

Re-learn

**A PROTOCOL
FOR EVALUATING HISTORICAL
CONTAMINATION OF MERCURY
IN THE FLORIDA PANTHER
AND OTHER FLORIDA WILDLIFE**

FINAL REPORT

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ABSTRACT

High levels of mercury have been found in the Florida panther, a top carnivore of the Everglades. Similarly high levels have been found in other mammals, birds, and fish from the Everglades and other parts of South Florida. The principal source of mercury contamination in the panther is from the aquatic food web. The time period and the source or sources of this contamination are not known.

The purpose of this study is to investigate the historical contamination of mercury in the Florida panther and other wildlife species by correlating the historical record of mercury content in wildlife tissues (i.e., hair and feathers) with anthropogenic activities involving mercury in Florida.

Fourteen bioindicator species have been identified for tissue sampling and analysis. Six historical periods will be sampled, each reflecting a period of generally different anthropogenic activities potentially contributing to mercury contamination in South Florida. For mammals, hair samples will be obtained by combing museum specimens. Protocol studies indicate that there is no significant difference in the mercury content in hair from different body locations. For birds, body feathers and primary feathers will be analyzed.

The report that follows presents the protocol development and specific details of the sampling protocol.

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1.0 INTRODUCTION

Alarmingly high levels of mercury have been found in freshwater fish and wildlife throughout the State of Florida, especially in the Everglades ecosystem of South Florida. Particular concern exists for the Florida panther (*Felix concolor coryi*), because very high levels of mercury [greater than 100 parts per million (ppm)] have been found in the liver of one specimen of this species. These levels have been correlated with high levels of mercury found in raccoons, which are considered a major prey species for at least one of the panther populations in the Everglades (Roelke, 1991; Roelke *et al.*, 1991). These reports indicate that mercury contamination in the aquatic food web has led to exposure in the Florida panther.

The length of time and the source or sources of contamination in the Florida panther and other species are unknown. Historical studies of mercury using museum specimens have correlated anthropogenic activities with mercury concentration, including one study on mercury content in feathers of Swedish birds (Berg *et al.*, 1966). In the Berg study, specimens over a 100-year time period were analyzed. Mercury concentration in feathers of several species of birds was 18 times higher during historical periods of mercurial pesticide use than in pre-use periods and declined after the use of mercurial pesticide stopped. The primary source of mercury contamination was the use of alkyl mercuric pesticides. A similar study (Johnels *et al.*, 1979) showed that mercury levels in the feathers of goshawks (*Accipiter gentilis*) and ospreys (*Pandion haliaetus*) were also higher (3 to 9 times) during periods of pesticide use than in pre-pesticide use periods.

The overall purpose of this study is to investigate the historical contamination of mercury in the Florida panther and other wildlife species. Specifically, this study will investigate whether the magnitude and spatial distribution of mercury contamination have changed in response to human activities. In addition, the study will investigate sources of mercury in the panther by attempting to correlate the historical record of mercury content in wildlife tissues with anthropogenic activities that may have released mercury into Florida's environment.

The project will evaluate whether a correlation exists between the timing of anthropogenic development in Florida and the methyl mercury levels detected in animal tissues. Museum specimens and recently collected specimens will be analyzed to determine the extent of mercury contamination over a period of 100 years, a period which reflects mercury use and mobilization in Florida. Particular emphasis will be given to the role of mercuric pesticides as a source of contamination.

The study is divided into two phases. Phase I involves a literature review and development of a mercury sampling protocol. Phase II involves a detailed investigation of the historical content of mercury in the Florida panther and other wildlife species. The Everglades will be the first area evaluated. Historical contamination will then be investigated in other regions of Florida with reported mercury contamination (i.e., Kissimmee valley, northwest coast up to Suwannee and Santa Fe Rivers, St. Johns River valley, and Suwannee River west to the Florida Panhandle) from periods preceding and following major development events.

An interim progress report (April 30, 1992) presented the literature review and conceptual approach to the sampling protocol. This report presents the results of the protocol development and a description of the sampling protocol.

2.0 MERCURY CONTAMINATION ASSESSMENT PROTOCOL

2.1 RATIONALE FOR PROTOCOL

The most direct way to evaluate the historical exposure of the Florida panther to mercury is to analyze preserved tissues, (i.e., hair samples) collected over time. Although recent panther tissue collections exist, the availability of hair samples from museum collections and from earlier time periods is limited for two reasons:

1. The number of panthers in museum collections is not sufficient for a detailed continuous historical analysis, and
2. Curators are reluctant to provide tissues for analysis.

The proximate source of mercury contamination for the Florida panther is through the aquatic food chain; therefore, the historical exposure of the Florida panther and Florida wildlife can be evaluated by using other species as bioindicators of historical mercury contamination. Other wildlife species are more accessible in museum collections and assure an adequate sample size due to a larger number of specimens available and a more complete historical record. The continuous record throughout Florida of available indigenous mammals and non-migrating birds from the nineteenth and twentieth centuries will facilitate the assessment.

2.2 PROTOCOL BIOINDICATORS

Two principal bioindicator groups have been identified to reflect aquatic food web exposure:

1. Aquatic carnivores known to be the prey of the panther [e.g., the raccoon (*Procyon lotor*)], and
2. Other wildlife species whose food habitats will be indicative of mercury contamination in the aquatic food chain [e.g., otters (*Lutra canadensis*) and piscivorous birds].

The selection of appropriate bioindicators for this study should meet several general criteria, including:

1. Recognizably distinct mammal and bird species or subspecies with geographical and historical representation similar to the panther;
2. Sedentary, non-migratory, or local breeding;
3. Relatively ubiquitous throughout habitat(s) for which adapted; and
4. Relatively common and not presently threatened or endangered.

Based on the review of literature and discussions with project committee staff (see Appendix A), fourteen bioindicator species have been chosen and will be used as bioindicators to assess the historical bioavailability of mercury and its potential impact on the Florida panther (See Table 2-1). Since the historical and geographical record may not be complete for any one species, multiple mammal and bird species will be sampled. Paired comparisons with present-day species will be used to evaluate significant historical differences.

Species have been selected based on aquatic and terrestrial food web exposure to mercury in South Florida and their likely availability in collections. Sampling of the hair from the Florida panther will be done on an as-available basis.

The protocol bioindicators are grouped into species reflecting aquatic food web exposure, including major prey species of the panther, surrogate mammalian predatory species, surrogate avian species, and analogous mammalian species.

Discussions with the advisory staff indicated that historical data is also needed to confirm the importance of aquatic versus terrestrial food web exposure to mercury. As a consequence, mammalian and avian species reflecting terrestrial food web exposure will also be sampled.

Of the two animal types, birds are more mobile than mammals, and the exposure location for birds may not be as certain as for mammalian specimens. To control for this uncertainty, birds will be sampled during life history stages (e.g., immature conditions) where their residency, food gathering behavior and, therefore, food web exposure are known.

2.3 PERIODS OF ANALYSIS

The study has been divided into six distinct periods encompassing potentially critical anthropogenic activities associated with potential mercury mobilization, use, and emissions in Florida. Samples from museum specimens from these various period will be analyzed and compared to present-day values to determine possible trends in mercury contamination and their association with the anthropogenic activities of each period.

Period 1 Prior to 1883, a period of no hydrological alteration

Period 2 1883 to 1910, a period of initial channelization and farming in the Everglades

Period 3 1910 to 1940, a period of major channelization

Period 4 1940 to 1970, a period of heavy mercuric pesticide use and initiation of drainage pumping and intense cultivation

Table 2-1. Protocol Bioindicator Species (Page 1 of 2)

Common Name	Scientific Name	Food Habits	Individuals To Be Sampled
Primary Prey Items of Panthers From Non-urbanized Areas of the Everglades and South Florida			
coons	<i>Procyon lotor</i>	Generally omnivorous, but animals found in aquatic environments feed primarily on aquatic vertebrates and invertebrates	Adults
er	<i>Odocoileus virginianus</i>	Plant material	Adults
al hogs	<i>Sus domesticus</i>	Plant material	Adults
Surrogate Mammalian Predatory Species Reflecting Aquatic Food Web Mercury Exposure From Non-urbanized Areas of the Everglades and South Florida			
rs	<i>Lutra canadensis</i>	Feeds on aquatic vertebrates and invertebrates	Adults
Surrogate Avian Species Reflecting Aquatic Food Web Mercury Exposure From Non-urbanized Areas of the Everglades and South Florida.			
-billed grebe	<i>Podilymbus podiceps</i>	Feeds on small fish and aquatic invertebrates	Summer adults, which are residents
kin	<i>Aramus quarauna</i>	Feeds on mollusc and aquatic invertebrates	Adults, regionally sedentary
nga	<i>Anhinga anhinga</i>	Feeds on fish	Immature or breeding adults
Blue Heron	<i>Ardea herodias</i>	Feeds on fish and other small vertebrates	Immature or breeding adults

Table 2-1. Protocol Bioindicator Species (Page 2 of 2)

Common Name	Scientific Name	Food Habits	Individuals To Be Sampled
Great Egret	<i>Casmerodius albus</i>	Feeds on fish and other small vertebrates	Immature or breeding adults
Green-backed Heron	<i>Bustroides striatus</i>	Feeds on fish	Immature and breeding adults
Mottled Duck	<i>Anus fulvigula</i>	Feeds primarily on vegetation, summer diet made up of aquatic invertebrates	Adults, resident species, post molting feathers reflects geographically specific conditions

D. Analogous Mammalian Predator Species Reflecting Mercury Exposure in Terrestrial Food Web

Bobcat	<i>Lynx rufus</i>	Feeds primarily on terrestrial vertebrates	Adults
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E. Analogous Avian Species Reflecting Mercury Exposure in Terrestrial Food Web

Northern Bobwhite Quail	<i>Colinus virginianus</i>	Feed primarily on grains, seeds, and insects	Adults, a resident species
Northern Cardinal	<i>Cardinalis cardinalis</i>	Feeds primarily on seeds, fruits, and insects when abundant	Adults

Period 5 1970 to 1980, a period of reduced mercuric pesticide use, initiation of operation of solid waste incinerators and medical waste incinerators, and increased use of fossil fuels

Period 6 1980 to Present, a period of operation of solid waste incinerators, medical waste incinerators, and fossil fuel utilization by power plants

The period prior to 1883 is considered a period free of anthropogenic activities that may have resulted in mercury release into the Florida environment. Any mercury found in tissues from this period is assumed to reflect background values. The periods from the 1880s to 1940 is a time when hydrological modification of the Everglades and intensification of agriculture in South Florida had the potential to mobilize naturally occurring mercury with subsequent methylation and food web contamination. From the 1940s to the present day, mercury has been introduced to Florida from a variety of human activities, such as mercuric pesticides and emissions from solid waste incinerators.

The initial geographical area of interest is South Florida as reflected by the general boundaries of the South Florida Water Management District.

2.3 TISSUE ANALYSIS CONSIDERATIONS

The tissues of the bioindicator species to be analyzed are hair and feathers. The use of hair and feathers as bioindicators of trace metals contamination, including mercury, has been well established (e.g., Jenkins, 1980).

Methyl mercury bioaccumulates in hair and feathers to a greater extent than in other tissues and, thus, can be used as a bioindicator of mercury exposure (Cumbie, 1975; Jenkins, 1980; Eisler, 1987).

Mercury concentrations in hair can be influenced by several factors. There are age-related differences: adult hair concentrations are higher than juvenile hair concentrations in raccoons (Cumbie, 1957). Species differences and trophic-level differences also exist. Ecological differences may include drought, which can affect food supply and change exposure conditions (Roelke, 1991). Cumbie and Jenkins (1974) and Roelke (1991) have shown that hair of carnivores has higher mercury concentrations than the hair of herbivores and omnivores. Studies by Roelke, Cumbie, and Jenkins on the hair of raccoons, deer, hogs, bobcats, and panthers indicate that geographical variation exists within a species, thus reflecting different exposures to mercury.

do not appear to be significant differences in the mercury content of hair from different parts of the body (Cumbie, 1975). This observation is confirmed in our study (see Section 3.0).

In mammals, the possibility of contamination by mercury due to the skin-tanning process in museum specimens exists. In order to test this possibility, a review of old tanning techniques was made. In the past, the techniques involved the use of alum salts, and mercury was not an ingredient. In response to our inquiries, the Chicago Museum identified a recipe used by the curators (see Appendix B). In addition, there is the possibility that mercury compounds may have been used to control pests such as moths or dermestids (see Appendix C).

To evaluate the potential for tanning techniques affecting mercury concentrations, raccoon skins will be analyzed for mercury under three scenarios: 1) no tanning, 2) old tanning methods, and 3) modern tanning methods. Tanning of samples is being performed by Sebring Custom Tanning in Sebring, Florida. Mercury analysis of tanned hair will be conducted to determine if any differences exist in mercury content results from tanning processes. Speciation of mercury content will be carried out on the museum sample collections to check for possible museum mercury contamination.

The concentration of mercury in hair appears stable. No differences in mercury content of hair are observed with different preparation procedures (e.g., washing with water, detergents, or organic solvents) (Cumbie, 1975).

Mercury is deposited in feathers only during feather growth (Goede and De Bruin, 1986). The excretion of mercury in feathers is considered the major excretory route for mercury in birds (Honda *et al.*, 1986). Up to 70 percent of the body burden of mercury exists in feathers. The mercury content can vary depending upon the type of feathers and certain ecological factors. Although different feather parts (e.g., the vane, shaft, distal, mid, and proximal portions) show some differences in metal content, metals such as mercury are accumulated in feathers more than in soft tissues. Whole feather analyses are the most commonly reported. Most contamination studies have chosen remiges or rectrices, normally primaries and secondaries or tail feathers, for analysis. We have assumed that these feathers are representative of the whole plumage or of the total mercury content of the bird.

Goede *et al.* (1986) studied the mercury content in several species of seabirds and showed that the variation due to molting is least in body feathers. Studies have shown variability in

mercury content in different feather types from the same species, although the variation is small compared to differences between feathers and other tissues. The best indication of geographical mercury exposure is from feathers that have recently molted (Applequist, *et al.*, 1984; Hanson and Jones, 1968).

Factors affecting the mercury content in feathers include species, age, and trophic levels. Differences do not appear significant (Hoffman and Curnow, 1979; Lindberg and Odsjo,

compared to other avian tissues, the mercury content in feathers is quite stable. Applequist *et al.* subjected feathers to ultraviolet light, heating [100 degrees Celsius (°C)], freezing, and weathering (rain, wind, and sun exposure) for 2 to 8 months. They found the loss of mercury due to exposure variation was less than 10 percent.

Studies have been conducted on the effects of different preparation techniques on mercury

Once in the feather matrix, mercury also appears to be quite stable and not dramatically affected by preparation techniques. Washing in distilled or fresh water is the best preparation technique, although tests have been conducted on washing in salt water, acids, and organic

Because of the volatility of mercury, air drying is used.

The possibility of mercury contamination during the preservation process is not as critical with feathers as with hair samples. This issue was investigated with respect to fumigants and chemicals used in bird study-skin preparation in both current and former practices. No source of contamination was uncovered from any substance or preparatory process either in recent times or from materials and methods used primarily in the first half of this century (personal communications, Dr. O. T. Owre, Curator of the University of Miami Reference Collection of Birds, and Dr. C. R. Hare, Associate Professor of Chemistry, University of Miami, July 1990).

Mercury concentrations in both hair and feathers can be correlated with concentrations of mercury in other tissues (e.g., muscle, kidney, liver, and bone) and with animal toxicity (Driver and Johnson, 1980; Vermeer and Armstrong, 1972; Cumbie, 1975). There appears to be up to a 10-fold difference of mercury content in feathers versus other tissues (Honda *et al.*, 1985; Heinz, 1979; and Forrester, 1991). Similar ratios have been reported for hair (Clark *et al.*, 1981; and Johnson, 1975).

2.5 SPECIMEN TISSUE AVAILABILITY

A sufficient supply of avian and mammalian specimens are available for conducting the proposed historical analysis. There are several sources for recently collected specimens, including the Florida Game and Freshwater Fish Commission (FGFWFC) collections of Dr. Roelke. She has panther and raccoon tissues from the late 1980s and early 1990s from various geographical locations in the Everglades as well as tissues from otters and bobcats. A reasonably complete record of Florida mammals and birds through this century is available from a combination of the collections. The following collections will be accessed:

- Museum of Natural History in Gainesville, Florida
- National Museum of Natural History (Smithsonian) in Washington, DC
- American Museum of Natural History in New York, New York
- Museum of Comparative Zoology at Harvard University in Cambridge, Massachusetts
- Field Museum of Natural History in Chicago, Illinois
- University of Miami Reference Collections in Miami, Florida

Dr. Earl Rich of the University of Miami and Dr. William Robertson of the National Park Service are responsible for identifying and obtaining museum samples. In evaluating specimen availability, emphasis will be placed on special collections of large numbers of individuals from one of the bioindicator groups.

3.0 RESULTS OF PROTOCOL DESIGN TESTING

To establish and confirm tissue analysis techniques and sources of variability, the following studies have been and are being undertaken as part of the protocol development phase of the project:

1. Sampling and analysis of hair from different parts of the body to confirm previously reported variability in other species,
2. Sampling of bird and mammal skins subjected to different preservation techniques to determine potential variability in mercury content due to preservation, and
3. Sampling and analysis of body feathers and primary feathers to confirm variation and establish baseline conditions for comparison with other studies and in other species.

Sampling and analysis of hair from raccoons and otters are complete. Hair samples were obtained from the FGFWFC collections of Dr. Melody Roelke. Raccoon samples represented animals from high- and low-exposure areas of the Everglades. Otters were from various locations in Florida. The statistical analysis of mercury in hair from different body locations (i.e., ventral, dorsal, general body combing) using a one-way or a two-way analysis of variance (ANOVA) revealed no significant difference in the mercury content in hair from different body locations for raccoons and otters (see Tables 3-1 and 3-2). This is consistent with Cumbe's (1975) findings for raccoons and bobcats.

The raccoon and otter data were used to calculate minimum sample sizes recommended for future collecting and hypothesis testing differences between populations (Zar, 1984). To distinguish 20 $\mu\text{g/g}$ differences between two raccoon populations, seven samples from each population should be obtained. Sample sizes of 12 and 15 from each raccoon population are recommended to distinguish differences of 15 and 10 $\mu\text{g/g}$, respectively. Mercury concentration variance in the otter population was lower than in the raccoon populations and resulted in fewer samples being required for hypothesis testing. Five samples from each otter population should be collected to distinguish 5 $\mu\text{g/g}$ differences and 12 samples to distinguish 2.5 $\mu\text{g/g}$ differences.

Sampling and analysis of different feather types is on-going. Similar analyses will be conducted to determine the minimum sample size for birds. An analysis of the observed variability in previous studies of mercury in feathers of different species of birds from a 100-year period (Berg, 1966) indicated a minimum sample size of 18 samples per period would be appropriate. Sample variability should be lower since individual life history information will be controlled for in the statistical analysis to facilitate data handling a time-series regression analysis.

Table 3-1. Comparison of Mercury Concentration ($\mu\text{g/g}$ wet weight) in Raccoon Hair from Different Body Locations

Sample	Combed Whole Pelt	Ventral	Dorsal
R11	3.91	4.31	3.78
R12	3.26	8.00	6.60
R13	3.36	4.32	6.09
R14	4.20	3.87	6.11
R15	12.58	9.52	12.25
R16	14.53	13.43	16.71
R61	55.49	65.84	59.55
R62	90.25	106.83	98.07
R63	50.43	48.16	50.41
R64	33.02	32.21	32.18
R65	31.41	41.87	35.15
R66	59.80	41.96	49.01

Source: KBN Engineering and Applied Sciences, Inc.

Table 3-2. Comparison of Mercury Concentration ($\mu\text{g/g}$ wet weight) in Otter Hair from Different Body Locations

Sample	Combed Whole Pelt	Ventral
86F404	5.30	4.64
86F473	0.83	1.22
87F720	3.76	4.03
N9103	8.04	6.28
N9104	7.45	4.51
N9109LCA	3.82	3.70
N9111LCA	23.40	16.67
N9112LCA	2.98	2.25
N9201	6.41	4.25
LCA931	3.48	2.92

Source: KBN Engineering and Applied Sciences, Inc.

4.0 RECOMMENDED PROTOCOL

4.1 TISSUE TYPE AND COLLECTION PROCEDURES

For mammals, both guard hair and fur will be analyzed for total mercury (and methyl mercury, depending upon the uncertainty of contamination during preservation).

Unless requested by curators to do otherwise, scientists will obtain hair by a general combing using a "slicker brush" similar to those used in dog and cat grooming. The brush will be cleaned of any residual hair after each specimen collection. If cutting of hair is required, a razor blade will be used to cut or scrape hair down to the skin. Care will be used to avoid collecting skin samples. Hair then will be weighed. At least 50 mg (preferably 100 mg) is required, and all collected hair will be wrapped in tissue paper (KimWipes) or other suitable material.

Samples will be labeled and stored in closed containers. Labels will include animal number (i.e., its museum number), sex, age (if known), weight, date and location of collection, how sample was obtained (i.e. combing versus cutting) and other pertinent data that may be available.

For birds, body feathers from the abdominal area will be collected. When appropriate and if available, primaries also will be collected.

At least 50 mg (preferably 100 mg) of feathers will be obtained by plucking. Samples will be labeled with information, including the animal number (i.e., its museum number), sex, age (if known), weight, date and location of collection, reproductive condition (if known), molting state, and other pertinent data that may be available. Samples will be placed in cellophane envelopes or other suitable material.

4.2 ANALYTICAL PROCEDURES

Methods recommended by the U.S. Environmental Protection Agency (EPA) and the Florida Department of Environmental Protection (FDEP) will be used to analyze for total and methyl mercury. Representative split samples will be sent to the U.S. Fish and Wildlife Service (USFWS) Patuxent Laboratory for quality assurance and quality control. Non-destructive techniques for panther hair are being investigated.

Hair and feathers will be analyzed for total mercury. Because of the possibility of mercury contamination in preservation, speciation of the mercury content will be carried out on representative samples from each museum collection group.

4.3 DATA ANALYSIS

Appropriate statistical analyses, including time-series regression analysis, will be used to correlate observed mercury concentrations within given time periods for the bioindicator species. Factors to be analyzed are those that contribute to variability, including time, species, geographical location, sex, age, trophic level, etc.

For mammals, efforts will be made to control for sample variability in mercury concentration by comparing samples for each period from animals at a similar life stage (e.g., adults) and the same geographical area. An attempt will be made to collect a sample size of 12 individuals for each species and historical period. This should allow for distinguishing a difference of 15 $\mu\text{g/g}$ between periods and geographical locations.

For birds, efforts will be made to control for variability by comparing samples collected from the same species, the same body location, similarly aged animals, representative geographical areas and similar locations, during the same season, and during similar reproductive and molt conditions.

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APPENDIX A
PROJECT COMMITTEE MEMBERS

APPENDIX A

HISTORICAL ANALYSIS OF MERCURY IN WILDLIFE PROJECT
COMMITTEE/PARTICIPANTS

Dr. Thomas Atkeson	—	Florida Department of Environmental Regulation
Dr. Craig Barber	—	U.S. Environmental Protection Agency
Dr. James Brady	—	Florida Game and Freshwater Fish Commission
Dr. Joseph Delfino	—	University of Florida
Dr. Charles Facemire	—	U.S. Fish and Wildlife Service
Dr. James Newman	—	KBN Engineering and Applied Sciences, Inc.
Dr. Earl Rich	—	University of Miami
Dr. William Robertson	—	National Park Service
Dr. Jerry Stober	—	U.S. Environmental Protection Agency
Dr. Steve Sundlof	—	University of Florida
Dr. Michael Whitten	—	KBN Engineering and Applied Sciences, Inc.
Dr. Edward Zillioux	—	Florida Power & Light Company

APPENDIX B

CHICAGO MUSEUM TANNING RECIPE

Practical Chrome Tanning Mammal Skins With Hair On

By
Dominick Villa

The heavy hides should be tanned separately from small and medium sized skins. Chrome tanning is always done by immersion, and good strong, permanent, soft leather can be made by carefully following this method. It is very important to prepare the skins properly before tanning.

Do not tan skins for mounting with chrome. The color of chrome does not affect the hair even on white skins. The quantity of materials given here are for small and medium size skins, and are based on each gallon of water needed. Prepare the skins first in the usual way by scraping, fleshing, washing them clean from dirt, grease, salt, and blood. It might be necessary to wash the very greasy skins with soap and warm water and rinse them well in clear water after most of the grease has been scraped off. Then soak the skins in the following solution:

1 gal. water

8 oz. salt

4 oz. alum

1 teaspoonful carbolic acid

Do not use a metal container. Stir the skins occasionally.

After a day or two if any more scraping or shaving is necessary, let the skins drain over the tank, and do it at this time. Put the well prepared skins back into alum and salt solution. For each gallon of solution that you have, dissolve 1/2 oz. of "chrome tan" by boiling

in water for ten minutes, using one cup of water for each 1/2 oz. Add to the alum and salt solution in two portions of one day apart, without removing the skins, stirring frequently.

The next day after the last portion, for each gallon of the solution that you have, dissolve 8 grams of sodium carbonate using one cup of hot water for each 8 grams, and add to the solution very slowly by punching a small hole in bottom of an empty can fixed above the solution. Let the dissolved soda drip into the tanning solution in a small stream without removing the skins, and by stirring to prevent the foam and milk-like color formation. Leave the skins in the solution five or six days longer or more, stirring occasionally. In cold water chrome is slow to penetrate. Also the thicker ones will take longer to tan.

Now take the skins out and rinse them in clear water. Wring the water out, and while still wet give them a good coat of the following oil mixture:

Two parts of warm water by measure

One part of sulphonated neatsfoot oil by measure

One part of non sulphonated neatsfoot oil by measure

For each gallon of the mixture add 1/4 ounce carbolic acid by volume, hang the skins to dry on wooden poles or sticks. Let them dry completely so the oil can penetrate. Now take the dried skins down and using a sponge dampen them on the flesh side surface with water to which 1/4 ounce carbolic acid has been added per gallon. Fold them up and put them in an empty barrel or anything else that can serve

as a "damp box", and cover well using oil cloth or plastic sheet material. It is important to keep the moistened skins from drying out. The thicker ones might be necessary to dampen more than once.

The next day work them soft by stretching and pulling them over a dull "bench flashing knife." The large ones can be worked over the beam, with a dull scraping knife. This is an important part of the tanning work, and must be done right. In large tanneries it is referred to as "Staking." The idea of stretching them is to separate the fiber so the oil will get in between and the skins will no longer be hard. It will work very well provided the skins are in the right condition, that is, not too wet and not too dry.

Now let them run in the "Drum" with dry sawdust for two hours. It is much better to put many skins in the "Drum" at one time. Be sure they are only slightly damp at the time they are put in the "Drum." Cage or beat the sawdust out. Now they are ready for shaving.

Skins like foxes, rabbits, opossum etc., should be fleshed before tanning. But others, like deer flat skins, raccoon, wolves, pumas, small calves and bear skins, etc. should be scraped and partly shaved on the thicker places only before tanning, and completely shaved after the tanning. Soak the flesh side surface with carbolic ~~and~~ water using a sponge. Fold them up tight and place them back in "damp box." Keep them well covered so the water can penetrate. The next day shave them down all over on the beam, using a hand shaving knife. (Currie's knife.)

After they are well shaved all the skins must be neutralized by soaking and washing them for half hour in solution of 1/2 ounce of Borax for each gallon of luke warm water needed. Rinse in clear water. Wring the water out, and nail the skins on the stretching board, stretching them as much as possible. Dry the skins in this position, with the exception of the ones that have to be used for fur. These can be dried by hanging them on sticks. Now finish all the dried skins by running them in the "Drum" again for about two hours with new dry sawdust.

Heavy skins like cow hides can be chrome tanned with the same procedure as for small skins, but be sure they are first well shaved to the proper thickness.

The quantity of chrome for heavy hides should be increased from 1 to 1 1/2 ounces per gallon and added to the solution one third at a time of one day apart, the soda should also be increased to 1/2 ounce per gallon and must be added very slowly using half one day and the remainder the next day. The quantity of salt is the same as for small skins, the alum should be 6 oz. per gal. Be sure the heavy skins are left in solution long enough. It will take about three weeks in order for heavy hides to be well tanned. Stir occasionally during the time they are in solution.

Note: Chrome tanning is very popular and the above method should be of interest to the skin and fur dressers. With chrome a hide can be tanned which resists shrinking or hardening in boiling water, so it must be good. Skins for mounting or to be made into fips furs are

not supposed to be tanned into a real leather. However, some nice skins to be used around the house as throw rugs will come out much better and in a nice flat shape if they are tanned in the above manner, and they will not retain the odor of wild animals or the tanning oil. As for the Museum's study collection, it is of the utmost importance that the skins be tanned so they can be preserved in a good shape for a very long time. The neutralizing and washing explained above should leave the skins free from any chemical or oil which can harm the hair or the leather in the future. The quantity of chrome is adjusted according to the type of tanning to be done and is not by any means for a full chrome tanning.

Chrome tanned skins will not turn out right unless they are tacked and dried on the stretching board as explained above. "Krome-tan" is a trade name like "Tanolin", "Koreon" etc. for a series of commercial "One Bath" chrome tanning materials. "Krome-tan" is sold by the Northwestern Fur Co. Carbolic acid is not a tanning material. It is a strong preservative and will keep a solution or wet skins from getting sour.

APPENDIX C

**NATIONAL MUSEUM OF CANADA METHODS OF COLLECTING AND
PRESERVING VERTEBRATE ANIMALS**

DEPARTMENT OF THE SECRETARY OF STATE

NATIONAL MUSEUM OF CANADA

Bulletin No. 69

Biological Series No. 18

Methods of Collecting and Preserving
Vertebrate Animals

BY

Rudolph Martin Anderson

(Fourth Edition Revised)

Issued under the authority of the
Secretary of State

1965

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kept stirred while a skin is being dipped, will do very well. Sodium arsenite diluted with water is better, as it penetrates the skin and is soluble. The sodium arsenite solution is made as follows:

Arsenic trioxide (white arsenic, As_2O_3).....	1 pound
Carbonate of soda (washing soda, Na_2CO_3).....	1 quart
Water	1 quart

Boil for an hour, stirring occasionally (Rowley, 1925).

If the skin softens after dipping, it should be re-salted and thoroughly dried out again.

This formula may be used to poison the exterior of mounted specimens that are infected with moths or dermestids, after spraying or painting the hair with gasoline (applied outdoors). Poison preparations on the inside of the skin help to keep out insect pests and protect the skin itself, but unfortunately there is no substance yet known that will work through the skin and positively protect the hair or feathers. The old method of spraying or brushing the outside of a specimen with corrosive sublimate or bichloride of mercury ($HgCl_2$) dissolved in alcohol is effective enough as far as insects are concerned, but is extremely dangerous and is not recommended, as persons handling the specimen later are very apt to suffer from mercuric poisoning. In dusting such specimens the invisible bichloride powder may be inhaled or absorbed by the skin.

The development of the use of DDT (dichloro-diphenyl-trichloro-ethane) solution about 1944 for control of various insect pests, including moths and beetles which damage or ruin skins, furs, and woollen fabrics, has been very successful and may supersede some of the older insecticides and repellents. It may be dusted on as a powder, or sprayed in a solvent or emulsion. C. R. Twinn (*in litt.*, 1947) cites experiments by Jensen and Holdaway (1946) who conducted experiments to determine the comparative efficiency of various materials in protecting rabbit hides from larvae and adults of two species of *Dermestes*. They found DDT to be outstandingly superior to all others. When applied in spray and dust form a single treatment gave excellent protection for at least 371 days, and hides treated while fresh were as well protected for extended periods as were hides treated after they had been dried. Two forms of DDT were used, namely, dusts and sprays. He recommends spraying with 5 per cent DDT in deodorized kerosene, provided that a light application of kerosene would not harm the specimens. DDT itself has been used successfully as a moth-proofing agent on various kinds of textiles. For this purpose it is efficient against clothes moths as well as *Dermestid* beetles.

Conditions in Canada are such that if fresh specimens are carefully watched in the field and are clean when shipped, hair poisoning is seldom necessary, as the specimens can be treated in the museum before serious damage is incurred. If a large skin, or mounted mammal or head, has been treated with hair poison, it should be marked "Poisoned with" for the benefit of persons who have to handle it afterwards.

SPEED AND EFFICIENCY IN TECHNIQUE

Getting into the haunts of big game at the present time in North America usually involves long travel and heavy expense, and frequently when the game is stalked and killed the end of the day is near and time