parenchyma as compared to when it was present in a patchy distribution such as occurred following administration on particles. These results are consistent with those of earlier experiments utilizing higher doses (*7, 9*). With similar nanocurie amounts per instillation, ^{210}Po administered in saline (Table 2) yielded a somewhat lower radiation dose than did ^{210}Po administered on particles (Table 1). This is because the soluble ^{210}Po was more efficiently cleared from the lung in the early time periods, particularly by way of the bloodstream.

Doses in the range of several thousand to 10^5 rads have generally been necessary for the induction of experimental lung cancer by beta or gamma radiation (*5*). In two other studies, however, lung tumors have been induced by relatively low doses of alpha radiation. Yuile *et al.* (*10*) found primary lung tumors (mostly epidermoid carcinomas) in 3 to 13 percent of rats which received 71 to 538 rads from ^{210}Po inhaled in a sodium chloride aerosol. These results were complicated somewhat by the presence of acute and chronic pulmonary infection which was endemic in their rat colony. Recently, Sanders (*11*) has reported inducing lung cancer (primarily bronchioloalveolar carcinomas) in 6.6 to 25 percent of rats receiving 9 to 375 rads from inhalation of an aerosol of "soluble" ^{238}Pu (^{238}Pu emits alpha radiation similar to that of ^{210}Po). In his lowest exposure group, bronchioloalveolar carcinomas were found in 2 of 30 rats which received only 9 rads to the lungs from an initial lung burden of 5.0 nc of inhaled ^{238}Pu. One lung tumor, a large cell undifferentiated carcinoma, was found among 92 control (untreated) rats.

In these studies, as in ours, the estimated lifetime radiation dose was averaged over the entire lung volume. Local doses to small tissue volumes where the radioactivity may preferentially accumulate or concentrate, such as occurs in bronchial epithelium of cigarette smokers' lungs (*3*), may have been significantly higher. This would certainly be the case following intratracheal administration of ^{210}Po on ferric oxide carrier particles. Following administration of ^{210}Po in saline, however, there was no autoradiographic evidence of inhomogeneities in the microdistribution of radiation dose throughout the bronchiolar-alveolar region of the lung (the target tissue) (*9*). Local tissue doses would thus be expected to approach the whole lung average. Such would also appear to be the case for the inhalation of soluble ^{238}Pu (*11*).

From these considerations, it appears reasonable to conclude that the local alpha radiation doses associated with the induction of lung cancer in some experimental animals may be within the same general order of magnitude as those received by cigarette smokers to small areas of the bronchial epithelium from deposited ^{210}Po. The total amount of ^{210}Po administered to our lowest exposure group in Table 1 (3.75×10^3 pc) is roughly one-fifth the amount inhaled by a heavy cigarette smoker (two packs per day) during 25 years (*2, 12*). In addition, not only does cigarette smoke contain small amounts of many chemical carcinogens which may be acting synergistically with the alpha radiation, but the respiratory tract is also unusually vulnerable to infection which may enhance the carcinogenic effect of radiation (*13*).

These results tend to support the hypothesis that ^{210}Po or ^{210}Pb in cigarette smoke may be a significant factor in the initiation of lung cancer in smokers. They are, moreover, components which are well characterized, do not contribute to "flavor," and should be relatively easy to remove from cigarette smoke (*14*).

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Tetrachlorodibenzodioxin: An Accidental Poisoning Episode in Horse Arenas

Abstract. *Tetrachlorodibenzodioxin was identified as the apparent cause of an outbreak of poisoning in humans, horses, and other animals. Exposure was related to the spraying of contaminated waste oil on riding arenas for dust control. The contamination resulted from the improper disposal of a toxic industrial waste. The pathologic effects and chemical identification of tetrachlorodibenzodioxin are described.*

2,3,7,8-Tetrachlorodibenzodioxin (TCDD) is a particularly toxic compound; the oral LD_{50} (lethal dose to 50 percent of a test group) for many animal species is in the range of micrograms per kilogram (*1*). It is a very stable compound, with a half-life in soil of about 1 year (*2*). It is only gradually excreted in the feces and urine of mammals, and its toxic effects suggest that it accumulates in body tissue after recurrent exposure (*1, 3*). The known toxic effects of TCDD include anorexia, severe weight loss, hepatotoxicity, hepatoporphyria, vascular lesions, chloracne, gastric ulcers, teratogenicity, and delayed death (*1*).

Chlorinated dibenzodioxins include a large number of compounds, some extremely toxic and others essentially nontoxic. They have no known use, but occur as contaminants in technical products such as tri-, tetra-, and pentachlorophenol and a number of other related compounds (*4*). The most toxic of the chlorinated dibenzodioxins, TCDD has been associated with several industrial accidents in which the subsequent cleanup of the area contaminated by this compound created major problems (*5*). This report describes the first recognized incident in which significant poisoning resulted from the improper disposal of waste residues containing TCDD.

Waste oil sludge is frequently used to control dust on riding arenas and dirt roads. On 26 May 1971, a salvage oil company sprayed an arena for this purpose on a horse breeding farm in eastern Missouri. Three days after the oil sludge spraying, sparrows and other birds that normally populated the barn rafters were found dead on the arena floor. Over the next several weeks, hundreds of birds, several cats and dogs, and numerous rodents died after being exposed to the arena (*6*). Of the 125 horses on the farm at the time of spraying, 85 were exercised for varying periods within the arena. Sixty-two of those exposed became ill, and 48 died. The first horse death occurred on 20 June, and despite removal of soil from the arena in October 1971 and April 1972, horses continued to die as late as January 1974. All of

these deaths occurred among horses exposed to the sprayed arena soil in the summer of 1971. Among the signs of toxicity in the affected horses were chronic emaciating weight loss, loss of hair, skin lesions, dependent edema, intestinal colic, dark urine, gross hematuria, conjunctivitis, joint stiffness, and laminitis. In addition to the laminae, the soles and frogs of the horses' feet were particularly inflamed.

Within 3 weeks after spraying this arena, the same salvage oil company sprayed two additional horse arenas and a road on a farm in eastern Missouri with sludge oil. Several horses died of similar illnesses in these arenas. Seventy chickens that were exposed to the sludge oil on the farm road died within 2 weeks after the spraying.

Human illnesses were less severe, but included one case of hemorrhagic cystitis in a 6-year-old girl who frequently played in the arena soil. Cystoscopy confirmed this diagnosis, and a retrograde pyelogram revealed signs of focal pyelonephritis. Bacterial and viral cultures were negative. Three other children and one adult frequently exposed to the arena complained of skin lesions. In at least two of the children, the lesions described were consistent with chloracne. Intermittent arthralgias have been associated by two adults with previous exposure to the arena.

Initial analysis of an arena soil sample revealed a complex mixture of organic compounds. The detection of colorless needle-shaped crystals in a condenser, which were subsequently identified as 2,4,5-trichlorophenol (TCP), led to the identification of TCDD and related chemicals. Although TCP has limited toxicity, its presence suggested that TCDD, a known contaminant in TCP production, might also be in the soil.

To test for TCDD and to confirm the toxicity of the arena soil, a rabbit ear bioassay was performed using adult New Zealand rabbits. Using a Soxhlet extractor, the neutral fraction from a dichloromethane extract was prepared and concentrated to a dark, oily residue. A dose of 0.2 ml of this material was applied daily for 3 days to the inner aspect of one ear on each of four rabbits. A second experimental group of four rabbits received a similar 0.2-ml application of a concentrated acetone extract of the arena soil daily for 5 days. A third group of four rabbits served as controls; 0.2 ml of refined salvage motor oil of known composition was applied daily to one ear of each rabbit in this group for 5 days.

While no toxic effects were observed in the control group, one rabbit in the first and two rabbits in the second experimental group died within 7 days of the first soil extract application (*7*). All surviving rabbits were killed 7 days after the first application. Skin from the treated ears and liver tissue were obtained for microscopic examination and stained with hematoxylin and eosin. The histopathologies of the two experimental groups were identical (see Figs. 1 and 2). The epithelium of the ears had undergone marked proliferation with widened hair follicles filled with keratin plugs. Sebaceous glands were virtually absent. The subcutis was edematous with an inflammatory reaction.

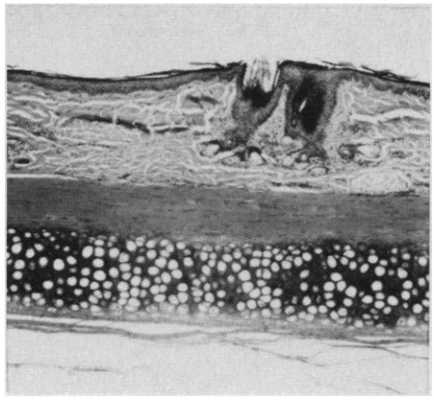

Fig. 1. Section of normal rabbit ear skin stained with hematoxylin and eosin (× 40).

Liver histology was abnormal in both experimental groups. In seven of the eight rabbits, extensive necrosis of the liver with associated hemorrhage was noted. The eighth rabbit liver had degenerative and necrotic changes in individual hepatocytes. Multinucleated hepatocytes were observed. Bile duct epithelium and occasionally small blood vessel endothelium appeared degenerated. In some sections, early fibrosis and bile duct proliferation were evident, and sinusoids contained inflammatory cells. All control rabbits had normal histology.

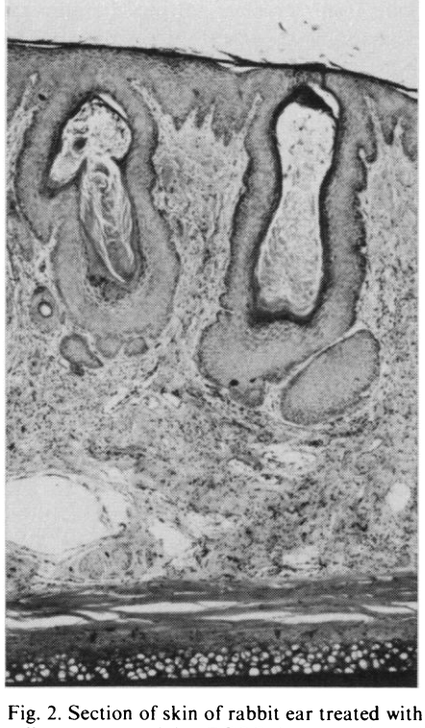

Fig. 2. Section of skin of rabbit ear treated with soil extract containing TCDD; hematoxylin and eosin stain. Note the increased thickness of the skin, pronounced proliferation of the epithelial cells, absence of sebaceous glands, and increase in keratin (× 40).

The liver necrosis and skin lesions of the rabbits' ears represent a typical response to TCDD and some related compounds (*1*). To substantiate the presence of TCDD, the neutral fraction of the soil extract was concentrated and analyzed by an adaptation of a method described by Firestone *et al.* (*8*). For gas-liquid chromatography a Tracor 220 gas chromatograph was employed, which was equipped with a 1.8-m glass column (inner diameter, 2 mm) packed with 3 percent OV-17 and a Coulson conductivity detector. The column oven was programmed from 180° to 225°C at a rate of 4°C per minute. The TCDD was determined quantitatively by methods of addition using standard TCDD (*9*).

Additional confirmation was obtained using an LKB 9000 gas chromatograph–mass spectrometer with a glass column (7.32 m by 0.63 cm) packed with 5 percent TABSORB on Chromosorb W(HP) (80 to 100 mesh) held isothermal at 225°C. The TCDD was identified by comparing retention times and mass spectra of sample components to those of reference standards. The mass spectra of the unknown toxic component from the arena soil showed a base peak at a mass-to-charge ratio (m/e) of 320. A set of five peaks was obtained at m/e 320, 322, 324, 326, and 328 with relative intensities of approximately 75 : 100 : 50 : 10 : 1, which indicated a tetrachloro compound. Mass fragment ion peaks at m/e 285, 257, and 194 and a tetrachloro m^{2+} pattern at m/e 160 were obtained.

The horse arena soil was found to contain 31.8 to 33 μg of TCDD per gram (parts per million) (*10*). The neutral fraction extract of this soil used in the rabbit ear assay had 61 μg of TCDD in each 0.2-ml application.

Since commercial-grade products do not contain such high concentrations of TCDD, an industrial waste by-product of chlorinated aromatic compounds was suspected as the original source. Further investigation revealed that the sludge used to spray all three arenas came from a common storage tank at the salvage oil company. Salvage oils in this tank had been collected from a large variety of sources in eastern Missouri. Although none of the sources given by the salvage oil company could be associated with waste products

containing TCDD, a review of chemical industries revealed a hexachlorophene producer in southwestern Missouri that accumulated distillate residues containing TCDD during TCP production in 1970 and 1971. The hexachlorophene producer contracted disposal of these residues during 1971 to a chemical distributor. The distributor subcontracted this disposal to the salvage oil company that sprayed the three affected arenas. Between February and October 1971 the salvage oil company hauled the residue, totaling approximately 68,000 liters, in six separate trips. These residues were kept in the storage tank from which the sludge for spraying the three arenas was obtained. The hexachlorophene plant discontinued operations in late 1971, leaving the tank originally used to store the distillate residue at the plant site undisturbed. The remaining residue in this tank was sampled in 1974 and analyzed by the methods described above. A portion of the oily residue was diluted with petroleum ether, washed with dilute sodium hydroxide, and then chromatographed on an alumina column. The TCDD was quantitated by methods of addition, and identification was confirmed by mass spectrometry. The waste residue contained TCDD in concentrations of 306 to 356 μg/g.

The investigation demonstrates that the improper disposal of toxic chemical wastes may have serious consequences. Human illnesses and the death of a number of valuable horses occurred when industrial wastes containing TCDD were mixed with reclaimed motor oils and lubricants. TCDD or other chlorinated dibenzodioxins or chlorinated dibenzofurans may be present in the waste products from chlorination of benzenes, phenols, and polyphenyls, since traces have been found in the commercial products (*1, 11*). Companies responsible for disposal of such wastes should be aware of the toxicity of some of these chemical waste products and of the proper methods of disposal. More extensive regulation of disposal of toxic industrial wastes may be necessary to prevent similar or more serious occurrences.

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Mechanism of Insulin-Induced Paralysis of Muscles from Potassium-Depleted Rats

Abstract. *Zinc-free insulin elicited a reduction in the potassium conductance of muscle fibers from potassium-depleted muscle, which led to depolarization, blockade of action-potential mechanism, and paralysis. These changes are proposed as the mechanism of insulin-induced paralysis in patients with hypokalemic periodic paralysis. A similar effect by concanavalin A suggests that the effect may be mediated through the insulin receptors.*

Patients with hypokalemic periodic paralysis episodically develop muscle weakness associated with a decrease in the concentration of K^+ in the serum and a depolarization of skeletal muscle cells (*1*). Although there is evidence that an elevation of serum insulin may be responsible for the triggering of these episodes (*2*), the underlying mechanism remains unclear. One hypothesis is that the paralysis results from sarcolemmal inexcitability (*3*). Gordon *et al.* (*4*) proposed that the inward rectification characteristic of resting K^+ conductances (g_K) of the sarcolemmas might contribute to the development of sustained depolarization and inexcitability when the K^+ concentration in serum is low. A second hypothesis is that excitation-contraction uncoupling may be primarily responsible for the weakness (*5*).

For more detailed studies of this problem, the K^+-depleted rat appeared to be a useful animal model because their muscles possess characteristics similar to those seen in the patients' muscles: depolarization and paralysis induced by Zn^{2+}-insulin (*6*), lower intracellular K^+ concentration and higher Na^+ concentration than normal (*7*), and morphologically altered membranous organelles (*8*). Since Zn^{2+} has a marked effect on muscle g_K (*9*), we investigated the effects of Zn^{2+}-free insulin on the electrical membrane properties of diaphragm from K^+-depleted rats. Our results indicate that in these muscles insulin alone causes a decrease in g_K, leading to depolarization, inexcitability, and weakness.

Small strips of diaphragm from rats that had been kept on a K^+-deficient diet (*6*) for 4 to 8 weeks were mounted at room temperature in low-K^+ (0.5 m*M*) Tyrode solution with 3 mg of tubucurarine chloride per liter. The serum concentration of K^+ in rats ranged from 1.5 to 2.5 m*M*. The solution was oxygenated and buffered at *p*H 7.4.

After the addition of 1 to 5 mU of Zn^{2+}-free insulin per milliliter (Lilly, lot IDG 0497193), the maximum twitch tension elicited by direct stimulation started to decrease in 30 minutes and by 1 hour had usually fallen to less than 10 percent of its original magnitude. Increasing the concentration of K^+ in the bath to 5.5 m*M* fully restored the twitch tension in 15 minutes, and insulin did not change this in either normal or K^+-depleted muscle in the solution with 5.5 m*M* K^+.

Our next step was to locate the deficit in muscle activation produced by insulin. Contractures evoked by 20 m*M* caffeine increased slightly in amplitude after insulin-induced paralysis of twitch, indicating an intact response of the contractile filaments to the release of Ca^{2+}. Contractures evoked by 120 m*M* K^+ were either unchanged or only slightly decreased, even when the twitch response was completely paralyzed by insulin. This suggests that the loss of sarcolemmal excitability rather than a deficit in excitation-contraction coupling is responsible for the insulin-induced paralysis.

To study the sarcolemmal excitability, we made intracellular recordings of the same identified single fibers of K^+-depleted muscle, both before and 1 hour after addition of insulin (5 mU/ml) in the 0.5 m*M* K^+ solution. Each surface fiber