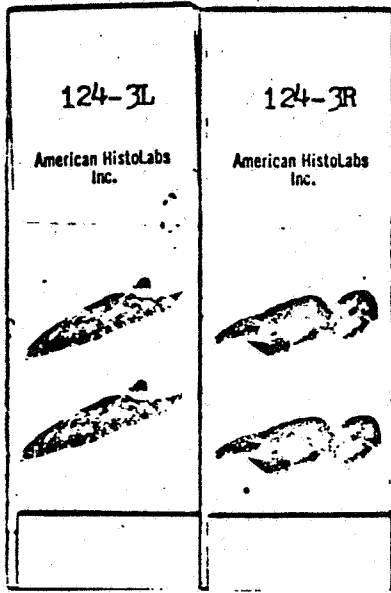
[These totals are corrected for original (nonstep-sectioned) slides which had multiple sections of lung tissue present. In those cases, the total area of lung tissue actually available for examination was considered to be the area of only one of the multiple sections. This was due to the fact that the multiple sections are virtually identical histologically, having been cut sequentially at the time of the initial tissue processing.]

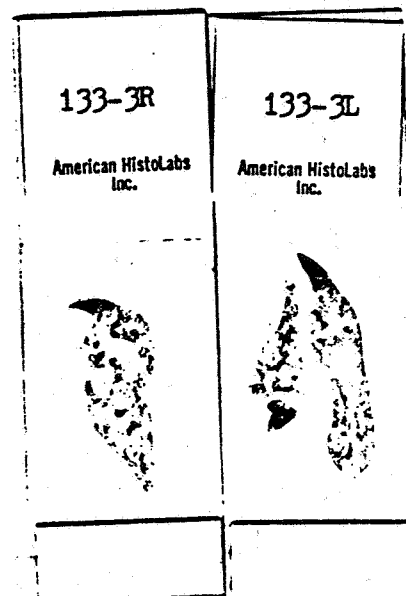
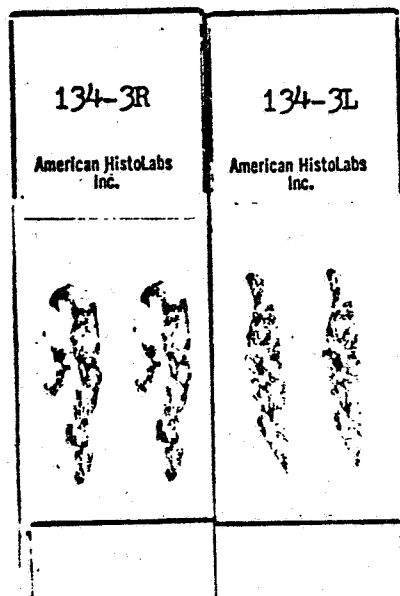
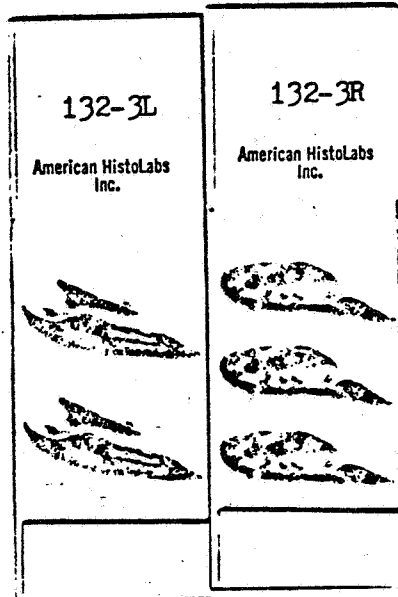
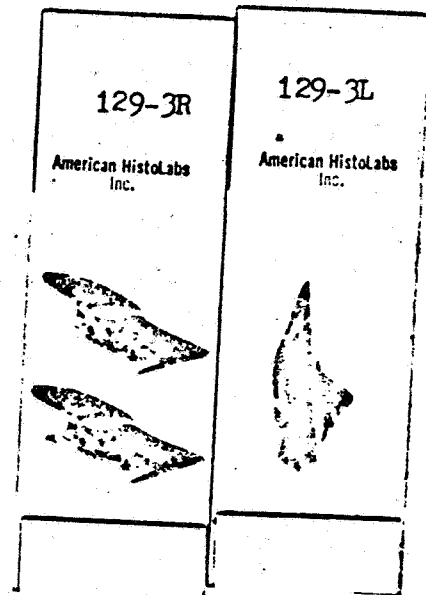
The number of male animals bearing bronchio-alveolar tumors has been diagnosed as 8/60, 6/56, 12/59 and 11/59 for control, 20 ppm, 100 ppm and 500 ppm groups, respectively, based on the diagnoses of Dr. Busey (original slides) and Dr. Billups (step-sectioned slides). However, as is shown in Table II, the total area of lung examined was substantially greater in the control group compared to treated groups.


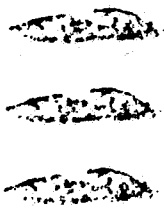
If it can be assumed that the likelihood of discovering a tumor is related to the amount of tissue examined, an attempt can be made to artificially adjust the tumor incidence, based on area examined, to the hypothetical tumor incidence which would be expected if all groups had equal areas of lung tissue examined. In the following table, the number of lung tumor-bearing animals was adjusted by multiplying the number of tumor-bearing animals actually observed in each treated group by the ratio of the areas (as shown in Table 2) of control and each of the treated groups.

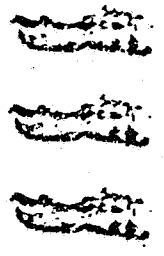












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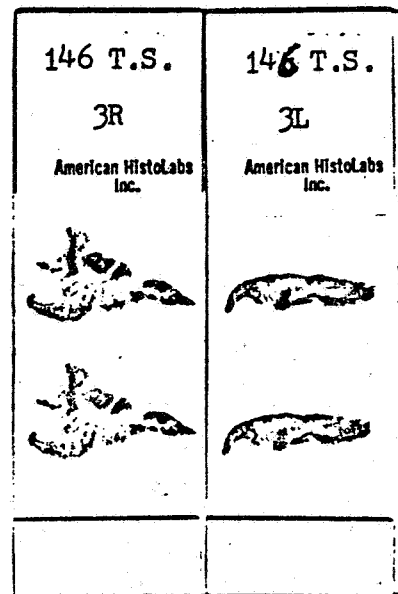
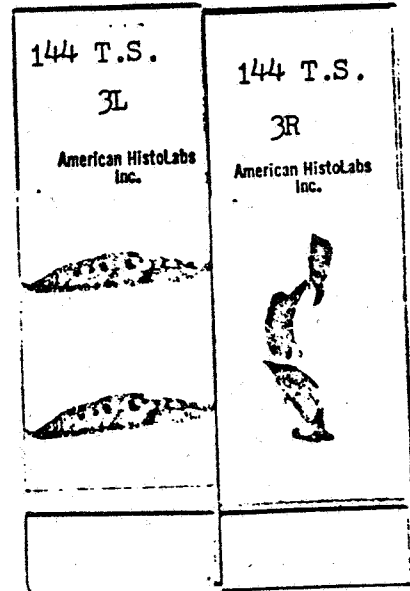
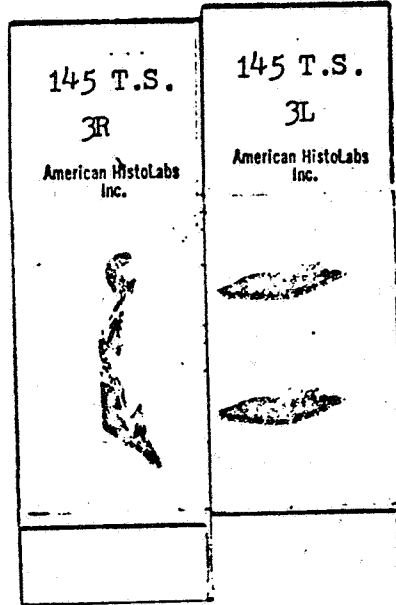
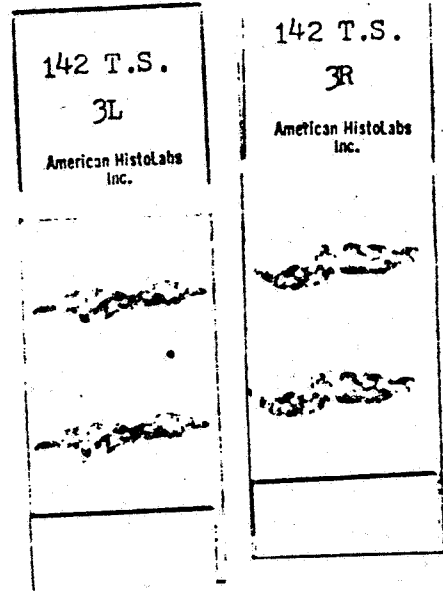
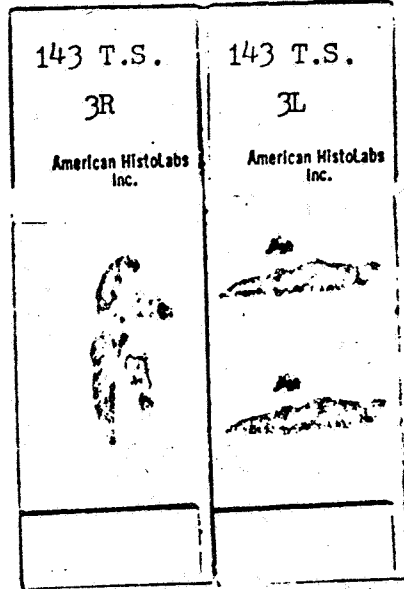
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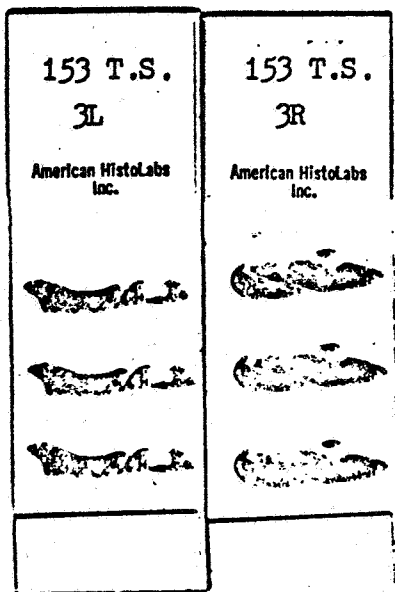
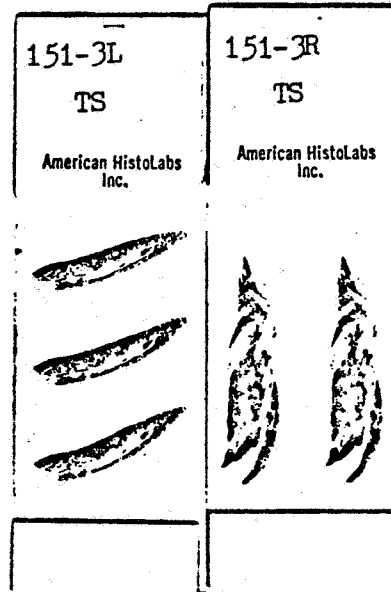
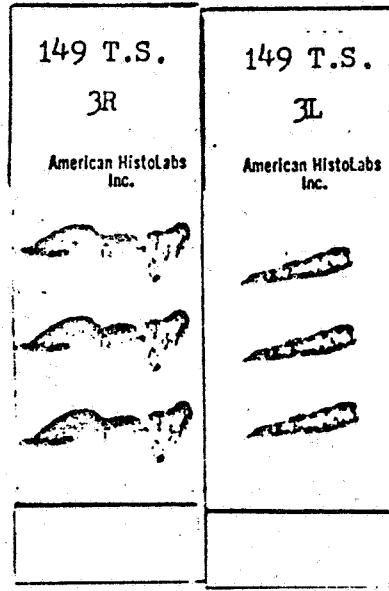
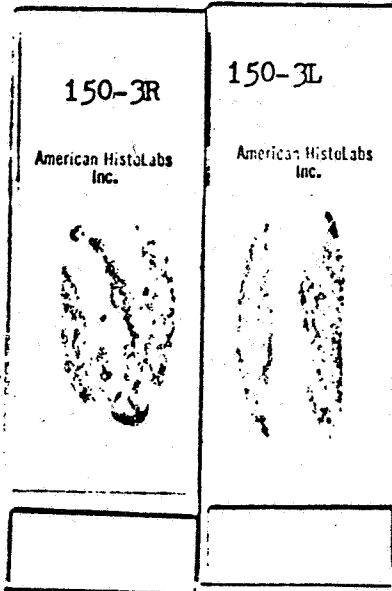
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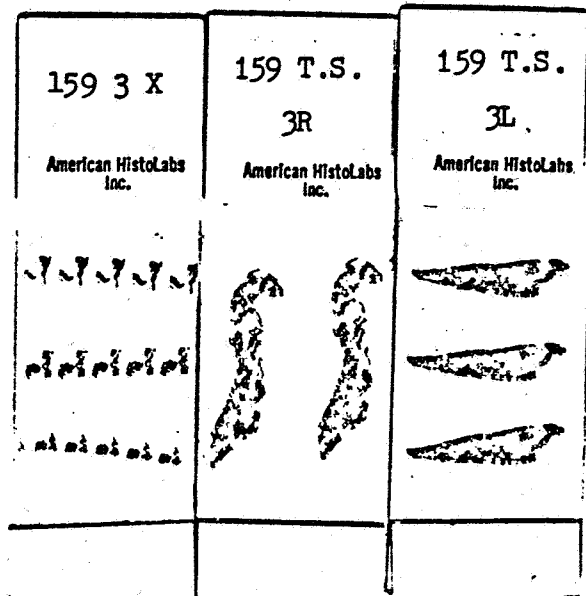
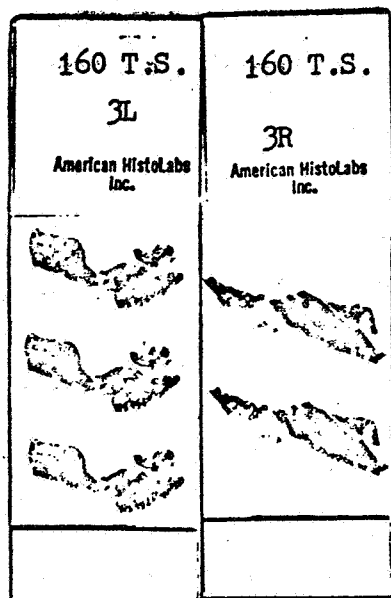
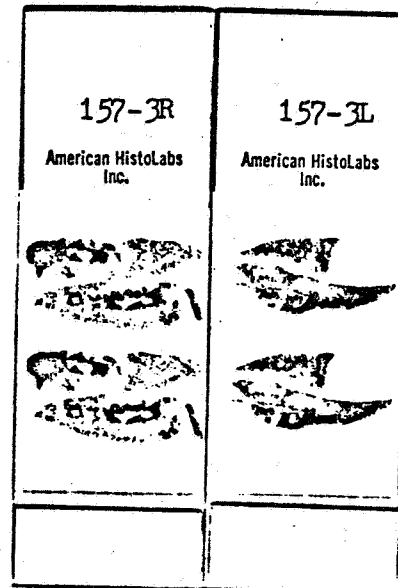
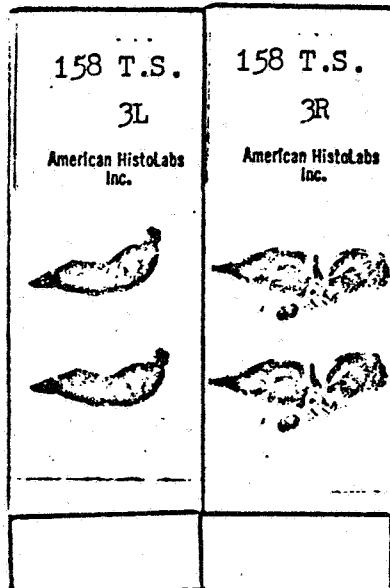
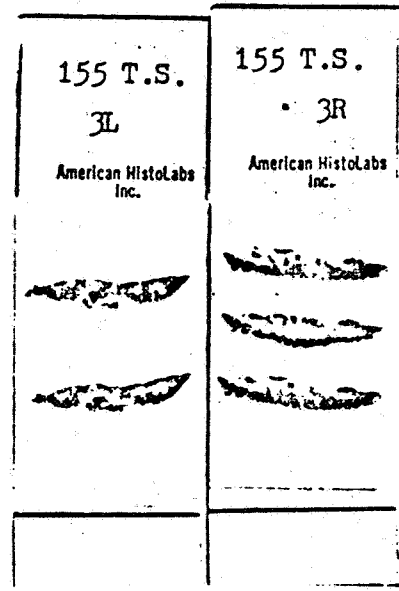
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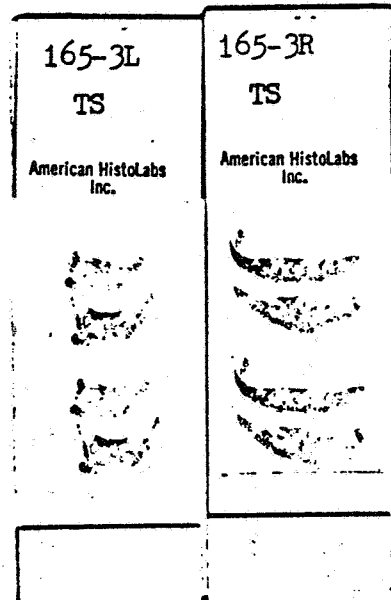
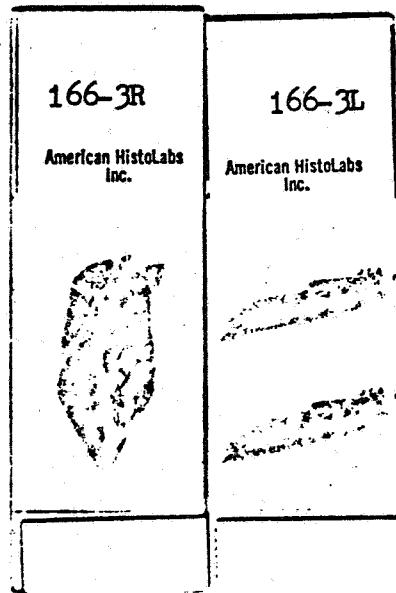
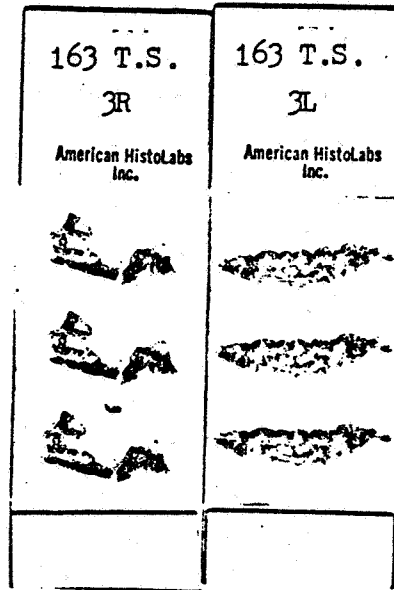
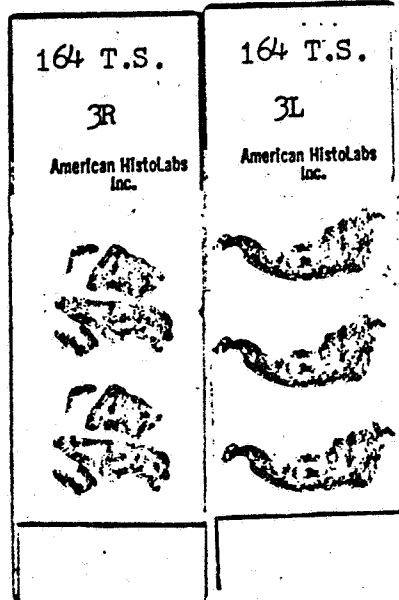
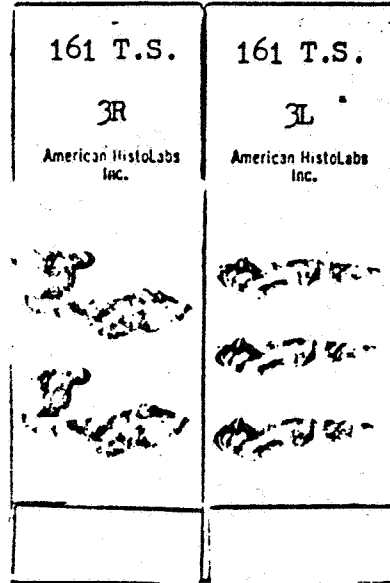
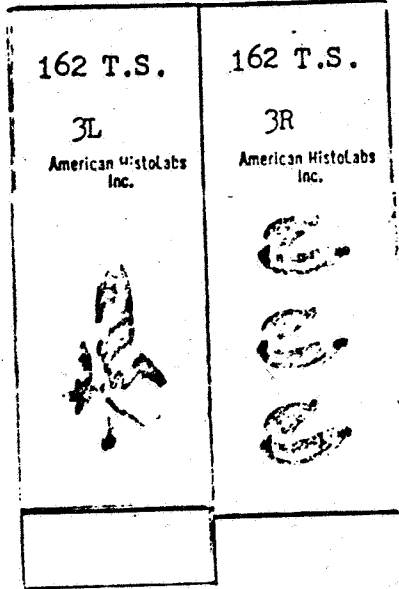
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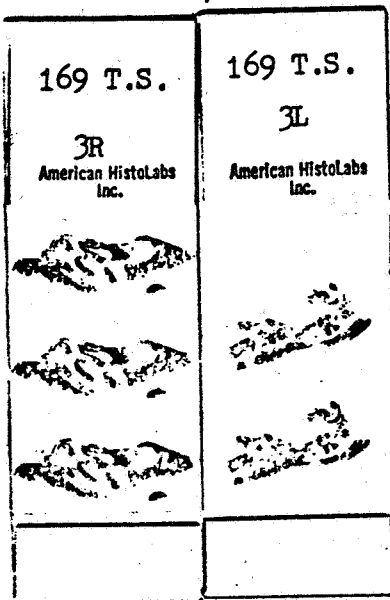
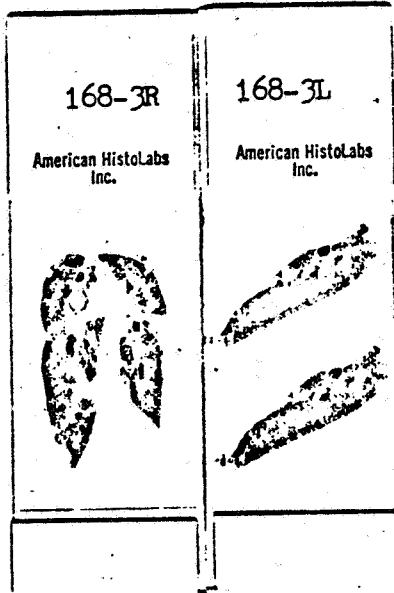
140-3R TS American Histolabs Inc.	140-3L TS American Histolabs Inc.
	

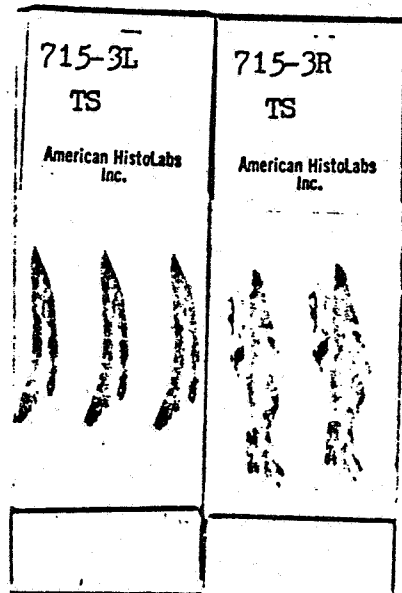
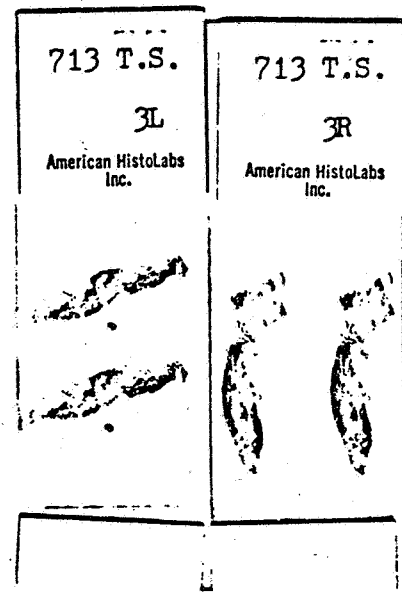
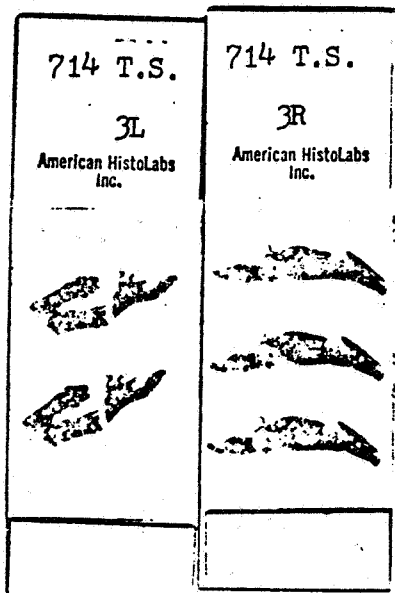
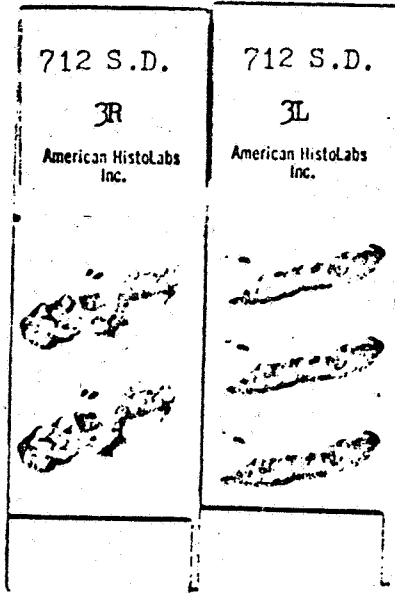


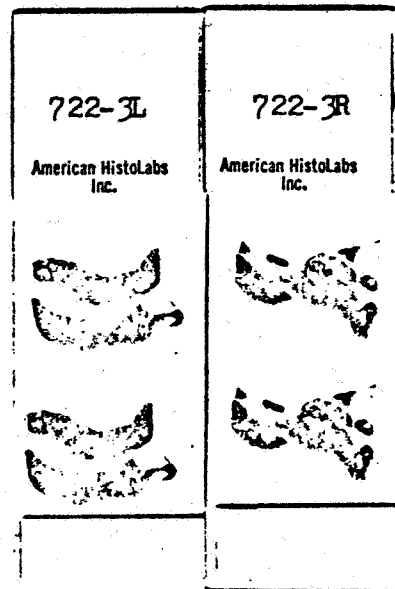
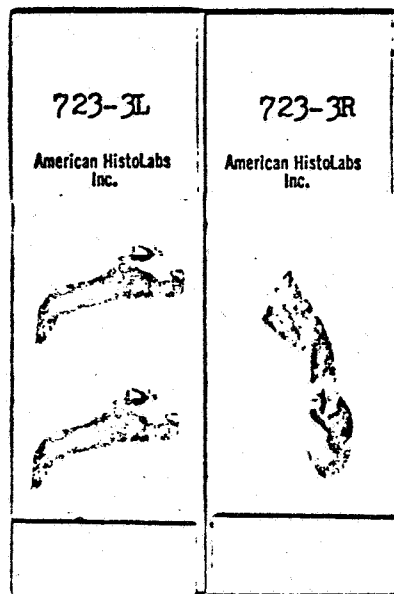
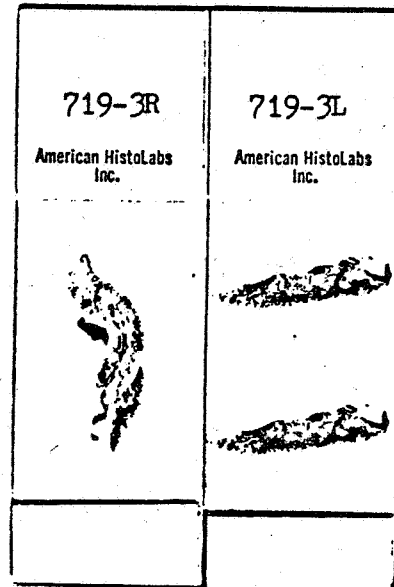
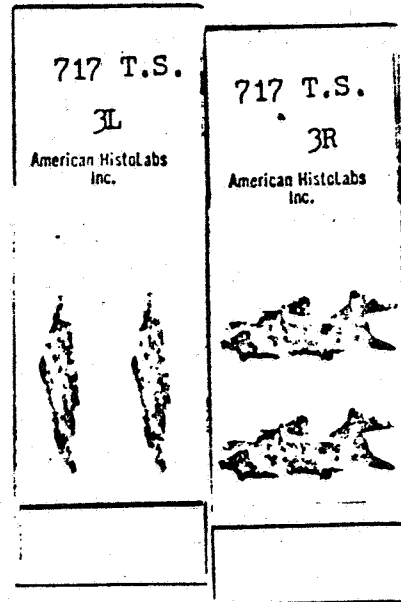
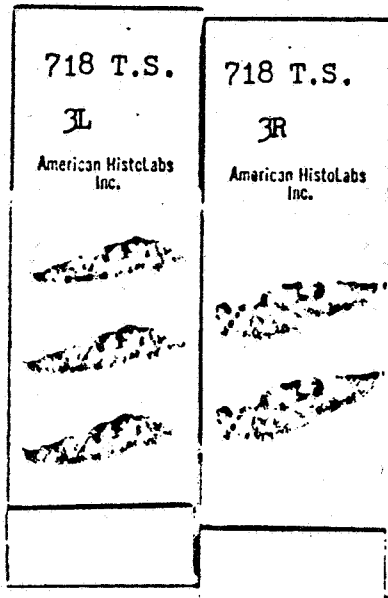


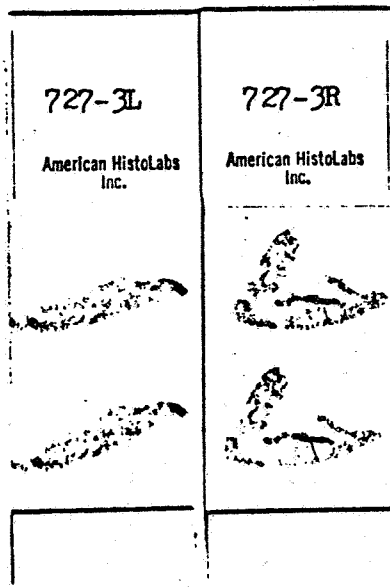
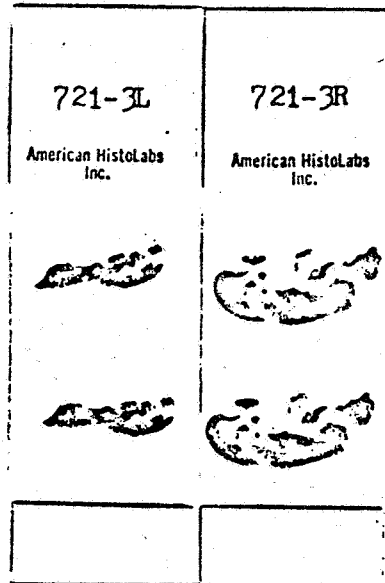
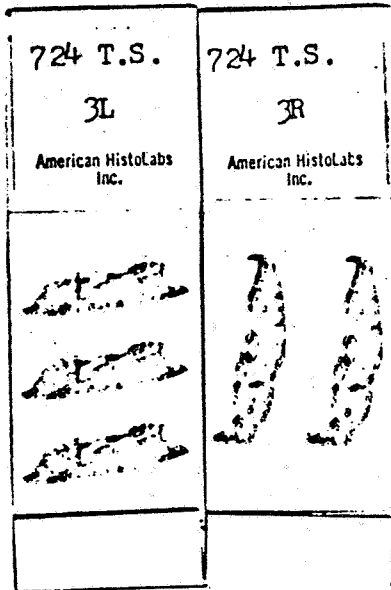


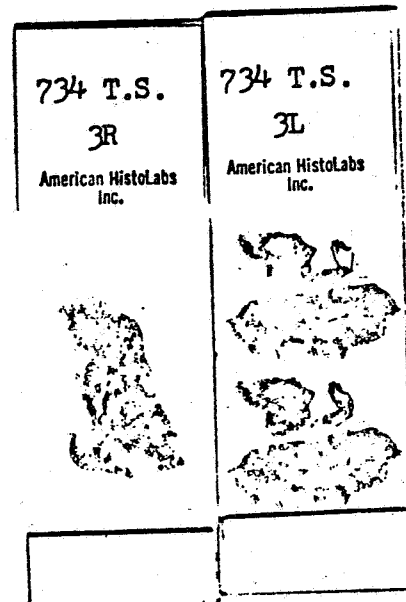
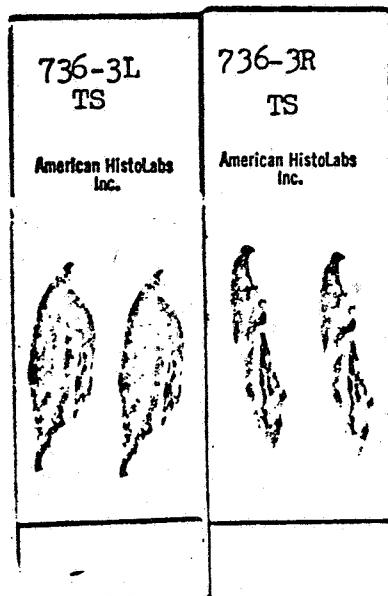
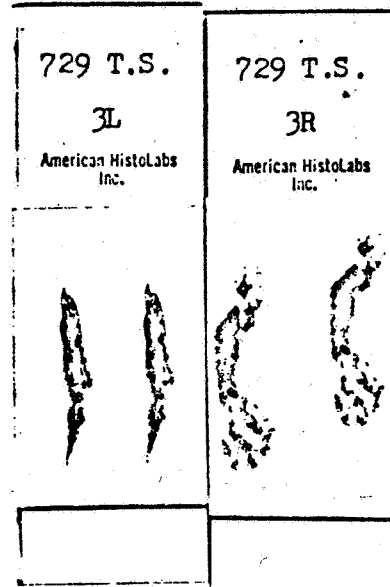
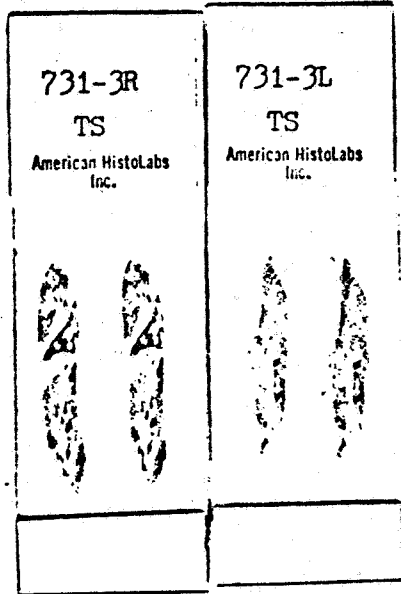


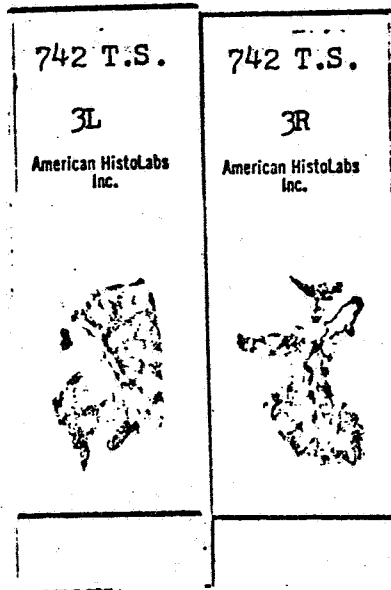
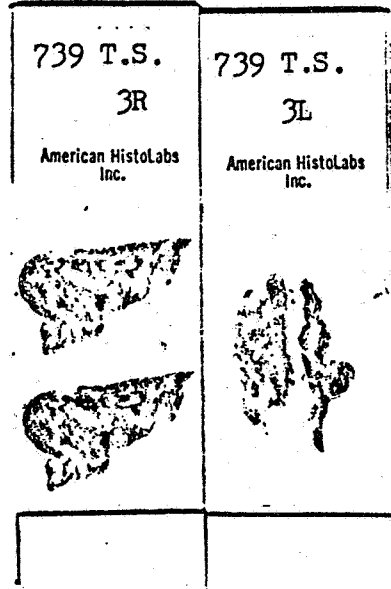
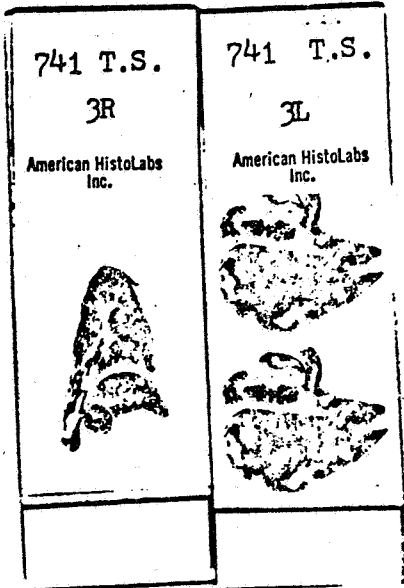
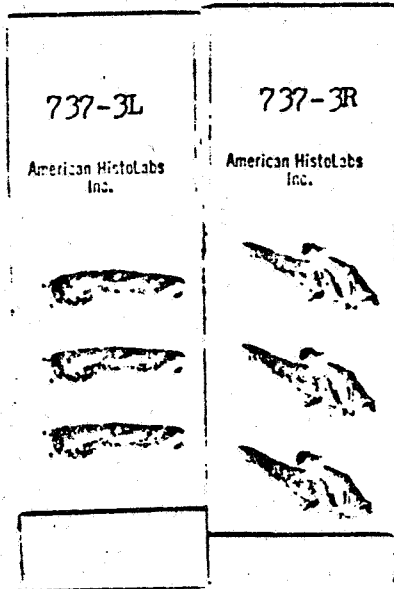
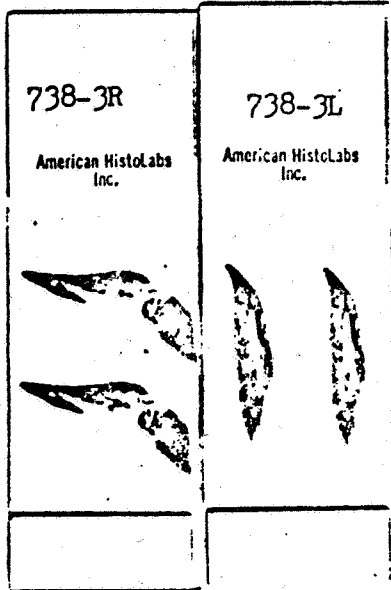


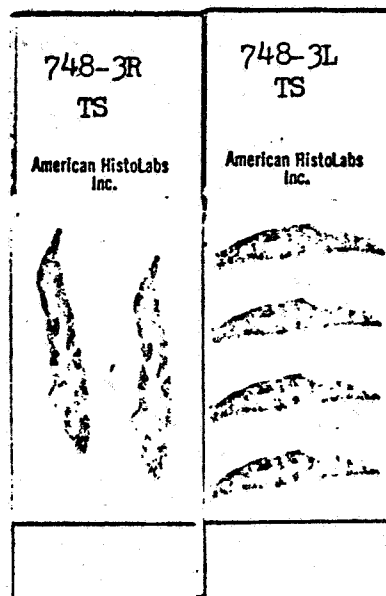
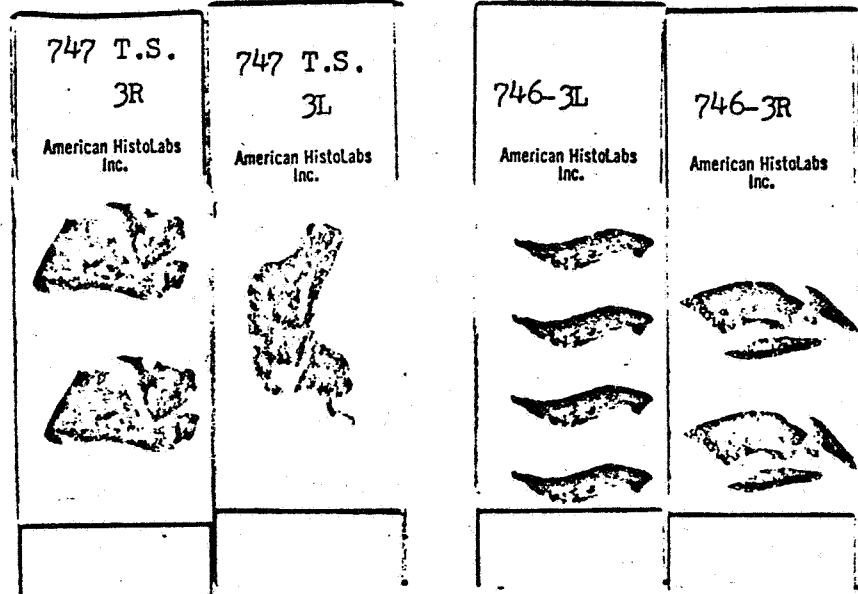
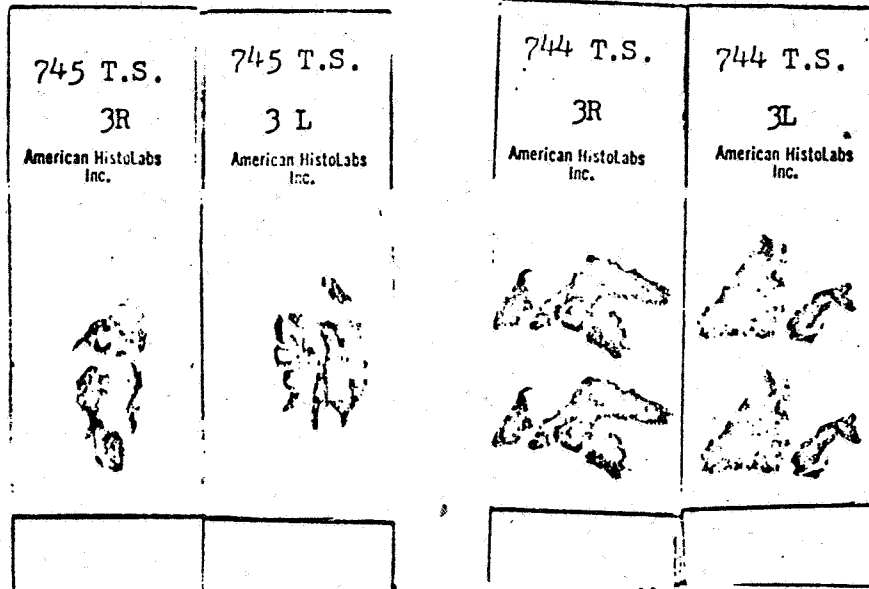


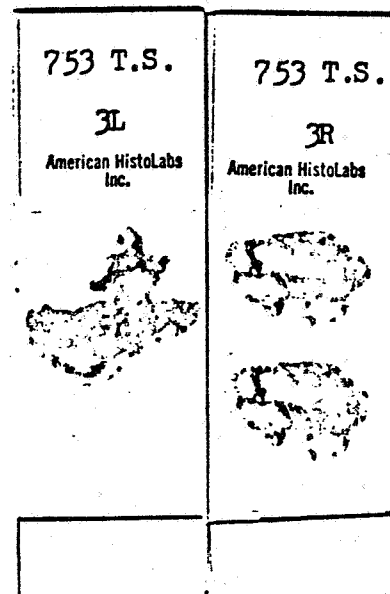
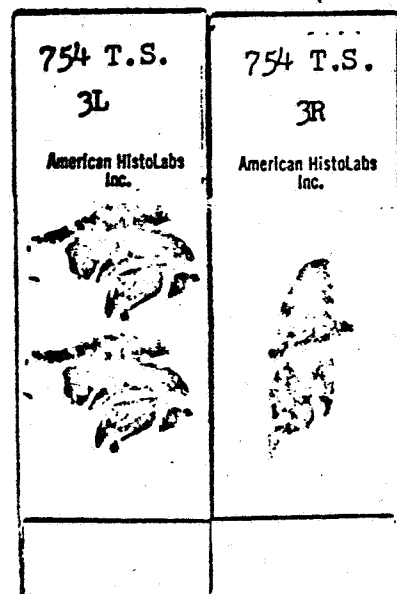
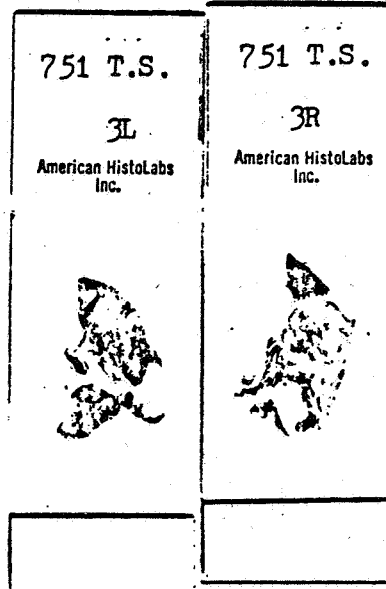
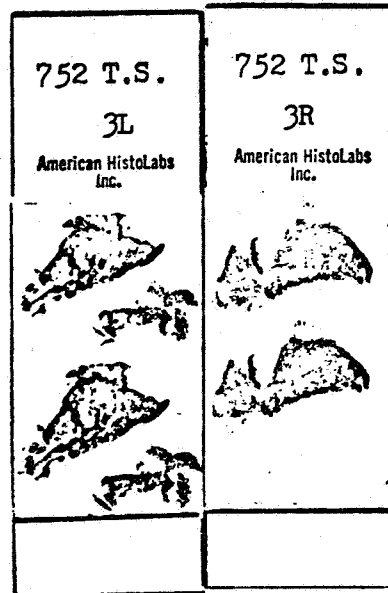
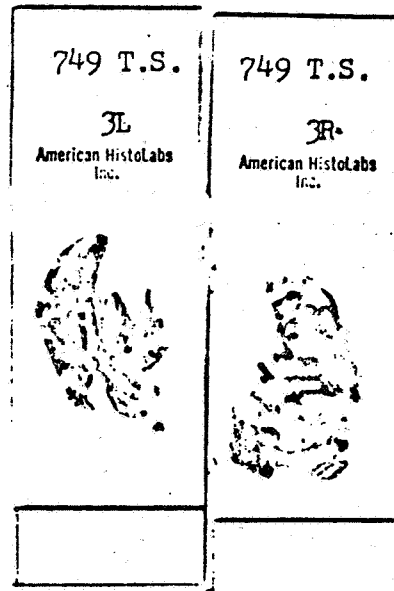












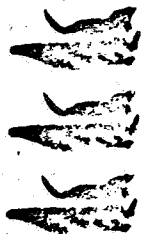
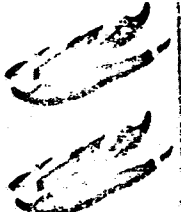




756 T.S. 3L American HistoLabs Inc.	756 T.S. 3 R American HistoLabs Inc.
	

755-3R TS American HistoLabs Inc.	755-3L TS American HistoLabs Inc.
	


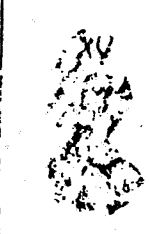
758-3R American HistoLabs Inc.	758-3L American HistoLabs Inc.
	



757 T.S. 3L American HistoLabs Inc.	757 T.S. 3R American HistoLabs Inc.
	

761-3R American HistoLabs Inc.	761-3L American HistoLabs Inc.
	



759-3R American HistoLabs Inc.	759-3L American HistoLabs Inc.
	

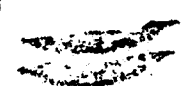

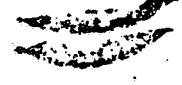
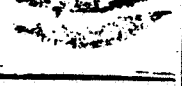
764 S.D. 3L American Histolabs Inc.	764 S.D. 3R American Histolabs Inc.
	




763 T.S. 3L American Histolabs Inc.	763 T.S. 3R American Histolabs Inc.
	

762 S.D. 3L American Histolabs Inc.	762 S.D. 3R American Histolabs Inc.
	

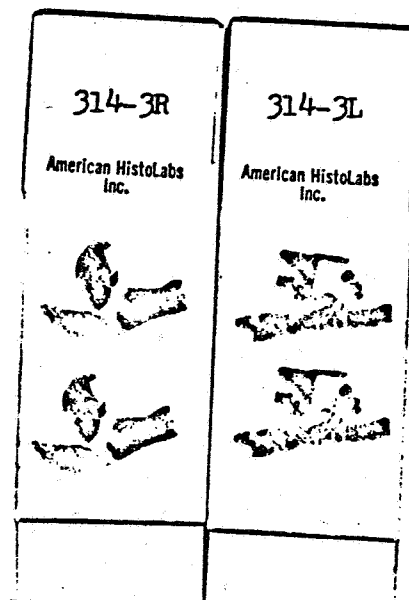
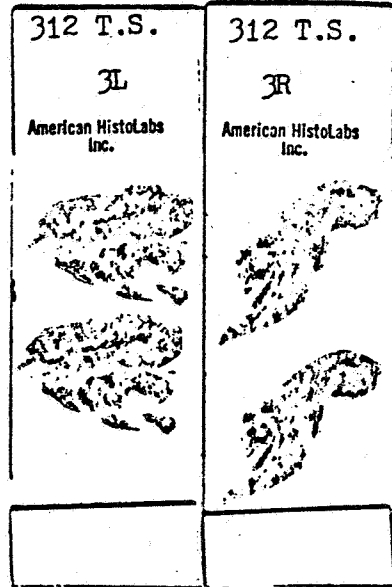
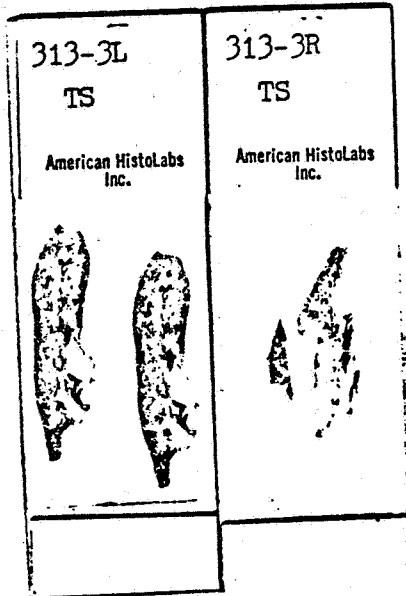
767 T.S. 3L American Histolabs Inc.	767 T.S. 3R American Histolabs Inc.
	
	

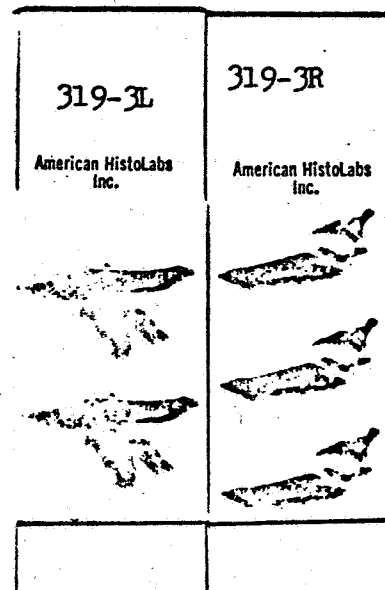
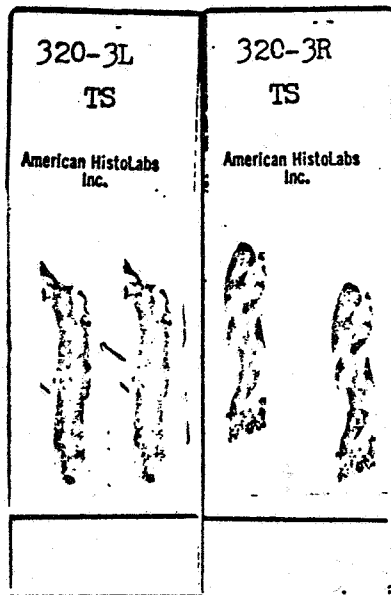
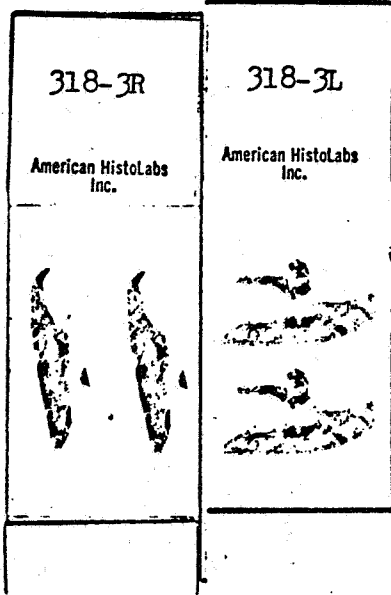
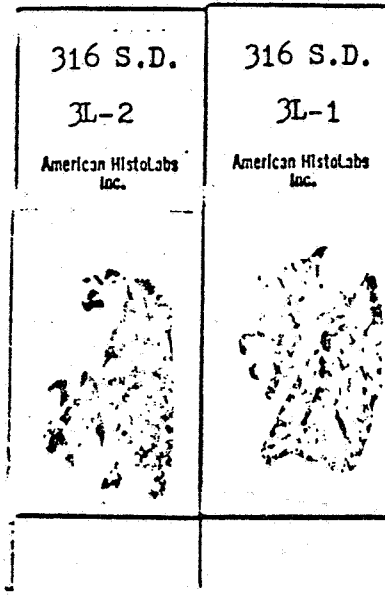
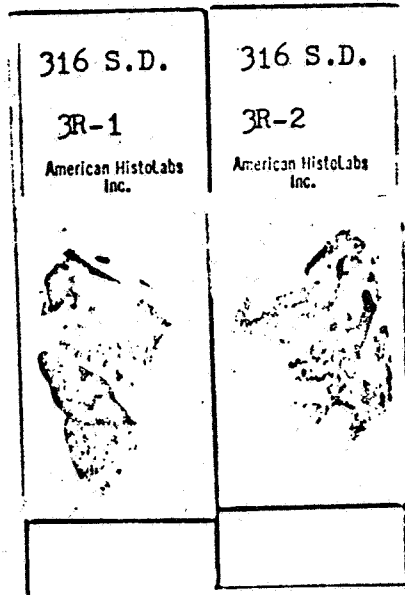
766 T.S. 3L American Histolabs Inc.	766 T.S. 3R American Histolabs Inc.
	
	

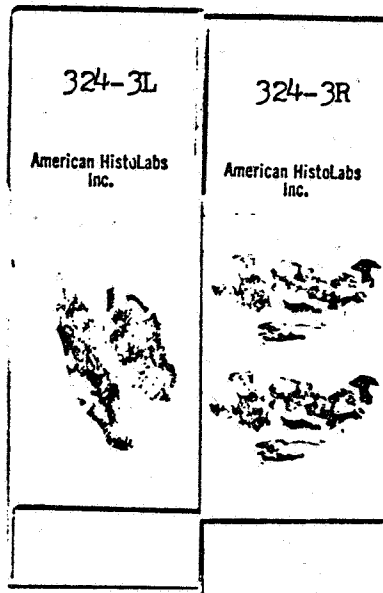
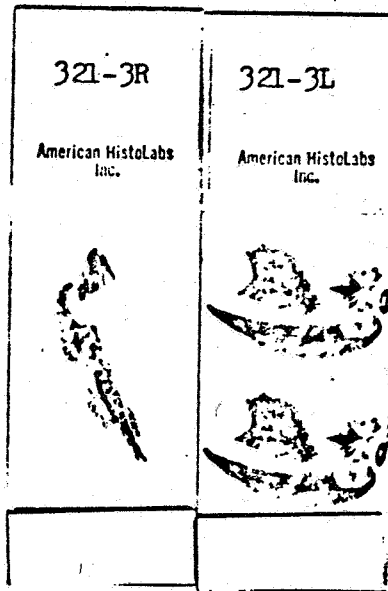
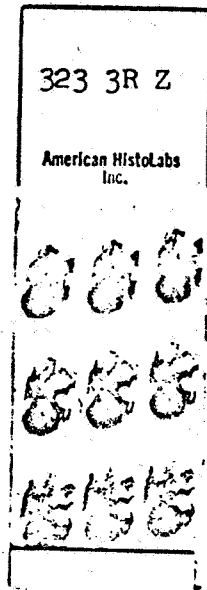
765-3L American Histolabs Inc.	765-3R American Histolabs Inc.
	
	
	

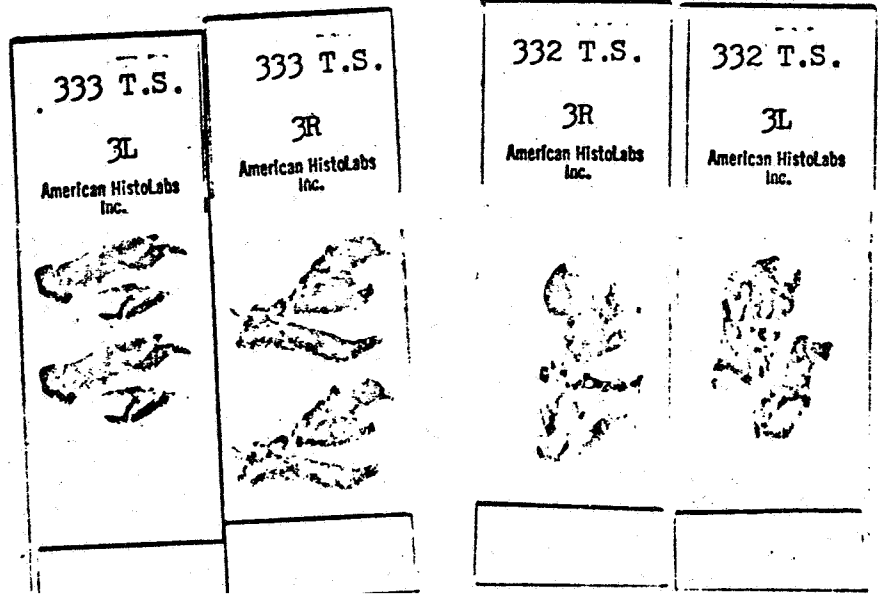
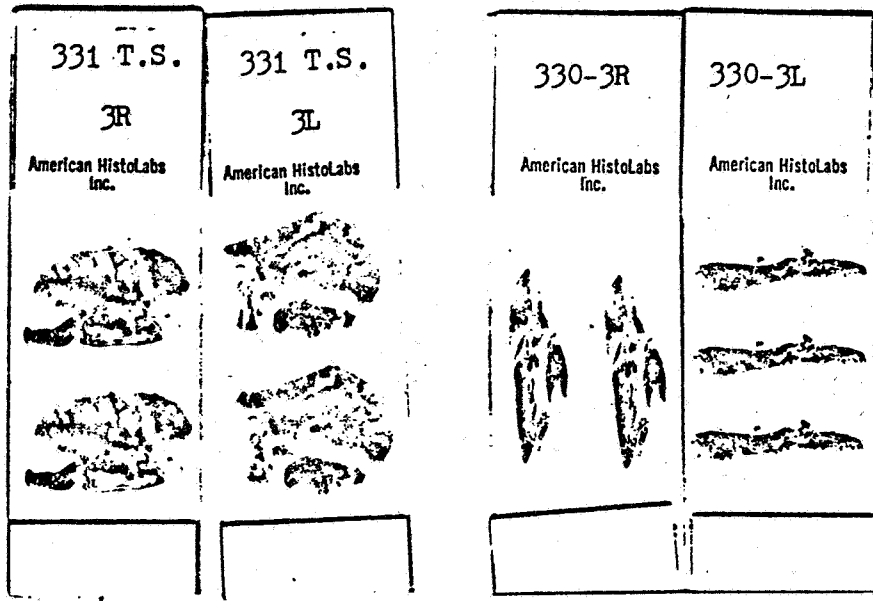
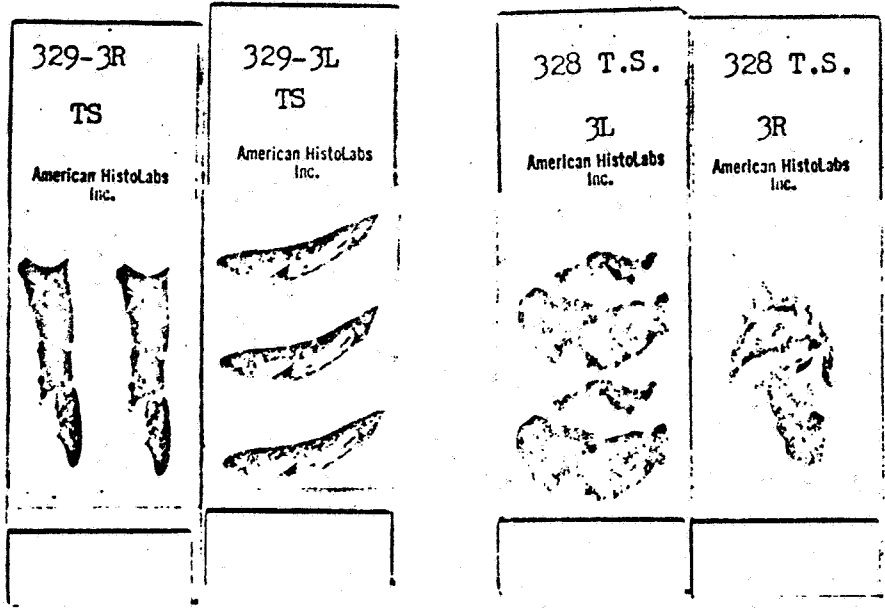
769 T.S. 3R American Histolabs Inc.	769 T.S. 3L American Histolabs Inc.
	
	

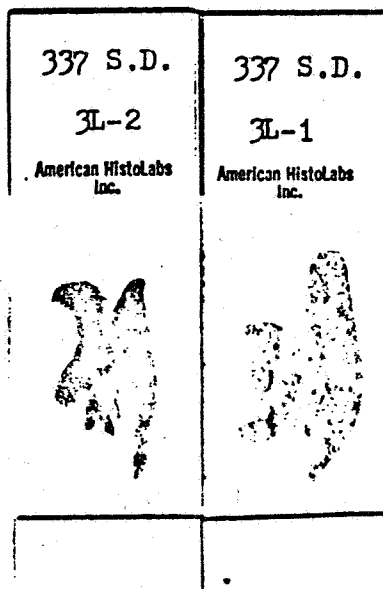
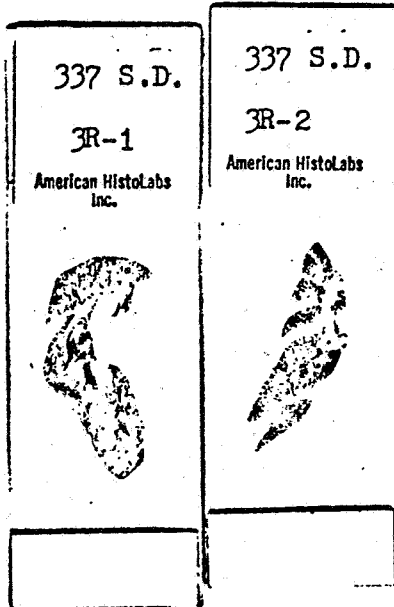
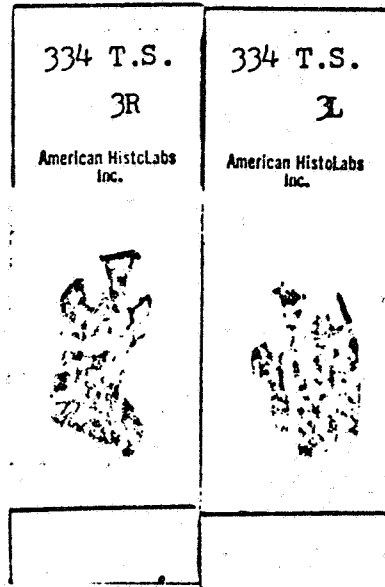
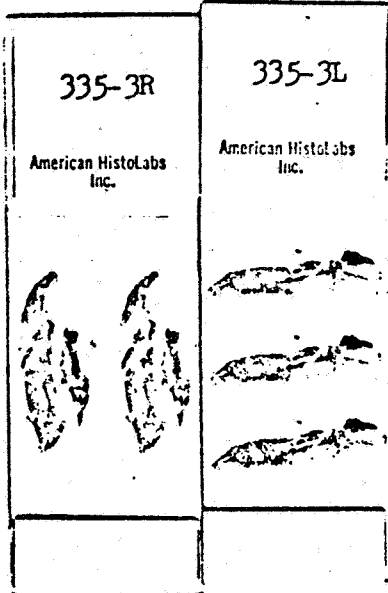
768 T.S. 3R American Histolabs Inc.	768 T.S. 3L American Histolabs Inc.
	
	











340 T.S. 3L American Histolabs Inc.	340 T.S. 3R American Histolabs Inc.

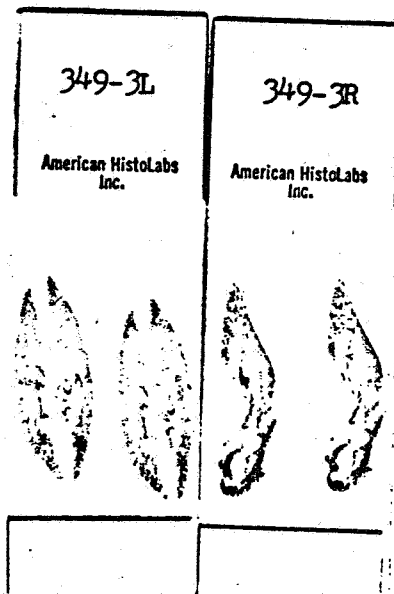
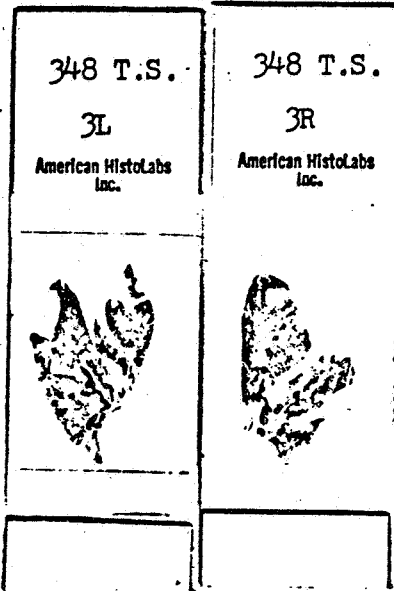
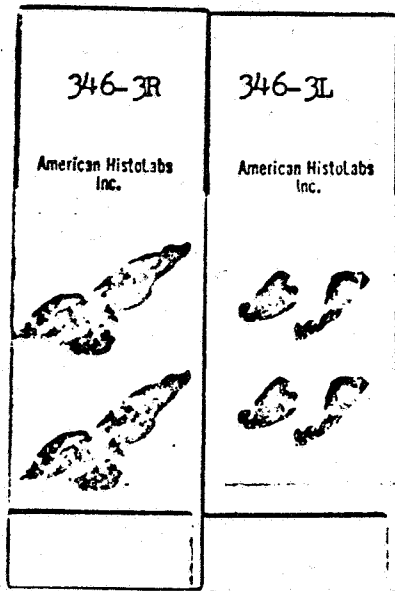
339 T.S. 3L American Histolabs Inc.	339 T.S. 3R American Histolabs Inc.

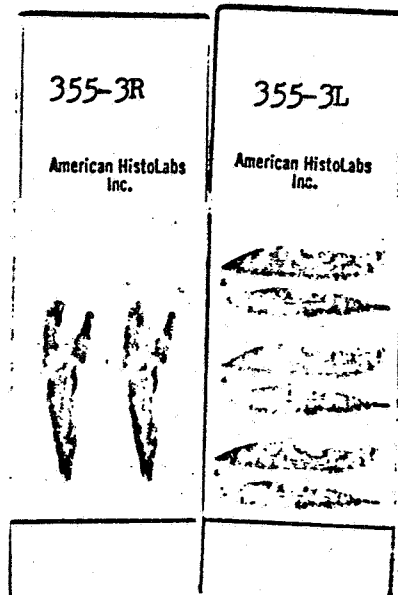
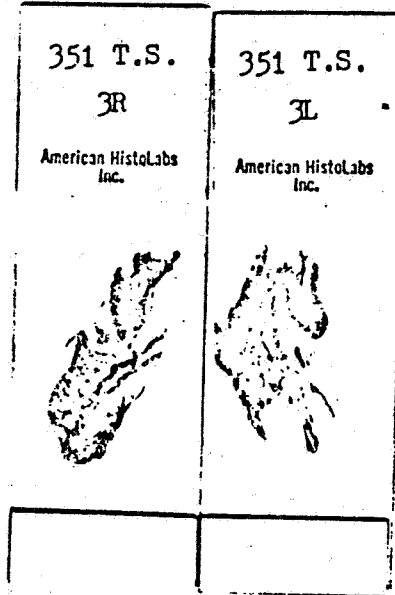
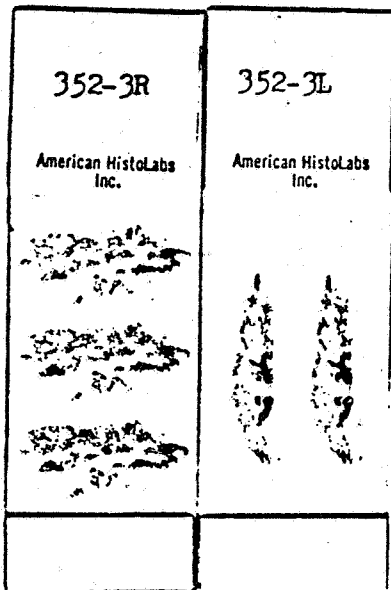
342 T.S. 3L American Histolabs Inc.	342 T.S. 3R American Histolabs Inc.

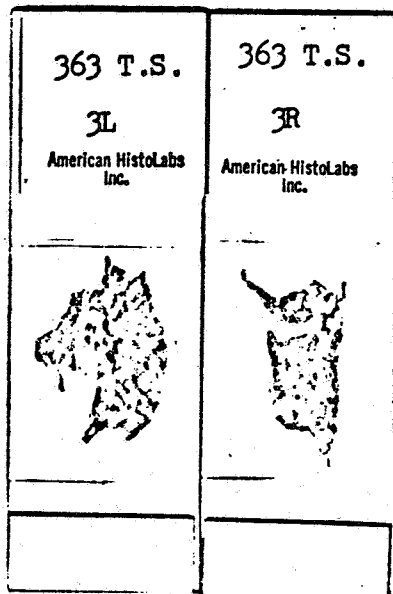
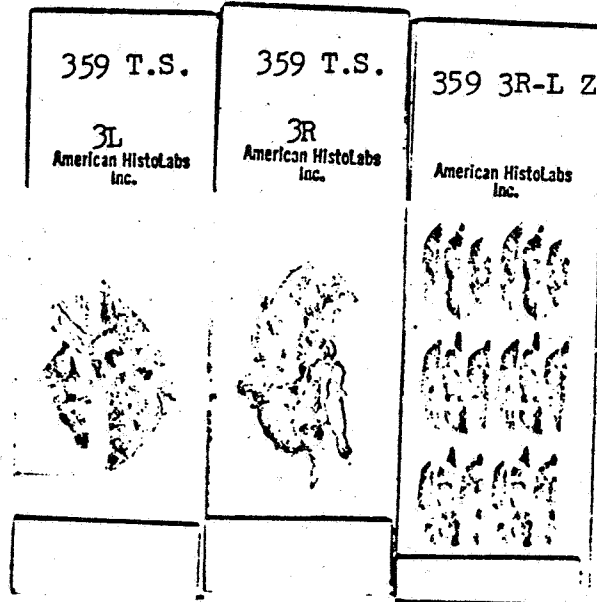
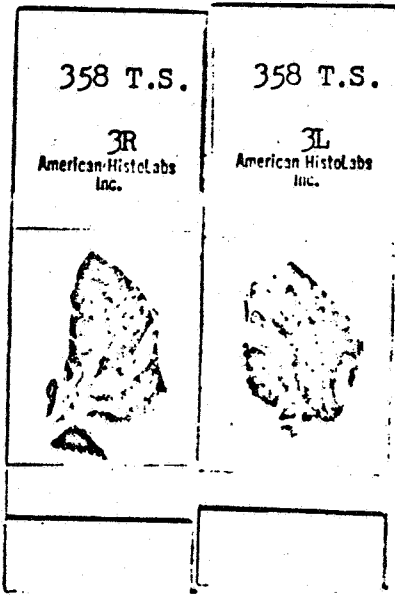
341 T.S. 3L American Histolabs Inc.	341 T.S. 3R American Histolabs Inc.

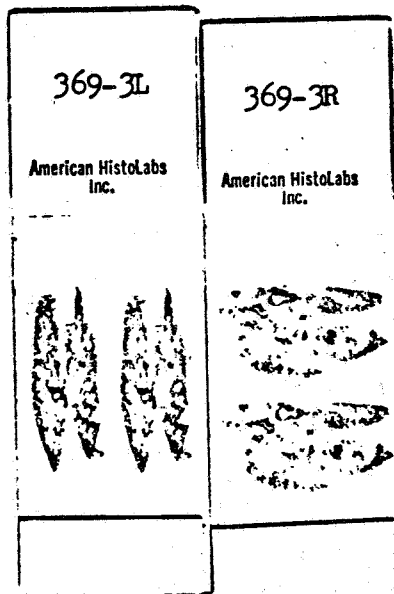
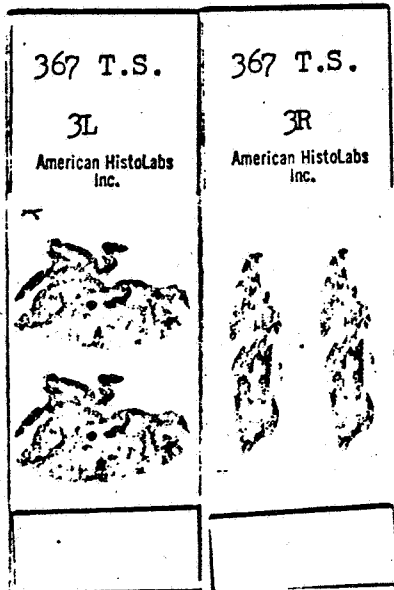
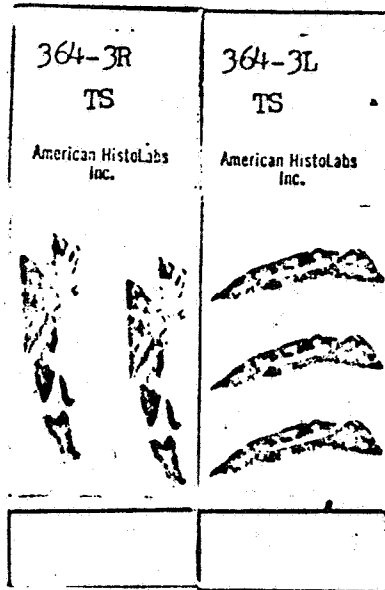
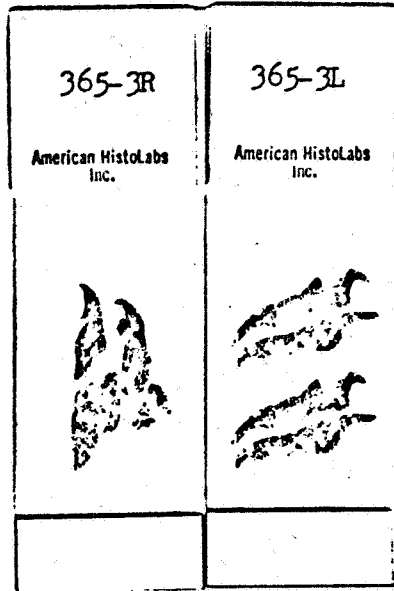
344-3R TS American Histolabs Inc.	344-3L TS American Histolabs Inc.

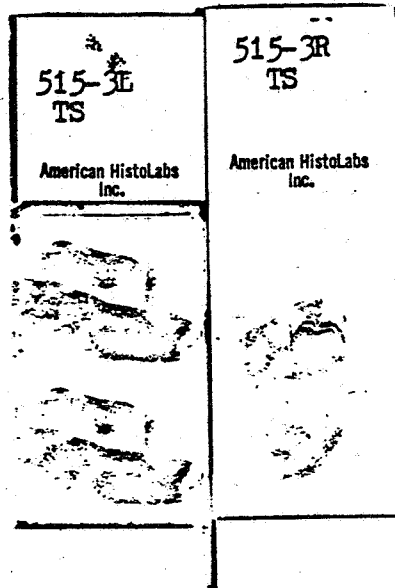
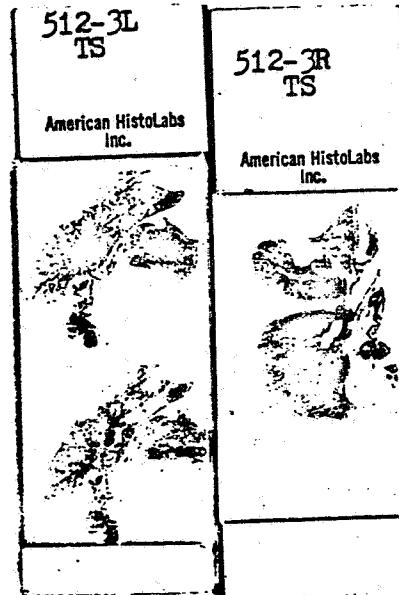
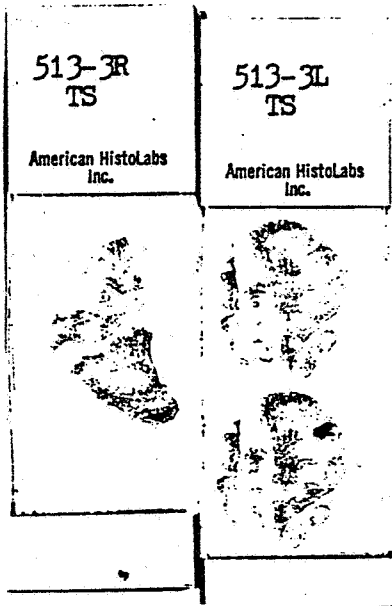
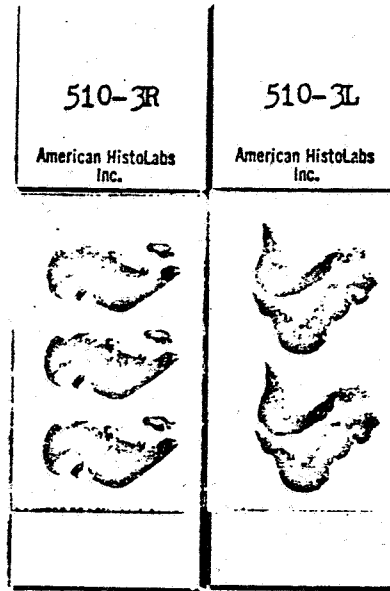
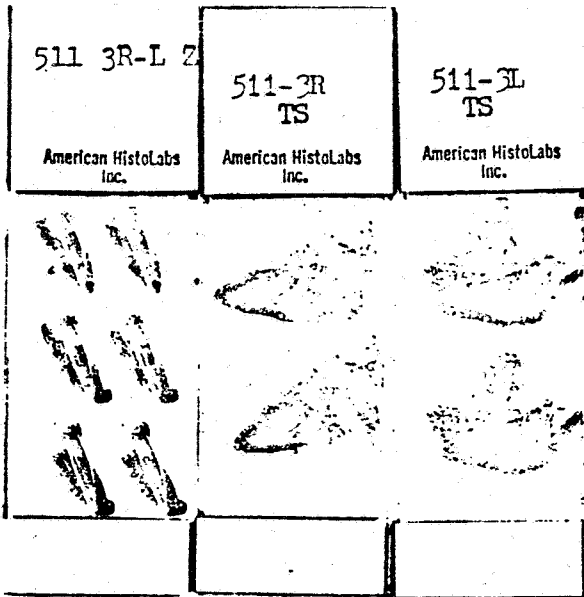
343 T.S. 3R American Histolabs Inc.	343 T.S. 3L American Histolabs Inc.

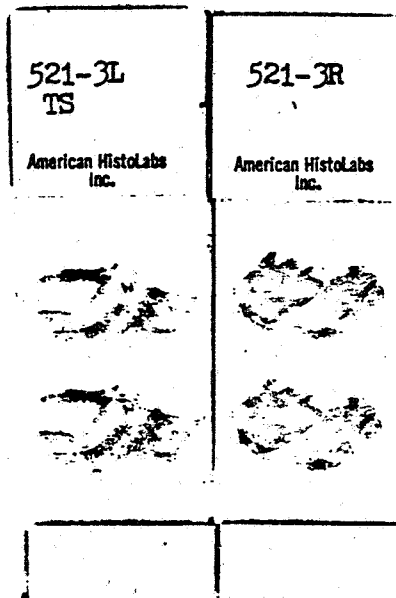
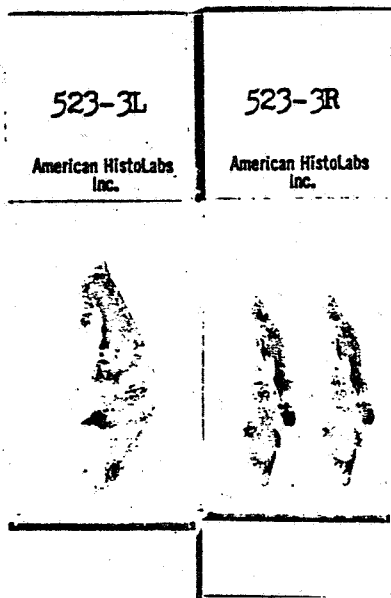
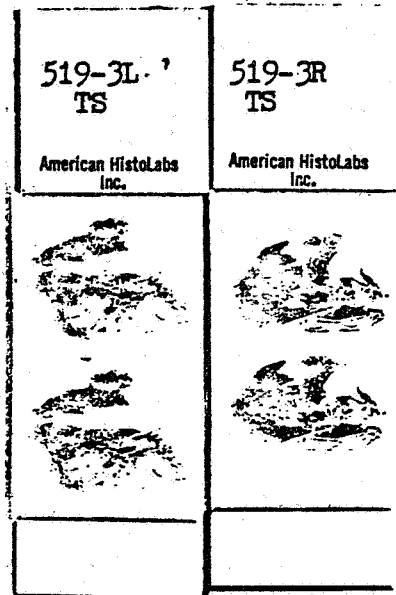
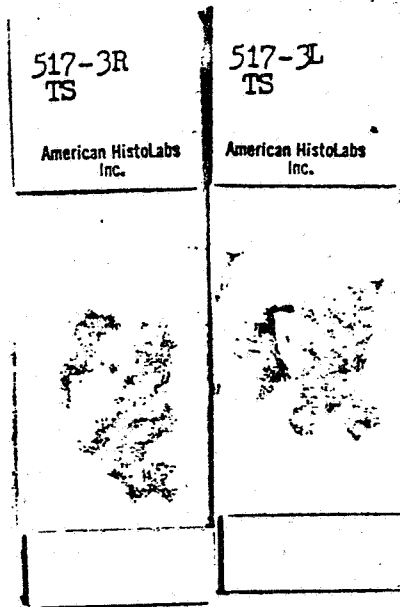
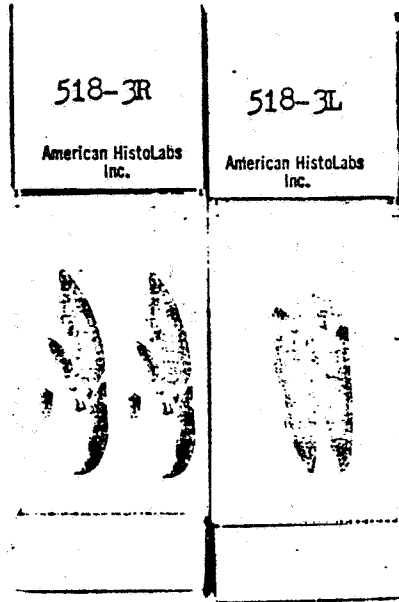


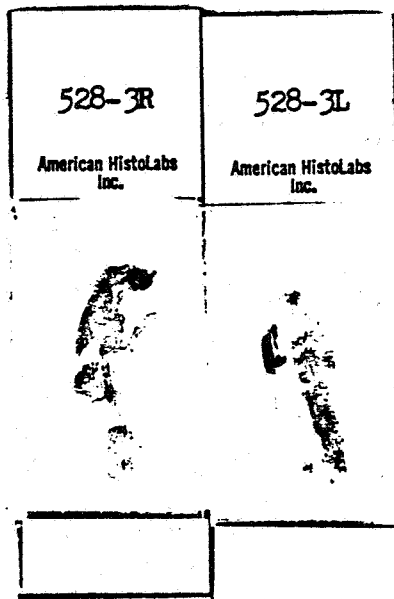
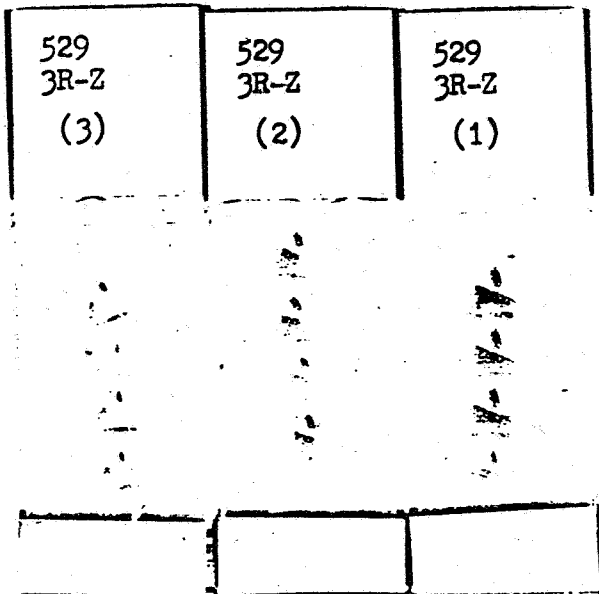
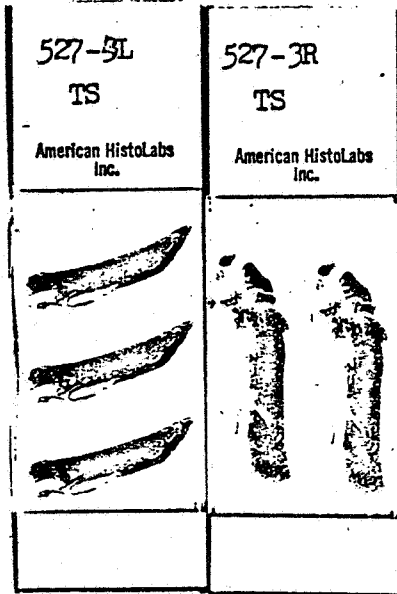
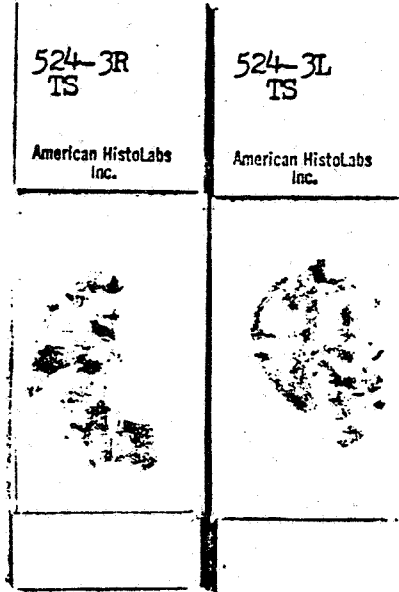
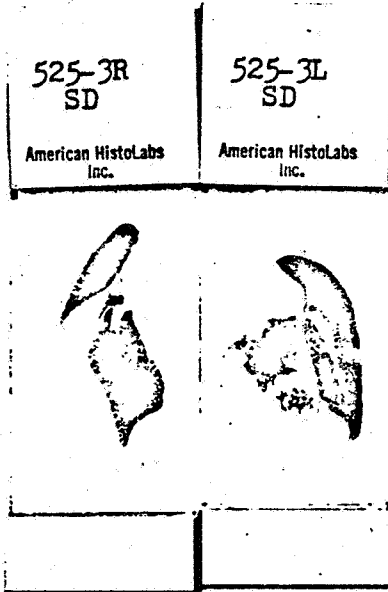


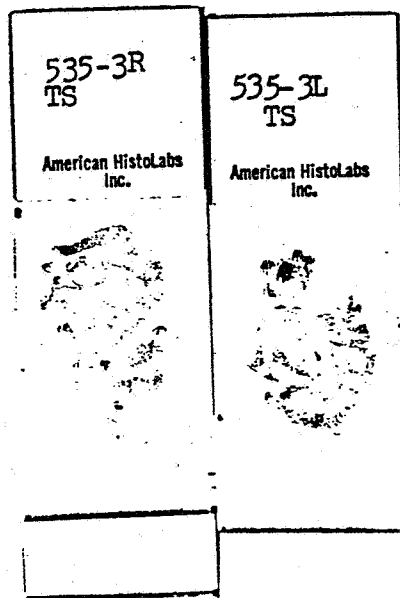
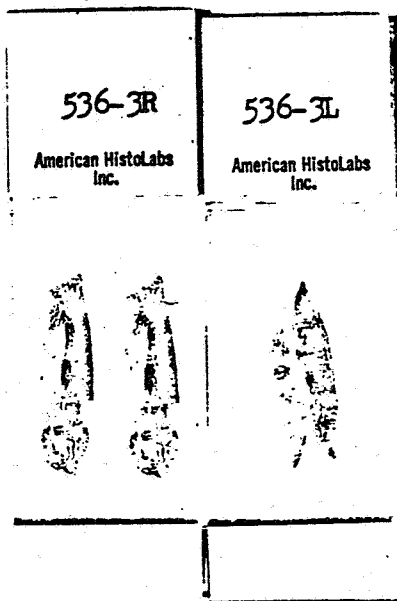
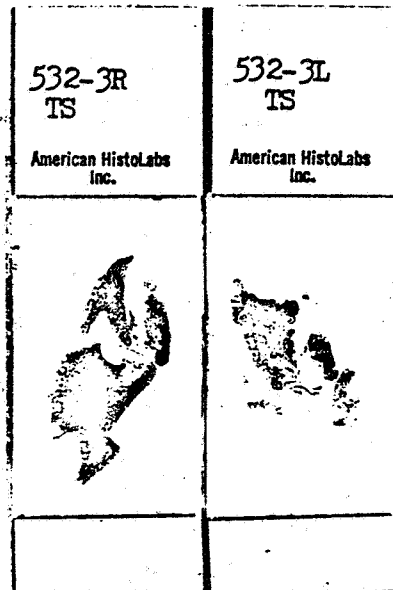
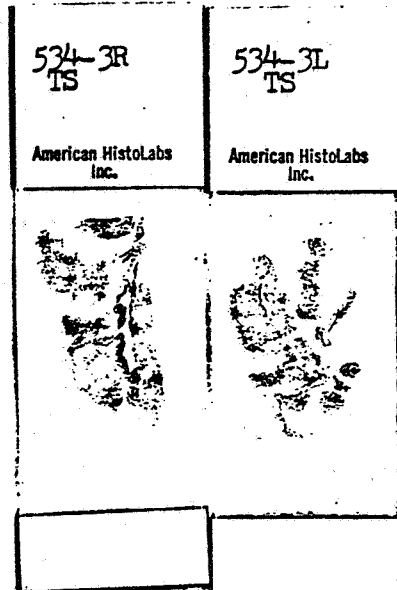
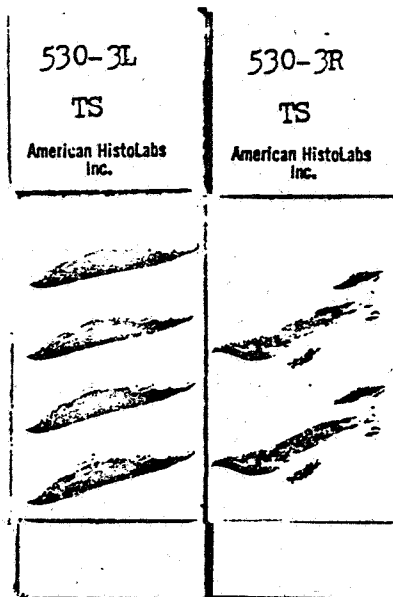
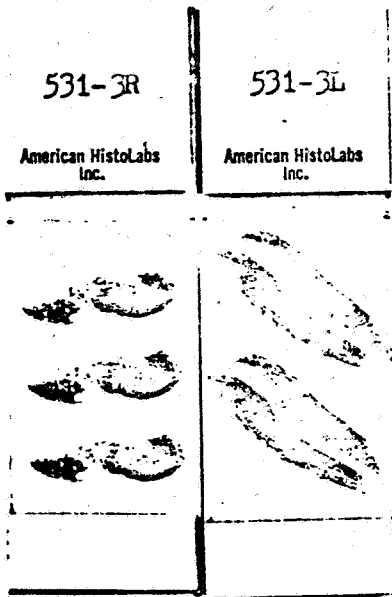


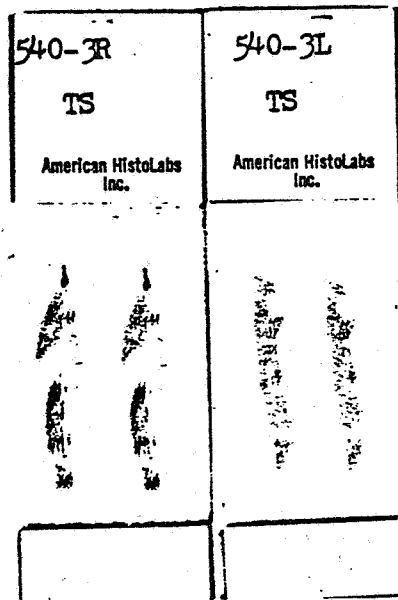
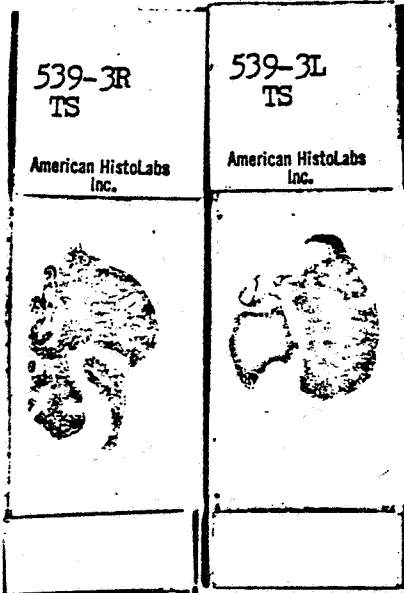
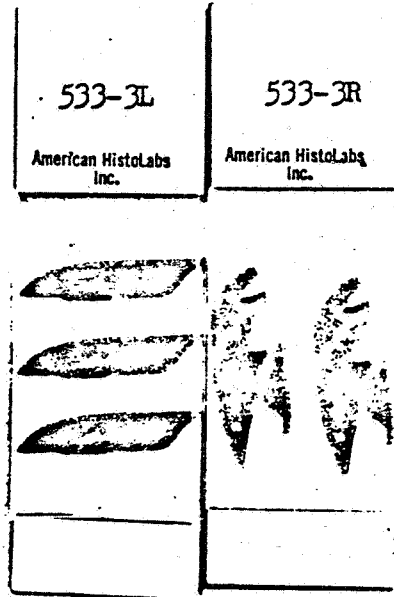
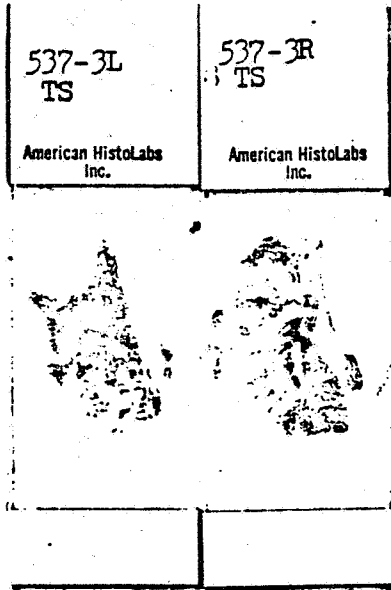

















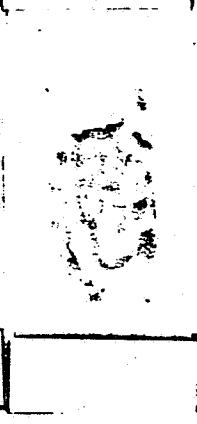


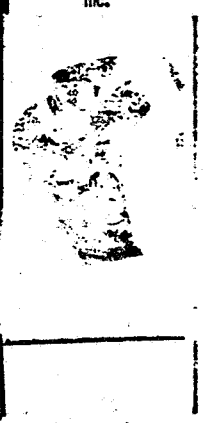
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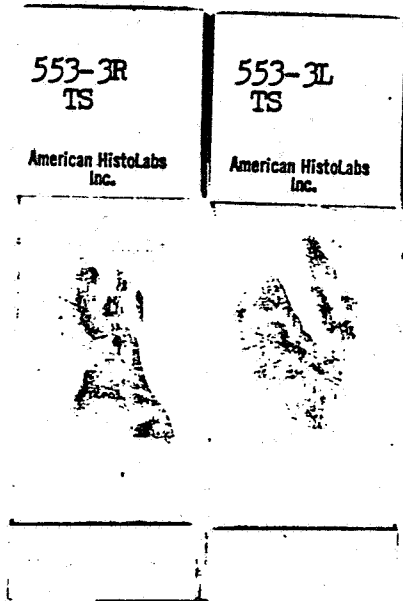
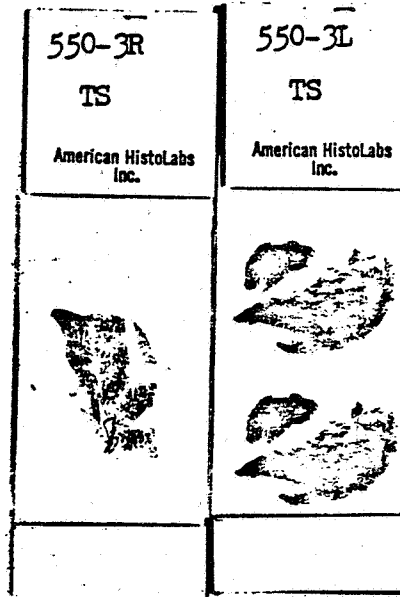
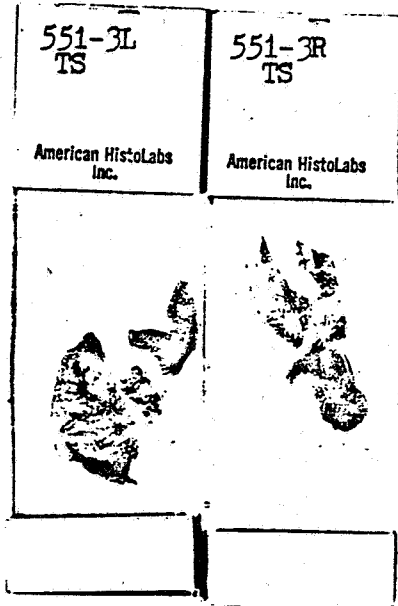
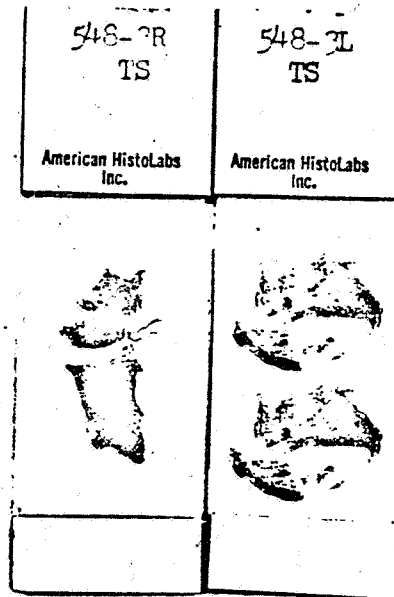
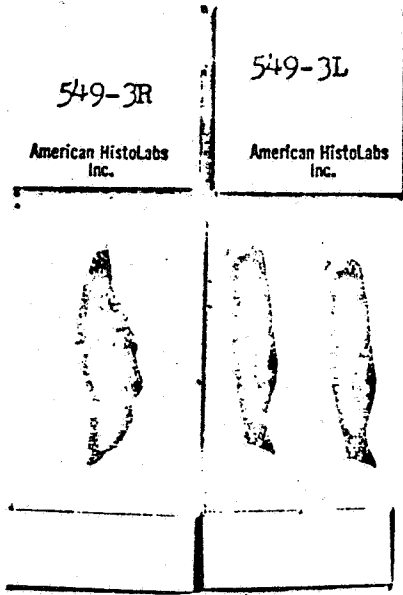
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<p>545-3R</p> <p>American Histolabs Inc.</p>	<p>545-3L</p> <p>American Histolabs Inc.</p>
	

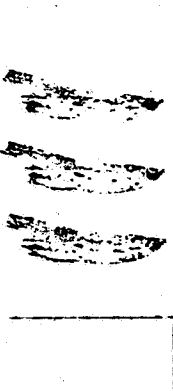
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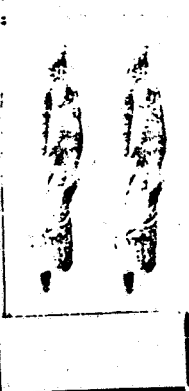
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556-3L
TS
American HistoLabs Inc.



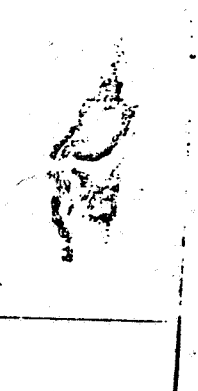
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American HistoLabs Inc.



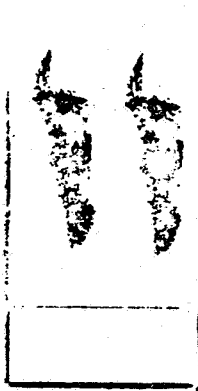
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American HistoLabs Inc.



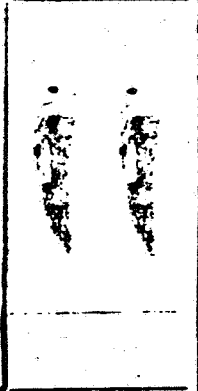
555-3R
TS
American HistoLabs Inc.



554-3R
American HistoLabs Inc.



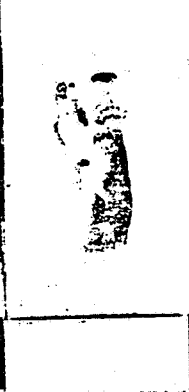
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American HistoLabs Inc.



559-3L
TS
American HistoLabs Inc.



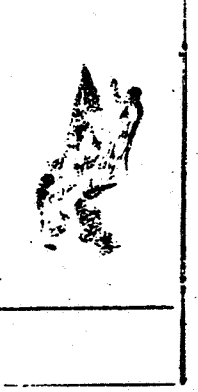
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American HistoLabs Inc.



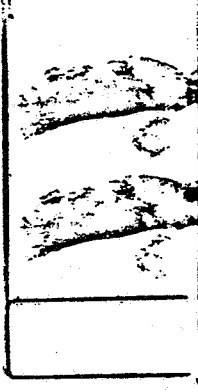
558-3L
TS
American HistoLabs Inc.



558-3R
TS
American HistoLabs Inc.



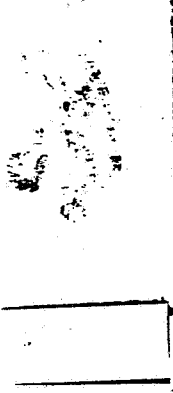
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American HistoLabs Inc.



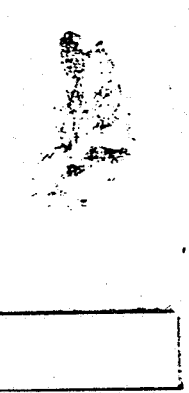
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TS
American HistoLabs Inc.



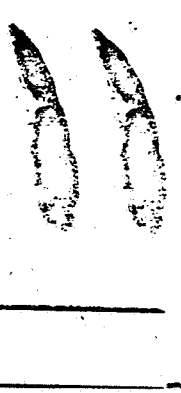
562-3L
TS
American HistoLabs Inc.



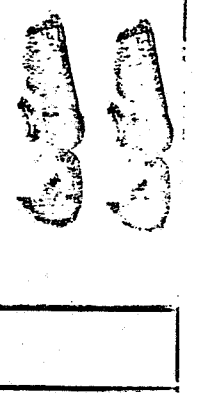
562-3R
TS
American HistoLabs Inc.



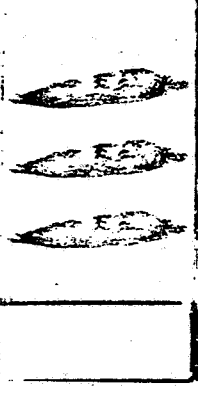
561-3L
American HistoLabs Inc.



561-3R
American HistoLabs Inc.



560-3L
American HistoLabs Inc.



560-3R
American HistoLabs Inc.



564-3L TS American Histolabs Inc.	564-3R TS American Histolabs Inc.

563-3R TS American Histolabs Inc.	563-3L TS American Histolabs Inc.




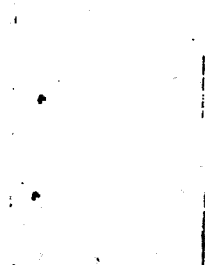

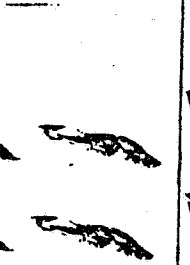
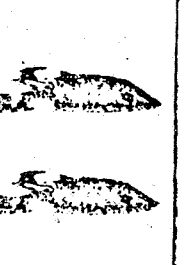
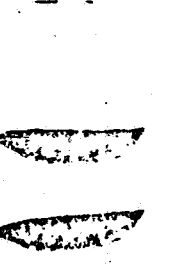
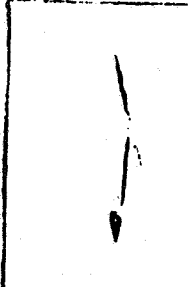
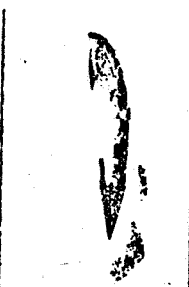
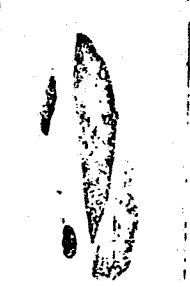
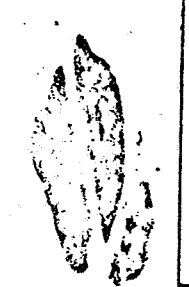

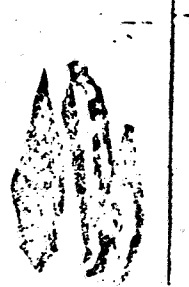
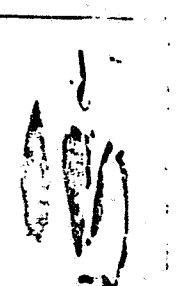
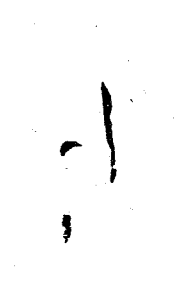



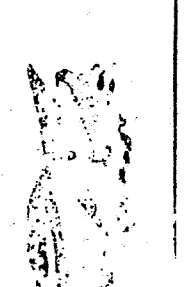
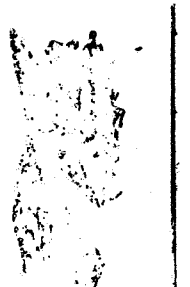
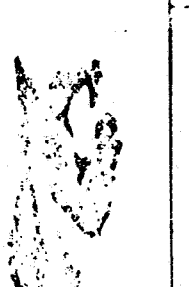
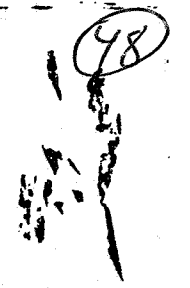
566-3L American Histolabs Inc.	566-3R American Histolabs Inc.

565-3L TS American Histolabs Inc.	565-3R TS American Histolabs Inc.

569-3R TS American Histolabs Inc.	569-3L TS American Histolabs Inc.













567-3L TS American Histolabs Inc.	567-3R TS American Histolabs Inc.

STEP-SECTIONING OF ANIMAL NUMBER 538

538-3R Step 5 	538-3R Step 3 	538-3R Step 1 	538-3L Step 9 	538-3L Step 7 	538-3L Step 5 	538-3L Step 3 	538-3L Step 1 
	538-3A Step 13 	538-3A Step 11 	538-3A Step 9 	538-3A Step 7 	538-3A Step 5 	538-3A Step 3 	538-3A Step 1 
538-3B Step 15 	538-3B Step 13 	538-3B Step 11 	538-3B Step 9 	538-3B Step 7 	538-3B Step 5 	538-3B Step 3 	538-3B Step 1 

98

STEP-SECTIONING OF ANIMAL NUMBER 757

757-3R Step 11	757-3R Step 9	757-3R Step 7	757-3R Step 5	757-3R Step 3	757-3R Step 1
					
757-3L Step 11	757-3L Step 9	757-3L Step 7	757-3L Step 5	757-3L Step 3	757-3L Step 1
					
		X	X	X	X

BIO/BIOMEDICALS INC. POST-MORTEM EXAMINATION RECORD

Sponsor F-1

Test Substance FMC-33297

Anesthetic: Pentobarbital, Sodium

Chloroform

Ether

Other: _____

Prosector(s): D. Bohm

I.D. Markings: Correct

Sacrifice: Interim

Terminal

Spontaneous

Moribund

Other: _____

Species: Rat

Rabbit

Mouse

Dog

Monkey

Other: _____

Path. No.	_____
An. No.	<u>742</u>
Project No.	<u>74R-1022</u>
Sex	<u>♂</u>
Group	<u>II</u>
Dose	<u>20 ppm</u>
Date Death	<u>7/1/77</u> <small>7-1-77</small>
Test Day	<u>736</u>

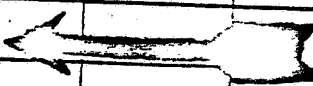
Term. Body Wt.: 565.0g

Clinical Signs: Chronic S(B), (S) Dry scales, (S) A G Stomach

Tissues Not Preserved: _____

Remarks Pit. Wt. - 0.0130g

Gross Pathology: (R=Right; L=Left; B=Bilateral)

Tissue and/or Lesion	R/L/B	Color	Shape and Surface	Consistency	Size (cm)	Weight (gms)	Other (Check and Describe Below)
<u>Liver</u>							
<u>Adrenal</u>	<u>(L)</u>	<u>TAN</u>	<u>with a black focus present</u>				<u>(1)</u>
<u>Kidneys</u>	<u>(B)</u>	<u>Reddish pink color</u>	<u>slightly pitted</u>				<u>0.04</u>
<u>H to 10 B</u>							

Other (Further remarks or other description of tissue checked above):
The left lateral lobe of the liver was adhered to a lobe below it and the cardiac portion of the stomach was adhered to this area. Liver is firm in the area of adhesion.

Photographs: Number(s) Taken: _____

See Over

POSTMORTEM EXAMINATION RECORD

Sponsor 701
 Test Substance FAL - 32297

Sacrifice: Interim
 Terminal
 Spontaneous
 Moribund

Path. No.	
An. No.	<u>352</u>
Project No.	<u>74R-1022</u>
Sex	<u>♂</u>
Group	<u>III</u>
Dose	<u>100 ppm</u>
Date Death	<u>5/27/57</u>
Test Day	<u>701</u>

Anesthetic: Pentobarbital, Sodium
 Chloroform
 Ether
 Other: _____

Other: _____
 Species: Rat
 Rabbit
 Mouse
 Dog
 Monkey
 Other: _____

Prosector(s): Kidney Histology

I.D. Markings: OK

Term. Body Wt.: 356 g

Clinical Signs: M - AG Blood little slow AG M - red nasal

Tissues Not Preserved:

Remarks: (circled) 2/1

Gross Pathology: (R=Right; L=Left; B=Bilateral)

Tissue and/or Lesion	R/L/B	Color	Shape and Surface	Consistency	Size (cm)	Weight (gms)	Other (Check and Describe Below)
<u>Kidneys</u>	<u>B</u>	<u>Brown Black</u>	<u>E Pitted</u>				
<u>Pancreas</u>		<u>Blue-green</u>					<u>✓</u>
					<u>1.0 x 1.8</u>	<u>1.38</u>	
<u>IT 10</u>							
<u>Total 12 Blocks</u>							

Other (Further remarks or other description of tissue checked above):
1 Kidney has fluid-filled cyst 0.6 x 0.5 cm

Photographs: Number(s) Taken: _____

See Over:

AFFIDAVIT

STATE OF

Michigan

COUNTY OF

Van Buren

Before me, D. M. Erspamer, an employee of the Department of Health, Education, and Welfare, Food and Drug Administration, designated by the Secretary, under authority of the Act of January 31, 1925, 43 Statutes at Large 803 (5 U. S. C. 521); Reorganization Plan No. IV, Secs. 12-15, effective June 30, -1940; and Reorganization Plan No. 1 of 1953, Secs. 1-9, effective April 11, 1953, to administer or take oaths, affirmations, and affidavits, personally appeared Dr. Mohammad Asif in the county and State aforesaid, who, being duly sworn, deposes and says:

I am presently employed by International Research and Development Corporation Mattawan, Michigan, as Chief Histologist. A copy of my C.V. is attached to this affidavit. From June 1975 to August 1978 I was employed by American Histo-Labs Incorporated, Rockville, Maryland. My primary duties with American Histo-Labs included trimming of tissues, microtoming and staining of tissues.

During my employment at American Histo-Labs one of the animal studies I worked on was the FMC Chronic Rat Study, with Permethrin, NCT #549.32. I had primary responsibility for the trimming of tissues on this study.

On 10-23-81 I was interviewed by EPA representatives Dr. Adrian Gross, and Gary Burin, and FDA investigator D. M. Erspamer. I was shown original necropsy sheets from the above study, and recognized my own handwriting on some of the necropsy sheets, for example animals #110 through #115. I was also shown some of the slides of lung tissue from male animals on this study. I explained the manner in which I collected sections of lung tissue to be embedded. The method of sectioning is illustrated on the attached diagram, and is described as follows:

In general, for the left lung, a transverse or (cross) section of the lung was

AFFIANT'S SIGNATURE AND TITLE

Handwritten signature

FIRM'S NAME AND ADDRESS (Include ZIP Code)

Handwritten address

Subscribed and sworn to before me at Mattawan, Michigan (City and State)

this 23rd day of October, 19 81.

Handwritten signature of D.M. Erspamer
(Employee's Signature)

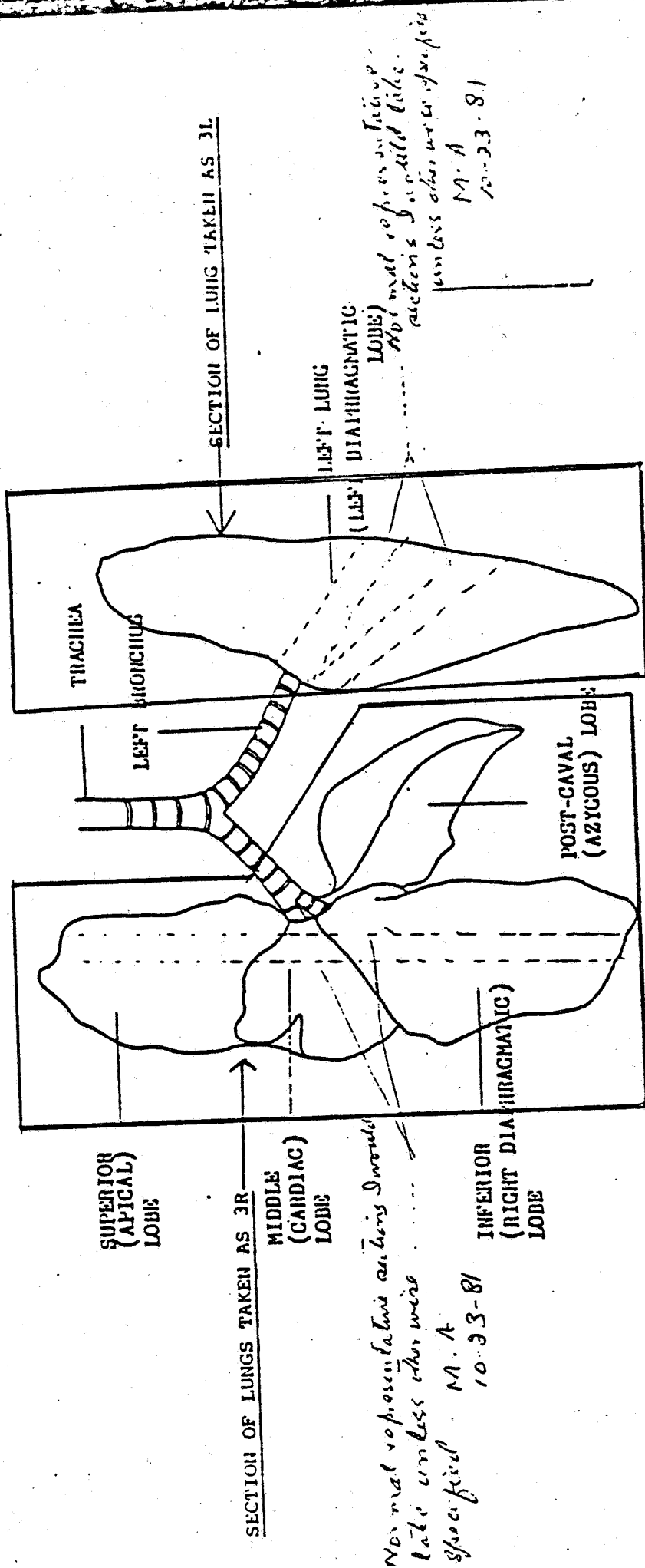
Employee of the Department of Health, Education, and Welfare designated under Act of January 31, 1925, Reorganization Plan IV effective June 30, 1940; and Reorganization Plan No. 1 of 1953, effective April 11, 1953.

Handwritten number 52

AFFIDAVIT		SAMPLE NO.
<small>STATE OF</small> Michigan	<small>COUNTY OF</small> Van Buren	
<p>Before me, <u>D. M. Erspamer</u>, an employee of the Department of Health, Education, and Welfare, Food and Drug Administration, designated by the Secretary, under authority of the Act of January 31, 1925, 43 Statutes at Large 803 (5 U. S. C. 521); Reorganization Plan No. IV, Secs. 12-15, effective June 30, -1940; and Reorganization Plan No. 1 of 1953, Secs. 1-9, effective April 11, 1953, to administer or take oaths, affirmations, and affidavits, personally appeared <u>Dr. Mohammad Asif</u> in the county and State aforesaid, who, being duly sworn, deposes and says:</p> <p>collected for sectioning. For the right lung, where there are several distinct lobes of the lung, usually a longitudinal cross section was collected ^{SC 117 8} as to provide a representative section of each of the lobes. The remaining portions of the lung were placed back in the original wet tissue containers, and were retained.</p>		
<small>AFFIANT'S SIGNATURE AND TITLE</small> <u>Dr. Mohammad Asif</u>		
<small>FIRM'S NAME AND ADDRESS (Include ZIP Code)</small> <u>10-23-81</u>		
Subscribed and sworn to before me at <u>Mattawan, Michigan</u> <small>(City and State)</small>		
this <u>23rd</u> day of <u>October</u> , 19 <u>81</u> .		
<u>D. M. Erspamer</u> <small>(Employee's Signature)</small>		
<small>Employee of the Department of Health, Education, and Welfare designated under Act of January 31, 1925, Reorganization Plan IV effective June 30, 1940; and Reorganization Plan No. 1 of 1953, effective April 11, 1953.</small>		

53

FIGURE #2 - RAT LUNGS - TAKEN AS HALVES



ALL LOBES DRAWN APART FOR IDENTIFICATION
(VENTRAL VIEW)

AFFIDAVIT

Attachment 7, p. 1

STATE OF

Maryland

COUNTY OF

Montgomery

Before me, Rodney T. Allnutt, an employee of the Department of Health, Education, and Welfare, Food and Drug Administration, designated by the Secretary, under authority of the Act of January 31, 1925, 43 Statutes at Large 803 (5 U. S. C. 521); Reorganization Plan No. IV, Secs. 12-15, effective June 30, 1940; and Reorganization Plan No. 1 of 1953, Secs. 1-9, effective April 11, 1953, to administer or take oaths, affirmations, and affidavits, personally appeared Richard J. Verfuert in the county and State aforesaid, who, being duly sworn, deposes and says:

I am presently Technical Advisor for American HistoLabs, Inc. Prior to this, I was Director of Laboratories at this firm from October 15, 1973 until September 11, 1981. In addition, I was Vice President of this corporation from October 15, 1973 until December of 1975, when I was appointed President of this corporation. I served as President from December 1975 until September 11, 1981, when Mr. Lee G. Luna took over the office of President and assumed sole ownership of this firm.

On September 21st, 1981 FDA Investigator Rodney T. Allnutt accompanied by Gary J. Burin and M. Adrian Gross of the Hazard Eval. Div., Office of Pesticide Programs, EPA, visited the firm where I am employed. In response to their inquiries, I provided the following information relative to a rat study on Permethrin carried out for FMC by Biodynamics Inc. of Princeton, NJ, whose tissues were processed by my firm, American HistoLabs.

Approximately on ^{RTV} ~~Early~~ 1977 Dr. Schoening of FMC contacted me for the purpose of exploring the processing of these tissues.

I modified ^{THE RTV} a mouse tissue disposition form ^{FROM THE STUDY FROM THE COMPANION MOUSE STUDY RTV} to include the tissue handling he requested, a copy was provided to the Investigator.

Sometime after delivery of these tissues and subsequent to their examination by Dr. Leonard Billups of Rockville, MD, Dr. Schoening contacted me again.

Dr. Schoening informed me that Dr. Billups' examination raised certain concerns relative to lesions in the lungs of the male animals; he requested that the entire remaining lung tissue be examined. I suggested a step sectioning plan to Dr. Schoening. He accepted this plan and his acceptance is confirmed in Dr. Schoening's letter of ^{RTV} MAY 1, 1978. A copy of Dr. Schoening's letter was provided to the investigator.

Page I of XII

AFFIANT'S SIGNATURE AND TITLE

Richard J. Verfuert

FIRM'S NAME AND ADDRESS (Include ZIP Code)

AMERICAN HISTOLABS INC.
ROCKVILLE, MD.

4540 WYACONDA RD.
20852

Subscribed and sworn to before me at Rockville, MD. (City and State)

this 23rd day of September, 1981.

Rodney T. Allnutt
(Employee's Signature)

Employee of the Department of Health, Education, and Welfare designated under Act of January 31, 1925, Reorganization Plan IV effective June 30, 1940; and Reorganization Plan No. 1 of 1953, effective April 11, 1953.

55

55

AFFIDAVIT

STATE OF

Maryland

COUNTY OF

Montgomery

Before me, Rodney T. Allnutt, an employee of the Department of Health, Education, and Welfare, Food and Drug Administration, designated by the Secretary, under authority of the Act of January 31, 1925, 43 Statutes at Large 803 (5 U. S. C. 521); Reorganization Plan No. IV, Secs. 12-15, effective June 30, 1940; and Reorganization Plan No. 1 of 1953, Secs. 1-9, effective April 11, 1953, to administer or take oaths, affirmations, and affidavits, personally appeared Richard J. Verfuert in the county and State afore-said, who, being duly sworn, deposes and says:

Page II of III *RN*

Sometime ^{*PRIOR TO*} ~~after~~ delivery of these step sections of the lung of the male rats, ~~Dr. Schering requested~~ ^{*PROVIDED TO DR. SEMOUSE*} certain clarifications relative to differenced in total numbers of blocks and sections from each animal. I provided this to him verbally.

In June of this year, Dr. Donald Nye of FMC visited me here to discuss the manner in which the lung sections were prepared. I explained this to him and followed this up with a letter dated July 3rd, 1981. A copy of this letter was provided to the Investigator. Dr. Nye had suggested that a "~~strongly worded letter~~" would be most helpful and the letter be written as soon as possible. *A LETTER EXPLAINING THE METHODS BY WHICH THE RAT LUNGS WERE HANDLED.*

The investigators and I discussed the contents of this letter in great detail. During this discussion I examined grossly a number of stained sections of the lung prepared by my firm, American Histolabs which the investigators brought with them.

I told the visitors that although I was substantially involved with the trimming of the mouse tissue, my involvement with the trimming of the rat tissues was much more limited, at best. I told the investigators that Dr. Asif (currently with International Research and Development Corporation of Matawah, Mich., was the supervisor in charge of trimming the rat tissues. Others under his supervision and involved in trimming were: Marjorie Henney and Mohammed A. Ansari. I suggested that the investigators contact Asif for details concerning the trimming of the rat tissues.

I explained to the investigators that my letter to Dr. Nye was intended by me to convey a reasonable description of what I thought had actually occurred in the processing of these lungs. I realize now that my opinions were largely conjecture based on past experience that I have had trimming tissue over the years, but that what appears in the letter as a statement of fact

Page II of III

AFFIANT'S SIGNATURE AND TITLE

FIRM'S NAME AND ADDRESS (Include ZIP Code)

Richard J. Verfuert
AMERICAN HISTOLABS, INC.
ROCKVILLE, MD.

4940 WYACINDA RD
20852

Subscribed and sworn to before me at Rockville, Md. (City and State)

this 23rd day of September, 1981.

Rodney T. Allnutt
(Employee's Signature)

Employee of the Department of Health, Education, and Welfare designated under Act of January 31, 1925, Reorganization Plan IV effective June 30, 1940; and Reorganization Plan No. 1 of 1953, effective April 11, 1953.

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AFFIDAVIT

Attachment 7, p. 2

STATE OF

Maryland

COUNTY OF

Montgomery

Before me, Rodney T. Allnutt, an employee of the Department of Health, Education, and Welfare, Food and Drug Administration, designated by the Secretary, under authority of the Act of January 31, 1925, 43 Statutes at Large 803 (5 U. S. C. 521); Reorganization Plan No. IV, Secs. 12-15, effective June 30, 1940; and Reorganization Plan No. 1 of 1953, Secs. 1-9, effective April 11, 1953, to administer or take oaths, affirmations, and affidavits, personally appeared Richard J. Verfuert in the county and State aforesaid, who, being duly sworn, deposes and says:

Page III of III

TO MY RECOLLECTIONS RTJ

is in fact speculation. For this study, I never personally observed anyone actually trimming away periferal lung tissue of the rats as I described in my letter and as illustrated in the accompanying diagrams to that letter.

I wrote the letter wihtout having the benefit of consulting with Dr. Asif or anyone of the persons who actually performed the routine tissue trimming and blocking for this study, and I did not reexamine the slides for this study before writing the letter. I told the investigators that the initials of each individual who performed the blocking of tissues for each animal could be found on necropsy record forms which had been returned to FMC with the slides, blocks, and tissues. I also explained that all the blocks for one animal were identified in each individuals handwriting and that all blocks for one animal were usually processed by only one individual.

I provided the investigators copies of American HistoLabs, Inc. Invoices #1773, #1814, #1822, #1825, #1827, and #1837 which cover shipments of stained slides, unstained slides, and soft tissue billed to FMC Corporation, Agricultural Chemical Division in Middleport, NY 14105 and sent to Dr. Leonard H. Billups of Rockville, MD.

This is my own statement and in no way reflects opinions of American Hist Labs, Inc.

AFFIANT'S SIGNATURE AND TITLE

Richard J. Verfuert

FIRM'S NAME AND ADDRESS (Include ZIP Code)

AMERICAN HISTOLABS, INC.
ROCKVILLE, MD. 20852

4940 WYACONOA RD.

Subscribed and sworn to before me at

Rockville, Md.
(City and State)

this 23rd day of September, 1987

Rodney T. Allnutt
(Employee's Signature)

Employee of the Department of Health, Education, and Welfare designated under Act of January 31, 1925, Reorganization Plan IV effective June 30, 1940; and Reorganization Plan No. 1 of 1953, effective April 11, 1953.

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AFFIDAVIT

STATE OF

Maryland

COUNTY OF

Montgomery

Before me, Rodney T. Allnutt, an employee of the Department of Health, Education, and Welfare, Food and Drug Administration, designated by the Secretary, under authority of the Act of January 31, 1925, 43 Statutes at Large 903 (5 U. S. C. 521); Reorganization Plan No. IV, Secs. 12-15, effective June 30, 1940; and Reorganization Plan No. 1 of 1953, Secs. 1-9, effective April 11, 1953, to administer or take oaths, affirmations, and affidavits, personally appeared Kenneth A. Rawley in the county and State aforesaid, who, being duly sworn, deposes and says:

I am Senior Laboratory Supervisor at American HistoLabs, Inc. I have been employed here in a supervisory capacity since 1974. *FR March*

On September 21st, 1981 FDA Investigator Rodney T. Allnutt accompanied by Gary J. Burin and M. Adrian Gross of the Hazard Evaluation Division, Office of Pesticide Programs, EPA, visited the firm where I am employed. In response to their inquiries I provided the following information relative to a rat study on Permethrin carried out for FMC by Biodynamics Inc., for which the tissues from animals on that study were processed here.

As I recollect, my role was primarily that of quality control of the final product at the time of coverslipping the rat slides. I was not directly involved in the trimming of lung tissues - Dr. Asif had this responsibility.

The personnel that I remember, who were involved in the trimming of tissues for this study under Dr. Asif's supervision, were Mohammed Ansari, Mahboob Malik, Murtaza Malik, and Margie Henney. Having coverslipped the slides from this study, I feel that I can probably identify the person responsible for trimming and blocking the tissues for each animal because each block is numbered by that person doing the work, in their own handwriting. I also pointed out to the investigators that the blocking is recorded on necropsy records for each animal and that this is initialed by the person doing the work. Routinely, all blocks for one animal were prepared by the same person.

At the time I was working on the rat study slides, Mr. Verfuert was working almost exclusively on a mouse study involving this same test compound. He was responsible for trimming tissues for the mouse study and also for coverslipping and final quality control of that study.

AFFIANT'S SIGNATURE AND TITLE

Kenneth A. Rawley Lab Supervisor

FIRM'S NAME AND ADDRESS (Include ZIP Code)

AHL

Subscribed and sworn to before me at Rockville, Maryland (City and State)

this 23rd day of September, 1981.

Rodney T. Allnutt
(Employee's Signature)

Employee of the Department of Health, Education, and Welfare designated under Act of January 31, 1925, Reorganization Plan IV effective June 30, 1940; and Reorganization Plan No. 1 of 1953, effective April 11, 1953.

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