

## DISTRIBUTION OF CHROMIUM IN VEGETATION AND SMALL MAMMALS ADJACENT TO COOLING TOWERS

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### ABSTRACT

Surface contamination of vegetation by aerosol pollutants and subsequent ingestion by grazing vertebrates is a pathway for incorporation of toxic elements into food chains. Small mammals (herbivores) were live-trapped in a fescue-dominated field adjacent to large, mechanical draft cooling towers comparable to those utilized by power generation facilities. Cooling waters of the towers contain a chromate, zinc-phosphate compound to inhibit corrosion and fouling within the cooling system. A fraction of the cooling water becomes entrained within the exit air flow and is deposited as drift on the landscape. Resident mammals are subjected to increased chromium exposures both through ingestion and through inhalation pathways. Concentrations in vegetation ranged from 342 to 15 ppm at 15 and 130 meters downwind. Chromium distribution in mammals adjacent to the cooling towers was compared by organ analyses to corresponding organs and tissues of mammals collected remote from drift. Concentrations of chromium in pelt, hair, and bone of animals trapped near the cooling towers were significantly higher ( $P < 0.01$ ) than concentrations in tissues from control animals. Air concentrations of chromium had previously been determined to be relatively constant at  $50 \text{ ng/m}^3$  within 200m of the tower (Alkezweeny et al., 1975), and thus provided a potential pathway for increased chromium levels through inhalation. The fate of ingested chromium-contami-

nated vegetation and possible incorporation by body tissues was determined from a feeding experiment using radiolabelled chromium.

### INTRODUCTION

Chromium, an essential trace element found in almost every living organism, has been reviewed in detail as a potential environmental pollutant (National Academy of Sciences, 1974). As with many other trace elements or compounds, increased quantities often produce deleterious effects. Trace levels are considered essential to some mammalian species and irreversible metabolic damage may result from a long-term chromium deficiency. Chromium is a complex element occurring in several oxidation states. Hexavalent chromium (+6) is more toxic than the trivalent form (+3) because of its oxidizing potential and ease in penetrating biological membranes. Interaction of chromium oxide, dichromate or chromate compounds with organic matter can result in reduction to the inert trivalent form. Unreliable or non-reproducible analytical techniques have resulted in erroneous conclusions concerning the ambient or natural occurrence of the element and its effects on biota (National Academy of Sciences, 1974). Elevated levels of chromium have generally paralleled advances in technological and industrial uses of the element or compounds containing chromium. Such increased burdens in the environment may be derived from uses in plating and foundry applications, chemical manufacturing, and corrosion inhibition.

Deficiencies in extant data relative to biological effects and the cycling of chromium in the environment are evident in the lack of standards for maximum allowable concentrations (MAC) in ambient air, foods, dietary intake, or feeds for livestock. Standards, although questionable, do exist for drinking water (U.S. DHEW, 1962) and are recommended for atmospheric concentrations in industry (American National Standards Institute, 1971). Case studies utilizing appropriate analytical techniques, in environments receiving airborne chromium or release to surface streams, are necessary to quantify the magnitude of contamination from industrial sources and the transport of trace quantities to various trophic levels by food chains.

#### SCOPE OF STUDY

Field studies were limited to the landscape adjacent to the cooling towers of the uranium-enrichment facility of the Energy Research and Development Administration (ERDA) at Oak Ridge, Tennessee. Recirculating water of the towers utilizes significant quantities of chromate ( $\text{CrO}_4^{2-}$ ) to inhibit corrosion within the cooling condensers. The heat dissipated by the towers is comparable to that of 1000-2000 MW electric power generation stations. Data thus far have provided quantitative evidence of the transfer of significant amounts of chromium in drift to vegetation and soil (Taylor et al., 1975). The major concern of this study is the fate of ingested drift-contaminated vegetation and possible incorporation of trace quantities of chromium by small mammals.

#### METHODS

Cooling towers of the Oak Ridge Gaseous Diffusion Plant are situated on a north-south line, oblique to predominant northeast-southwest winds (Hilsmeier, 1963). Plant cover around the facility is maintained in grassland vegetation, with fescue (*Festuca arundinacea* Schreb.) the dominant species. Stability of the vegetation cover is controlled by periodic mowing and bush-hog maintenance procedures. Vegetation clip plots ( $0.1 \text{ m}^2$ ) were located along a distance gradient north and east of the tower in a 3.5-hectare area inhabited by cotton rats (*Sigmodon hispidus* Say and Ord). A trap grid was established parallel to the cooling tower (0-200 meters north) and extended perpendicular from the tower base beginning at 100 meters to a distance of 130 meters. The area closer than 100 meters was not suitable habitat for cotton rats and consequently was not trapped.

Cotton rats were live-trapped during the last week in June. Single samples of fescue grass and litter were collected on the last day of the capture period at the intersections of the grid lines. Total chromium in plant materials was determined by atomic-absorption spectrophotometry using preparation techniques of a previous study (Taylor et al., 1975). Controls for vegetation and litter were collected in areas remote from cooling tower drift, whereas control animals were from a contaminant-free laboratory population of the native species. In addition to the field study, twenty laboratory-born animals were fed fescue contaminated with radio-labelled chromium (as  $\text{Na}_2^{51}\text{CrO}_4$ ) and measured radiometrically for assimilation and retention estimates.

Organs and tissues of cotton rats were freeze-dried and ashed at  $450^\circ$  centigrade prior to analyses by automated colorimetric methods (Saltman, 1952; Gaddy, 1965) and atomic-absorption techniques for microdeterminations of chromium. Tissues or organs of animals exposed to drift were pooled into six samples of four animals each (except for hair, pelt and residual tissues, which consisted of one sample each of 24 animals). Tissues of control animals were not pooled, but were analyzed

individually with the addition of a graphite furnace attachment to the spectrophotometer. Tissue analyses were for one sample each of six animals (except for hair, pelt and residual tissues, which consisted of one sample each of three animals). One lobe of lung from each cotton rat was fixed and examined histopathologically.

#### RESULTS AND DISCUSSIONS

##### Vegetation

Most plants contain only trace amounts of chromium, ranging from 0.1 to 1 ppm (Saint-Rat, 1948; Hanna & Grant, 1962). Plant species having known concentrations are good indicators of foliar contamination or monitors of atmospheric transport from industrial sources. In a previous study, fescue grass (*Festuca arundinacea* Schreb.) was used to identify the quantity and the distribution pattern of chromium transported to terrestrial environments from cooling tower drift (Taylor et al., 1975).

The area most noticeably affected by drift from the cooling towers is within the first 300 meters. Chromium concentrations in grass are 2-5 times above natural levels at the distance of 2 kilometers from the towers. Orientation of the ORGDP mechanical draft towers perpendicular or oblique to prevailing winds magnifies the deposition of drift near the tower (Hanna, 1975). Concentrations were highest near the tower base, decreased exponentially with distance, and ranged from a mean of 342 ppm at 15 meters to 15 ppm at 130 meters downwind. In comparison, the mean chromium concentration in fescue in the environs remote from drift is  $0.55 \pm 0.03$  ppm ( $n=20$ ).

##### Mammals

Although the concentration of chromium in foliage within the first 100 meters is several orders of magnitude above natural concentrations, biological effects on natural vegetation are not apparent. The magnitude of contamination of foliage does, however, represent a potential pathway for incorporation of trace quantities in native mammals through ingestion. Additionally, air concentrations in the order of  $50 \text{ ng/m}^3$  (Alkezweeny et al., 1975) could result in further additions to the total body burden through inhalation. This latter consideration is important pathologically, as hexavalent chromium is a known carcinogen (National Academy of Sciences, 1974).

Twenty-four adult cotton rats were live-trapped along trap lines corresponding to the 100, 110, 120, and 130 meter horizontal transects used for vegetation collections. The primary food base of the cotton rats inhabiting these areas of the Oak Ridge Reservation is fescue grass (Digregorio et al., 1972). Each mammal was sacrificed and dissected into major organs or tissues. Analyses of chromium concentrations in pelt and hair ( $n=24$ ) were made according to the trap line position where the animal was captured. Concentrations in pelt ranged from  $0.9 \pm 0.3$  ppm to  $1.2 \pm 0.4$  ppm, while hair varied from  $3.9 \pm 1.2$  to  $4.8 \pm 1.4$  ppm along the distance gradient. An analysis of variance revealed that concentrations among the tissues of animals from the four trap lines were not significantly different. The similarity of concentrations in pelt and hair suggested that the animals ranged throughout the area of drift deposition

and that observed levels of chromium reflected an integrated average of concentrations in vegetation over the distance gradient. Whereas an animal inhabiting the areas of greatest contamination might ingest or accumulate significant quantities of chromium, the nature of its activities appears to limit the time in any particular area. The lack of statistical differences among these components between the trap lines provided a basis for pooling subsequent tissues and organs for microdeterminations of chromium. The pooling was considered essential with small organs to ensure adequate material for analytical procedures. Subsequent comparisons between organ and tissue concentrations are made between control animals and those which were exposed to chromium in cooling tower drift.

The lack of basic elemental data from wild mammals of other geographical regions limits adequate comparisons. Analyses of tissues from human autopsies (National Academy of Sciences, 1974) are useful to identify organs or tissues which may accumulate chromium and, in some instances, agree with data from analyses of native cotton rats. The distribution of chromium in animals exposed to chromate from cooling tower drift and controls is summarized in Table 1. Using statistics of fresh weights obtained from cotton rats captured on the Oak Ridge Reservation (O'Farrell et al., 1966), calculations are presented for total body burden of a "standard" adult animal. Total freeze-dry weights (standard animal) are calculated for each organ or tissue from the ratios of freeze-dry to fresh weights determined from animals trapped near the cooling towers. Total organ or tissue contents are expressed on a freeze-dry basis. Total chromium of an adult cotton rat is depicted in Table 1. The estimated whole body concentrations in background and exposed animals ranged from 0.2 to 0.4 ppm, respectively. Concentrations in organs of control animals ranged from 0.05 to 1.05 ppm. In contrast, concentrations in organs in animals exposed to drift ranged from 0.1 to 4.4 ppm (Table 1). Using an unpaired, pooled variance "t" test, significant differences ( $P < 0.01$ ) in concentrations of pelt, hair, and bone between control and treatment animals were identified. Hair accounted for only 3% of total animal weight (freeze-dry), whereas the fraction of total chromium was 9 and 34% for control and treatment of animals, respectively. Bone accounted for 16% of total weight, while pelt comprised 27% of total freeze-dry weight. Bones of cotton rats exposed to cooling tower drift contained 18% of total chromium in comparison to 13% in controls.

We hypothesize that the increased quantities of chromium in pelt and hair were derived from direct deposition of drift or removed from plant materials during feeding activities or during movements along runways. Increased chromium concentrations within tissues or organs are likely derived from dietary intake. Chromium is absorbed by the intestines and almost totally bound to transferrin, an iron-carrying protein in plasma (Hopkins & Schwartz, 1964). Iron is present in significant quantities in bone marrow. Thus, the strong affinity of marrow and blood-forming tissues for

transferrin may account for the significant differences in chromium observed in bone. This is further suggested in that the femurs, a major site of blood production, were used for elemental analyses of chromium. In another report, concentrations of both trivalent and hexavalent chromium were reported to be highest in bone (Shiraishi & Ichikawa, 1972).

Drift particles small enough to be inhaled would likely penetrate into the alveoli of the lung and become trapped in the tissue if insoluble. Measured air concentrations (Alkezweeny et al., 1975) within the trap area would suggest chronic exposures of mammals throughout their life span to increased levels of chromate and the possibility of lung carcinoma. Available evidence from occupational exposure to hexavalent chromium indicates the long-term effect to be a high incidence of lung cancer (National Academy of Sciences, 1974). Drift particles as a soluble hexavalent chromium would likely penetrate the cells, and be distributed by the blood throughout the body, or be reduced to the insoluble trivalent form and likewise become trapped. The lack of significant accumulations of chromium in the lungs suggests that either the dense vegetation cover and protection afforded by litter over runways intercepted the majority of drift and minimized the quantity inhaled or the fraction inhaled remained soluble as chromate and subsequently was redistributed throughout the body. One lobe of each lung from the cotton rats was examined histologically for evidence of pathological damage. In no animal was there evidence of edema, lesions, or carcinoma. Considering the relatively small concentrations of chromium in the lung (Table 1), this might be expected.

Even though three tissues showed statistically significant differences ( $P < 0.01$ ) in chromium concentration, the mechanism of incorporation or biological significance of the increased levels were not known. The significance of chronic exposure by ingestion to drift contaminated food was determined by feeding adult animals fescue grass labeled with isotopic chromium in a chemical form ( $\text{Na}_2^{51}\text{CrO}_4$ ) simulating the valence state of chromium as maintained in the recirculating cooling waters. Each animal was fed grass containing  $3.75 \mu\text{Ci}$  of Cr-51, and was measured radio-metrically for retention during 2 weeks post feeding. Initial whole-body radioactivity ( $A_0$ ) was  $0.0073 \mu\text{Ci/g} \pm 0.001 \text{ SE}$ . The elimination of Cr-51 by the cotton rats resulted in a two-component curve, which was resolved into a short component representing gut clearance and a long component illustrating loss of the assimilated fraction. The retention data were fitted to an exponential model (Van Hook & Crossley, 1969) described by the equation

$$A_t = A_0 (a_2 e^{-\lambda_2 t} + a_1 e^{-\lambda_1 t}), \quad (1)$$

where  $A_t$  represents whole-body activity at time  $t$ ,  $A_0$  is the initial whole-body activity, and  $a_2$  and  $a_1$  describe the assimilated and non-assimilated radionuclide eliminated at rates  $\lambda_2$  and  $\lambda_1$ , respectively.

The percent assimilated as determined from the intercept of the long component is 0.79 where  $a_2 = 1 - a_1$  or  $a_2 = 1 - 0.9921$ . Within 3 days post feeding,

greater than 99% of the initial whole-body radioactivity was eliminated through gut clearance ( $\lambda_1 = -1.94$ , whereas the remaining fraction (< 1%), representing loss by body tissues of the assimilated radionuclide, was eliminated at a much slower rate  $\lambda_2 = -0.001$ ). By 16 days post feeding, approximately 0.7% or 113 dpm/g of the initial body activity remained (Fig. 1).

The low assimilation (0.8%) and rapid initial loss of the hexavalent chromium suggest the nonessential nature of the element in cotton rats. The estimated fraction absorbed by man (ICRP, 1958) through ingestion (< 0.005) is similar to the fraction assimilated by the native rats (0.008). Similarly, the biological half-times of chromium assimilated by man and cotton rats are 616 and 693 days, respectively. The magnitude of the half-times suggests a high potential for biological interactions to chromium derived from a chromate. The retention data confirm the lack of any significant bioaccumulation as depicted in stable analyses (Table 1), and further suggest the reduced probability of a toxic effect through ingestion of drift contaminated vegetation or inhalation of drift contaminated air.

TABLE 1: Comparison of distribution patterns of chromium in cotton rats (*Sigmodon hispidus*) exposed to cooling tower drift and control animals.

Organ or Tissue	Fresh Weight Standard Adult <sup>d</sup> (g)	Freeze-Dry Weight (g)	Concentrations ( $\mu\text{g} \pm 1 \text{ SE}$ )		Total ( $\mu\text{g}$ )	
			Control	ORGDP <sup>a</sup>	Control	ORGDP <sup>a</sup>
Heart	0.42	0.08	0.105 $\pm$ 0.013	0.124 $\pm$ 0.016	0.008	0.010
Liver	4.24	0.89	0.046 $\pm$ 0.016	0.160 $\pm$ 0.039	0.041	0.142
Kidney	1.02	0.19	0.087 $\pm$ 0.014	0.124 $\pm$ 0.040	0.017	0.024
Spleen	0.13	0.02	0.493 $\pm$ 0.123	0.713 $\pm$ 0.084	0.010	0.014
Lung	0.55	0.10	0.289 $\pm$ 0.072	0.292 $\pm$ 0.063	0.029	0.029
Bone	8.09	5.02	0.160 $\pm$ 0.008	0.460 $\pm$ 0.024**	0.803	2.309
Muscle	55.29	13.82	0.234 $\pm$ 0.043	0.288 $\pm$ 0.029	3.234	3.980
G. I. Tract	7.20	0.43	1.046 $\pm$ 0.277	1.006 $\pm$ 0.183	0.450	0.433
Pelt	17.39	8.35	0.092 $\pm$ 0.007	1.056 $\pm$ 0.133**	0.768	0.818
Hair	4.08	0.98	0.395 $\pm$ 0.021	4.397 $\pm$ 0.555**	0.387	4.309
Residual <sup>b</sup>	9.80	1.67 <sup>c</sup>	0.200 $\pm$ 0.032	0.311 $\pm$ 0.025	0.334	0.519
Totals	108.21	31.55			6.081	12.587

<sup>a</sup> ORGDP = Oak Ridge Gaseous Diffusion Plant.

<sup>b</sup> blood, reproductive organs, brain, salivary and thyroid.

<sup>d</sup> O'Farrell et al. (1966).

<sup>c</sup> converted from mean ratio of freeze-dry to fresh weights of all other soft tissues.

\*\*P < 0.01 significant difference.

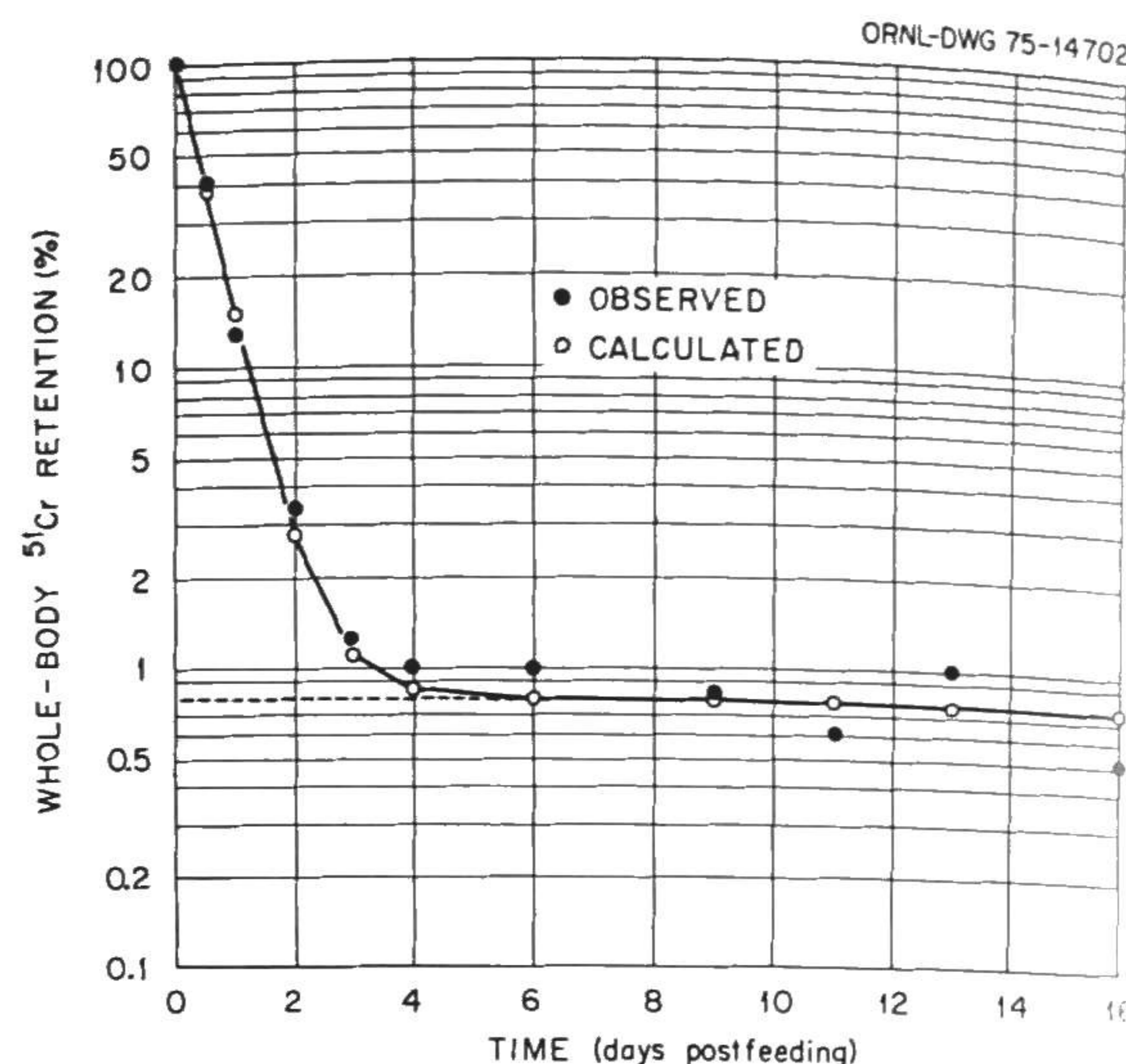


FIG. 1: Retention of chromium-51 by cotton rats following ingestion of contaminated fescue. Each observation represents a mean of twenty animals. The percent assimilated is determined from the intercept of the long component.

## CONCLUSIONS

The results summarized constitute a portion of a case study of some environmental aspects of drift transport from several large mechanical draft cooling towers of the Energy Research and Development Administration's uranium-enrichment facility at Oak Ridge, Tennessee. As these cooling towers are comparable to those used by 1000 MW electric generation stations, the data are valuable as guides to utilities using similar cooling devices. Measurements of drift loss (as chromate), deposition, and accumulations in natural vegetation and small mammal populations have provided quantitative data useful for the assessment of the potential environmental impacts of evaporative cooling towers. Vegetation analyses along distance gradients from the cooling towers identify areas of significant drift deposition, accumulation, and magnitude of atmospheric transport over the landscape. The elevated chromium concentrations in vegetation suggest a potential pathway for transfer of trace quantities to animal food chains. Elemental analyses of organs from cotton rats inhabiting the area nearest the tower identify the small mammals as useful indicators for assessing long-term accumulations in food chains. Similarities in chromium concentrations reflected the mobility of the animals within the trap grid, and indicated an integrated average between concentrations in vegetation over distance gradients. The low assimilation and rapid initial loss of hexavalent chromium by cotton rats suggests the nonessential nature of the element and indicate the lack of bioaccumulation. The data further suggest that similar studies are feasible for different tower designs or configurations where different biocides or inhibitors are used. Inventory data of concentrations in vegetation are sensitive as indicators of the efficiency and condition of the drift eliminators within the cooling towers.

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